ORIGINAL ARTICLE

CLOPIDOGREL RESISTANCE AND ITS RELATION WITH AGE AND GENDER

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Background: Clopidogrel is an anti-platelet drug used for ischemic heart disease patients, but it is not equally effective in all patients due to clopidogrel resistance. Clopidogrel resistance is a precursor to secondary adverse cardiac events. This study was conducted to assess the magnitude of clopidogrel resistance in Pakistan and to find its association with age and gender. **Methods:** This was a cross-sectional study conducted from 2015 to 2017 at Army Medical College, National University of Medical Sciences, Rawalpindi, Pakistan. We included 390 ischemic heart disease patients, who were taking clopidogrel 75 mg/day for at least 7 days. Blood samples of all these patients were taken and platelet aggregation studies were performed with the help of light transmission aggregometer using adenosine diphosphate as an agonist. **Results:** Our study demonstrates that 75.1% of the patients were clopidogrel responders while 24.9% patients were clopidogrel resistant. Mean platelet aggregation of clopidogrel resistant patients was significantly higher than clopidogrel responders (60.22±7.33% *vs* 27.73±12.17%, $p<0.001$). There was no effect of age and gender on clopidogrel response status with $p=0.266$ and $p=0.860$ respectively. There was no difference in mean platelet aggregation of both genders ($p=0.532$) and among different age groups ($p=0.234$). **Conclusion:** Clopidogrel resistance is quite common in Pakistan affecting a quarter of local population and is not specific to any age group or gender.

Keywords: clopidogrel resistance, clopidogrel responders, light transmission aggregometer, ADP

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INTRODUCTION

Clopidogrel is one of the principal anti-platelet drugs used in the management of ischemic heart disease (IHD). Prevalence of IHD in Pakistan has been recorded to be 26.9% with males and females equally affected, irrespective of age groups. IHD comprises of angina pectoris, myocardial infarction, acute coronary syndromes, and sudden cardiac death. Definitive treatment of IHD is coronary revascularization while medical treatment including drugs like anti-platelet drugs, fibrinolytics, lipid lowering drugs, beta blockers, angiotensin converting enzyme inhibitors etc. are also valuable.[1,2]

Platelet aggregation plays a vital role in the formation of thrombi. Upon rupturing of thin cap over the atherosclerotic plaque, the platelets start adhering to the rough surface and bind to subendothelial matrix. Subendothelial matrix contains substances like collagen, von Willebrand factor, vitronectin etc., exposure to which causes platelet activation. Adenosine diphosphate (ADP) is released from red blood cells, endothelium and platelets, along with thromboxane A_2, and fibrinogen, which collectively cause further platelet activation.[3]

Aspirin and clopidogrel are the most widely used anti-platelet drugs, generally used in combination. Clopidogrel is a thienopyridine derivative and it inhibits platelet aggregation by irreversibly blocking ADP $P2Y_{12}$ receptors on the surface of platelets. There are two types of ADP receptors, $P2Y_1$ and $P2Y_{12}$, blocking of either of these causes adequate platelet inactivation. Blocking of ADP receptors not only decrease platelet aggregation, but also causes reduction in p-selectin expression, thrombin production, pro-coagulant activity and release of inflammatory markers.[4]

Clopidogrel is a prodrug. Only 15% of the drug is activated in liver by cytochrome enzymes by a two-step oxidation process, rest of the drug is destroyed by esterases. Clopidogrel is not only used in emergency conditions but also for the prevention of secondary ischemic events and stenosis of the stents after percutaneous coronary intervention.[5] According to recent guidelines of American College of Cardiology and American Heart Association regarding dual anti-platelet therapy (DAPT) comprising of aspirin and clopidogrel, low dose aspirin should be continued indefinitely for lifetime while clopidogrel should be continued for at least 6 months after drug eluting stent and minimum 1 month after bare metal stent implantation in stable ischemic heart disease patients. The stable ischemic heart disease patients who tolerate clopidogrel well and are not at the risk of bleeding, can continue DAPT for longer duration. In patients of acute coronary syndrome, clopidogrel should be continued for at least 12 months after PCI, and can be continued further if there is no bleeding risk. After coronary artery bypass grafting, clopidogrel should be continued for at least 12 months. After treatment of patients with fibrinolytic therapy, clopidogrel should ideally be taken for 12 months.[6]

Variations to clopidogrel therapeutic response in terms of platelet inactivation are common as it is not equally effective in all patients due to clopidogrel

resistance which has been recorded as 5–70% in different populations. Clopidogrel resistance is associated with increased risk of secondary ischemic events and cardiovascular deaths.[7]

This study was designed to assess the prevalence of clopidogrel resistance in Pakistani population and to find its relation with age and sex of patients.

METHODOLOGY

The study was conducted at Department of Pharmacology, Army Medical College, National University of Medical Sciences, Rawalpindi, Pakistan, in collaboration with Armed Force Institute of Cardiology, and Armed Force Institute of Pathology. Study was approved by institutional ethical review board. It was a cross sectional analytical study. The study was conducted from April 2015 to May 2017. The inclusion criteria were ischemic heart disease patients taking clopidogrel for at least 7 days. Patients were of either sex, age >17 years and compliant to treatment plan. Exclusion criteria were lack of informed written consent, poor compliance, pregnancy, active hepatic or renal disease, malignancy, taking drugs that interact with platelets or clopidogrel like heparin, warfarin, omeprazole, eptifibatide or tirofiban within last 24 hours.

Sample size was calculated with the help of WHO sample size calculator. Latest study regarding clopidogrel resistance in Pakistan has shown anticipated value of 17% while their expected value was 30%. So keeping confidence interval at 95% and level of significance at 5%, sample size turned out to be 136. However, we increased the sample size to 390 to enhance the power of study.[8]

Blood samples (4.5 ml) of all the patients were taken and transferred into conical plastic tubes containing 0.5 ml trisodium citrate as anticoagulant. Platelet aggregation studies were performed within three hours of sampling on two chamber light transmission aggregometer (Chrono-Log corporation, Pennsylvania, USA) using ADP (10 µM) as agonist. This optical aggregometry works on the principle of optics that when the platelets are inactive and dispersed, less light pass through the plasma. On platelet activation with an agonist, platelets aggregate to form clumps, resulting in crossing of more light across the medium. The obtained blood was initially centrifuged at 800 rpm for 10 min to get platelet rich plasma (PRP) and later at 4,000 rpm for 5 min to get platelet poor plasma (PPP). Both PRP and PPP was placed in the chambers of aggregometer where PPP act as reference and light transmission across PRP was recorded in comparison to PPP with the help of Agro Link software.[9] Persistence platelet aggregation despite adequate dosing of clopidogrel can be called as clopidogrel resistance. Patients with <50% platelet aggregation were classified as clopidogrel responders, and patients with ≥50% platelet aggregation were classified as clopidogrel resistant.[10]

The data was analyzed using Microsoft Excel 2016 and SPSS-23. Numerical data was written as Mean±SD. Chi-square and *t*-test were applied to compare the mean platelet aggregation of two groups. One-way ANOVA and post hoc tukey test was applied to compare mean platelet aggregation of multiple groups. $p<0.05$ was considered significant.

RESULTS

Total 390 patients on clopidogrel therapy completed the study, of which 59.50% (n=232) patients were males and 40.50% (n=158) patients were females. Mean age of the patients was 53.5±11.9 years. Platelet aggregation studies showed that 75.1% (n=293) patients were clopidogrel responders, while 24.9% (n=97) patients were clopidogrel resistant. Table-1 is showing mean platelet aggregation of clopidogrel responder and clopidogrel resistant patients. Mean platelet aggregation of clopidogrel resistant patients was significantly higher than clopidogrel responders ($p<0.001$).

Figure-1: Platelet aggregation of clopidogrel responder and resistant patients (Mean±SD)

Response Status	Percentage	*p*
Responders	27.73±12.17	<0.001
Resistant	60.22±7.33	

Table-2 is a cross-tabulation between gender and clopidogrel response status. No significant difference was observed in clopidogrel response in either sex, $p=0.867$. There was no significant difference in mean platelet aggregation of male and female patients, 35.34±18.28% and 36.50±17.49% respectively, $p=0.532$.

Table-2: Cross-tabulation between gender and clopidogrel response status [n (%)]

Gender	Responders	Resistant	Total
Male	175 (75.4)	57 (24.6)	232
Female	118 (74.7)	40 (25.3)	158

On the basis of age, all the patients were divided into 6 groups and clopidogrel response status was assessed in each group as shown in Table-3. There was no significance difference in clopidogrel response status among different age groups ($p=0.266$).

Table-3: Clopidogrel response status of different age groups [n (% within group)]

Age (Years)	Responders	Resistant	Total
≤30	7 (87.5)	1 (12.5)	8 (100)
31–40	41 (78.8)	11 (21.2)	52 (100)
41–50	87 (79.8)	22 (20.2)	109 (100)
51–60	83 (74.1)	29 (25.9)	112 (100)
61–70	51 (65.4)	27 (34.6)	78 (100)
≥71	24 (77.4)	7 (22.6)	31 (100)
Total	293 (75.1)	97 (24.9)	390 (100)

Table-4 shows mean platelet aggregation of different age groups. One-way ANOVA and post hoc tukey tests showed that there was no significant differences in mean platelet aggregation among different groups (p=0.234).

Table-4: Mean platelet aggregation in age groups

Age (Years)	Mean Platelet aggregation (%)
≤30	28.13
31–40	34.65
41–50	34.35
51–60	35.9
61–70	39.95
≥71	34.13

DISCUSSION

Results of our study show that 75.1% of the ischemic heart disease patients were responders and 24.9% patients were resistant to clopidogrel, which is a significant percentage. Mean platelet aggregation of clopidogrel resistant patients was significantly higher than that of clopidogrel responders, (60.22% *vs* 27.73%, p<0.001), so the clopidogrel resistant patients have invariably greater chances of platelet adhesion and aggregation despite on antiplatelet treatment. No relation was found between clopidogrel resistance and any specific age group (p=0.266) or gender (p=0.532). Similarly, no significant difference was recorded in mean platelet aggregation of different age groups (p=0.234) or either gender (p=0.532). It shows that clopidogrel response is irrespective of age or gender.

Previously very scarce data is available regarding clopidogrel resistance in Pakistan as only couple of small scale studies were performed. Rizvi *et al*[8] conducted a study on 71 IHD patients on clopidogrel therapy in Lahore. They performed platelet aggregation studies and found 17% clopidogrel resistance in their study population. While comparing both studies by applying Chi-square test, no significance difference was found regarding clopidogrel response status between the two study populations. Comparing results of both genders showed that there was no significant difference in prevalence of clopidogrel resistance among female patients of our study group or their study group (25.32% vs 36.84% respectively) p=0.28, but there was significant difference in prevalence of clopidogrel resistance in male patients of both groups (24.57% vs 11.54% respectively) p=0.04. Rizvi *et al*[8] found that clopidogrel resistance was more prevalent in females, but stated that due to small sample size, this higher prevalence is inconclusive. This misperception is settled with results of our study that there is no priority of clopidogrel resistance in female patients.

Khan *et al*[11] conducted a double blind randomized controlled trial on 106 patients in Peshawar. Platelet functioning of the patients was checked using whole blood aggregometer and they found that 69.52% patients were responders, 24.76% were hypo-responders, and 5.71% were non-responders to clopidogrel. Assuming both hyporesponders and non-responders as clopidogrel resistant, results of our study are comparable to results of this study with no significant difference, p=0.245.[11]

Results of these studies show that significant number of ischemic heart disease patients, almost quarter of them, are resistant to the action of clopidogrel and are at risk secondary ischemic events.

Several factors can contribute to clopidogrel resistance which may be poor compliance, active hepatic or renal disease, clopidogrel drug interactions and pharmacogenetic factors. Such factors can result in lesser absorption, decreased metabolic activation and alteration in target site of clopidogrel. Mutation in genes encoding for ABCB1/MDR1 transporter, which is concerned with absorption of clopidogrel from gut, leads to poor absorption of the drug from gut, hence poor bioavailability and efficacy. Most important pharmacogenetic factor is variation in gene encoding for CYP2C19 enzyme, which is the primary cytochrome enzyme involved in the activation of clopidogrel prodrug. Other cytochrome enzymes involved in clopidogrel activation are CYP2C9, CYP3A4, CYP3A5 etc. but their association to clopidogrel resistance has not been proven yet. Regarding CYP2C19, carriers of non functional alleles of CYP2C19 (*2, *3, *4, *5, *6, *7, *8) have less than optimum conversion of clopidogrel to active metabolites, which leads to lesser clinical efficacy. Excessive platelet activation and aggregation can lead to thrombosis and subsequent cardiac events.[12] Clopidogrel is platelet ADP $P2Y_{12}$ receptor antagonist. T744C, G52T and C34T polymorphisms in the $P2Y_{12}$ ADP receptor gene can cause insufficient binding of clopidogrel to ADP receptor, resulting persistent platelet aggregation irrespective of adequate clopidogrel dosing.[13]

Various measures can be adopted to overcome clopidogrel resistance ranging from increasing its dose to replacing it with some other suitable anti-platelet drug. Increasing maintenance is not always beneficial as Fontana *et al*[14] found that doubling dose of clopidogrel from 75 to 150 mg/day, can decrease platelet aggregation by 20% in resistant patients, but even then, this platelet inhibition is not equivalent to clopidogrel action in normal responders.[14] Avoiding documented drug interactions with careful prescription can be useful in reducing clopidogrel resistance especially with proton pump inhibitors (PPIs) like omeprazole and esomeprazole which are potent inhibitor of CYP2C19. If PPI has to be used, then pantoprazole is the safer option with minimal CYP2C19 inhibitory activity.[15]

Other measures to decrease chances of clopidogrel resistance may include addition of cilostazol, an inhibitor of phosphodiesterase, along with standard dual anti-platelet regimen containing aspirin and clopidogrel after PCI. This addition has shown to reduce

the incidence of clopidogrel resistance and secondary ischemic events.[16] Next generation thienopyridine prasugrel, and cyclopentyl-triazolo-pyrimidine ticagrelor and cangrelor, are newer ADP $P2Y_{12}$ receptor antagonists, which were introduced to overcome clopidogrel resistance. These drugs are known to have better pharmacokinetic profile than clopidogrel, but chances of bleeding are higher. As ticagrelor is a non thienopyridine, it does not require hepatic activation to active metabolite. So, it has lesser interindividual differences in terms of clinical efficacy but chances of bleeding are higher.[17] Vorapaxar is protease activated receptor-1 antagonist, which is target site of thrombin and can be considered as alternative to clopidogrel, but problem is that it has not been proved to decrease the incidence of deaths due to myocardial infarction, stroke or recurrent ischemia and it is metabolized by CYP3A4, so has plenty of drug interaction.[18] New congeners of clopidogrel are in developmental phase which do not require metabolic activation in liver, such as Vicagrel, PLD-301, and W1. These novel drugs can be converted into clopidogrel thiolactone in the intestine by carboxylesterase 2 and alkaline phosphatase, thus bypassing cytochrome P_{450} mediated activation in liver.[19]

CONCLUSION

Prevalence of clopidogrel resistance is significant in Pakistani population and it is not related to any particular age or gender. Every patient on clopidogrel should undergo platelet aggregation studies to check its response, and necessary modifications in anti-platelet regimen should be made accordingly. Large scale studies for mapping of genes encoding for cytochrome enzymes involved in clopidogrel activation are future scope of research in Pakistani population.

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