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Original Article

Effect of Smoking on Antiplatelet Action of Clopidogrel in Patients with Ischemic Heart Disease

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ABSTRACT

Objective: To explore relationship between smoking and antiplatelet action of Clopidogrel in patients with ischemic heart disease (IHD).

Study Design: Quasi-experimental study

Place and Duration of Study: Department of Pharmacology, Army Medical College, National University of Medical Sciences, Rawalpindi, Pakistan from Aug 2015 to Dec 2019.

Methodology: The quasi experimental study was carried out on 390 patients with IHD recruited through convenience sampling. Patients were placed in two groups. Group-1 included smokers while group-2 patients were nonsmokers. Blood samples of smokers (n=151) and nonsmokers (n=239) IHD patients were taken. Platelet function testing of all the samples was done through light transmission aggregometer (LTA) using adenosine diphosphate (ADP) as an agonist.

Results: Among patients of IHD 82.12% of the smokers on Clopidogrel therapy were Clopidogrel responders and 17.88% were Clopidogrel resistant, while in nonsmoker group 70.71% patients were Clopidogrel responders and 29.29% were Clopidogrel resistant. There was a significant difference in Clopidogrel response status between smokers and nonsmokers, p=0.011. Furthermore, mean platelet aggregation of smokers was significantly lower than that of nonsmokers, p=0.004.

Conclusion: Smoking can enhance antiplatelet effect of Clopidogrel in IHD patients with greater platelet inhibition and lesser chances of Clopidogrel resistance.

Keywords: Clopidogrel, Platelet Aggregation, Smoking

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INTRODUCTION

Clopidogrel is an antiplatelet drug which is used for primary and secondary prophylaxis of ischemic heart diseases (IHD), ischemic stroke, peripheral vascular diseases etc. It is also used before and after invasive procedures like coronary angiography and angioplasty, coronary artery bypass grafting and carotid artery stenting.[1] It decreases cardiovascular morbidity and mortality through its platelet inhibition properties. As Clopidogrel is a prodrug, it is metabolized to active metabolite inside the body. While 85% of the drug is inactivated by plasma esterases, only 15% of the drug is converted to its active thiol metabolite by cytochrome enzymes. Metabolic activation of Clopidogrel is a two-step process and the cytochrome enzymes involved in these steps are CYP2C19, CYP1A2, CYP2B6, CYP3A4A5 and CYP2C9. Target sites of Clopidogrel are adenosine diphosphate (ADP) P2Y12 receptors, which are present on the surface of platelets.[2] Clopidogrel acts as an antagonist at P2Y12 receptors, forming a disulfide bond between the thiol group and two cysteine residues of the extracellular domains of P2Y12 receptors. Other antiplatelet drugs targeting these receptors include ticagrelor and prasugrel 1-2

Various factors including smoking status of the patient, can affect antiplatelet action of Clopidogrel.[3]

Ramotowski *et al*., investigated the platelet reactivity of patients who continue smoking and the patients who discontinue smoking 30 days post PCI (percutaneous coronary intervention) using VerifyNow P2Y12 assay. They found that the smokers had significantly lower PRU (platelet reactivity unit) as compared to smoking cessation group (118.4 ± 65.9 vs 150.5 ± 68.6,p=0.030). Also, the level of Clopidogrel active metabolite was decreased significantly in smoking cessation group (p = 0.072).[3]

Ferriero *et al*., analyzed CAPRIE (Clopidogrel Versus Aspirin in Patients at Risk of Ischemic Events) mega trial that compared Clopidogrel and aspirin monotherapy in patients with vascular diseases. They found that the current smokers on Clopidogrel therapy had reduced ischemic cardiac events as

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compared to the nonsmokers on Clopidogrel.[4] Some studies concluded that greatest clinical benefit of Clopidogrel in terms of reduced cardiovascular mortality, myocardial infarction and need of urgent revascularization was found among smokers who smokes more than ten cigarettes per day.[5]

Bliden *et al*., utilized light transmission aggregometer to demonstrate that current smokers on Clopidogrel therapy had lower mean platelet aggregation as compared to nonsmokers (32±12% vs 44±13%) using ADP as an agonist.[6]

Where some studies have claimed no association between smoking and antiplatelet action of Clopidogrel, others showed positive association (higher on treatment platelet reactivity) or negative association (lower on treatment platelet reactivity) between the two variables.[7-8] On the basis of platelet aggregation studies, patients can be classified as Clopidogrel responders or Clopidogrel resistant. Clopidogrel responders are the patients in which platelet aggregation is inhibited adequately, while Clopidogrel resistant patients have high-on-treatment platelet aggregation despite the therapeutic dosage of Clopidogrel.[9] The existing literature provides mixed results, however to optimize the Clopidogrel treatment in smoker/nonsmoker patients with IHD, further research can add lead to enhanced patient outcomes. With this aim this research study was carried out to investigate the relationship between smoking and antiplatelet action of Clopidogrel in ischemic heart disease patients.

METHODOLOGY

The quasi-experimental study was conducted between 2015 and 2019 at Department of Pharmacology, Army Medical College, National University of Medical Sciences, Rawalpindi, Pakistan. The study was given approval by the ethical board of the institution (ERC/SA-15/Dr Usman Nawaz). Informed written consent was obtained from all the patients.

Sample size was calculated with the help of World Health Organization sample size calculator, keeping anticipated population proportion of current smokers with Clopidogrel resistance as 32%, test value of the population proportion of nonsmokers with Clopidogrel resistance as 44%, population standard deviation of Clopidogrel resistance among current smokers and nonsmokers as 12.5%. Minimum sample size in each group was 23.6

For sampling of IHD patients, non-probability consecutive sampling method was employed.

Inclusion Criteria: Patients with IHD on Clopidogrel therapy 75 mg/day for at least 7 days were included in the study.

Exclusion Criteria: Patients with pregnancy, malignancy, bleeding disorder, hepatic or renal disease, or given glycoprotein IIb/IIIa inhibitor within 48 hours were excluded from the study. Ex-smokers were also excluded because of variable influence of smoking cessation on platelet aggregation and inconsistence interval between last cigarette smoke and sampling for this study.

The patients in the study were also receiving aspirin as component of dual antiplatelet therapy. In order stimulate the platelets and to counter platelet inhibitory effects of aspirin, ADP was used as an agonist at the dose of 5 µL (10 µM), because at this dose ADP can stimulate platelets irrespective of cyclooxygenase pathway which is blocked by aspirin.

Patients were categorized as smokers if they are currently smoking cigarettes or nonsmokers if they have never smoked cigarettes (Figure).

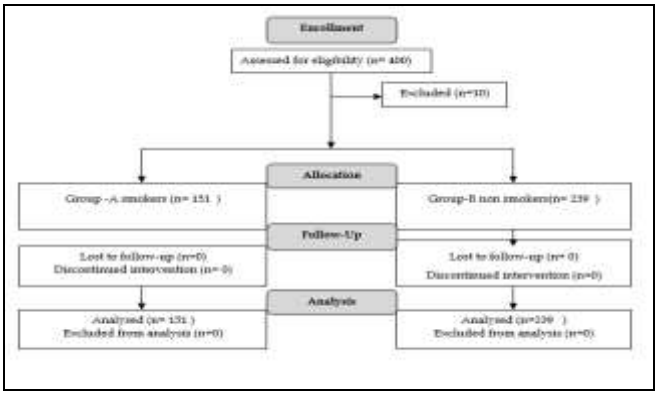

Figure: Patient Flow Diagram

Blood samples of all the patients were withdrawn through aseptic technique which was stored in conical tubes containing trisodium citrate as an anticoagulant. Blood counts and hemoglobin level of all the samples were measured using KX215583 Auto Hematology Blood Analyzer (Sysmex Corporation, China). Platelet aggregation studies were performed on all the blood samples with the help of Chronolog Light Transmission Aggregometer (Chrono-Log two chamber 490 Model, Chrono-Log Corporation, Havertown, Pennsylvania, USA). Platelet rich plasma (PRP) was obtained by centrifuging the blood

anticoagulant mixture at 800rpm for 10 min. PRP was separated and the remaining blood was again centrifuged at 4000 rpm for 5 min to obtain platelet poor plasma (PPP). PRP & PPP were loaded in the Chronolog cuvettes and placed in test chamber of the aggregometer. ADP was added as agonist to the PRP and light transmission across PRP was monitored keeping PPP as reference and the readings were plotted on graph paper using AGGRO/LINK® Opti8™ Software in computer. As a rule, greater light passes through the PRP with clumped platelets secondary to activation by ADP. Persistent platelet aggregation while on therapeutic doses of Clopidogrel can be regarded as Clopidogrel resistance. As per the LTA readings, patients with less than 50% platelet aggregation were categorized as "Clopidogrel Responders" while patients with 50% or more platelet aggregation were categorized as "Clopidogrel Resistant".[11]

The data was analyzed with the help of Microsoft Excel and Statistical Package for Social Sciences (SPSS) version 22. Descriptive data was expressed as frequencies and percentages. Continuous data was illustrated as Mean ± (Sd). Chi Square test was applied to check significance in distribution of categorical variables between two groups. Mean platelet aggregation was expressed using independent sample t-test. The *p* value of ≤ 0.05 was considered significant.

RESULTS

The study population comprised of 151 smokers and 239 nonsmoker patients with IHD on Clopidogrel therapy. Table-I is showing demographic profile and hematological indices of smokers and nonsmokers.

Mean platelet aggregation of smokers was significantly lower than that of nonsmokers' (32.57±16.45% vs 37.86±18.55%, *p*=0.004). Clopidogrel response status of the smokers and nonsmokers is shown in Table-II.

DISCUSSION

The study results shows that antiplatelet action of Clopidogrel is enhanced in IHD patients who are smokers as compared to nonsmokers, which is evident by the fact that the smokers had lower mean platelet aggregation than that of nonsmokers' (32.57±16.45% vs 37.86±18.55%). Furthermore, the proportion of Clopidogrel responders was greater in the smokers as compared to nonsmokers (82.11% vs 70.71%, *p*=0.011), with lesser chances of Clopidogrel resistance in smokers (17.88% vs 29.29%).

Results of our study are consistent with Park *et. al*., who used VerifyNow P2Y12 assay to demonstrate that smokers (n=249) on Clopidogrel therapy had lower on-treatment platelet reactivity (227.6±76.0 vs 244.9±79.7 PRU,*p*=0.001, OR 0.48; 95% CI, 0.31 to 0.74) as compared to nonsmokers (n=1182)12. Similarly, Cai *et al*., using light transmission aggregometry, have demonstrated that ADP induced platelet aggregation was lower in smokers (n=109) as compared to nonsmokers (n=195) on Clopidogrel therapy (44.97±20.05% vs 51.98±19.38%, respectively; *p*=0.018). This prospective study also established that smokers had lower incidence of secondary cardiovascular events (*p*=0.008).[8]

Our results are also consistent with Kang *et al*., who have demonstrated that there are lesser chances of Clopidogrel resistance among smokers in ischemic

Table-I: Demographic profile and Hematological Indices of smoker and nonsmoker IHD patients on Clopidogrel therapy (n=390)

	Smokers (n=151)	Nonsmokers (n=239)	*p*-value
Mean Age	53.83±12.34 years	53.29±11.58 years	0.665
Mean body mass index	24.27±3.78 kg/m2	25.01±3.52 kg/m2	0.049
Family History of IHD	16 (10.60 %)	31 (13.00 %)	0.483
Cardiac Failure	29 (19.20 %)	36 (15.10 %)	0.285
Bleeding Events	5 (4.00 %)	8 (3.30 %)	0.746
PCI/CABG	39 (25.81 %)	89 (37.21 %)	0.105
Hemoglobin	`	13.28±1.58 g/dl	0.926
Red Blood Cells	4.65±0.56 1012/L	4.70±0.61 1012/L	0.357
White Blood Cells	7.57±1.95 109/L	7.74±2.04 109/L	0.430
Hematocrit	39.50±3.97 %	39.85±4.98 %	0.475
No of Platelets	175.41±55.06 K/uL	181.88±58.13 K/uL	0.276
Mean Platelet Volume	8.56±1.58 fL	8.55±1.58 fL	0.945
Platelet Large Cell Ratio	17.57±8.94 %	17.94±10.96 %	0.731
Platelet Distribution Width	10.84±2.65 fL	10.77±2.48 fL	0.780

Table-II: Clopidogrel response status of Smokers And Nonsmokers (n=390)

	Clopidogrel Responders n (%)	Clopidogrel Resistant n (%)	*p* - value
Smokers (n=151)	124 (82.12%)	27 (17.88%)	0.011
Nonsmokers(n=239)	169 (70.71%)	70 (29.29%)	

stroke patients (odds ratio = 0.426, 95% CI 0.210-0.861), depicting protective effect of smoking in Clopidogrel treated patients.[13]

Berger *et al*., through a multicentric cohort study, concluded that current smokers are at increased risk of all-cause and cardiovascular mortality. However, use of Clopidogrel in current smokers is associated with reduction in all-cause and cardiovascular mortality (HR 0.68) as compared to former smokers (HR 0.95) and non-smokers (HR 1.14). But, interestingly, the effect of Clopidogrel on reducing cardiovascular secondary events like myocardial infarction and ischemic stroke did not differ in different smoking categories (current smokers HR 0.93, former smokers HR 0.83, never smokers HR 0.92).[14]

Smoking induces cytochrome enzymes mainly CYP1A2 and CYP2B6, both of which are involved in two step activation of prodrug Clopidogrel in the liver, intensifying its conversion to active metabolite, ultimately enhancing its antiplatelet action in smokers.[12] Dobrinas *et al*., assessed CYP1A2 activity in active smokers and in the subjects who abstained from smoking for four weeks and found that smokers had 1.55 fold higher CYP1A2 activity as compared to the subjects who abstained from smoking (p=0.001).[15,16]

Studies have suggested that high dose Clopidogrel or alternate and more potent P2Y12 inhibitors should be considered for the patients who have higher platelet reactivity after smoking cessation.[17-18]

LIMITATION OF STUDY

For determining better relation between smoking and Clopidogrel response, the sample population should be studied longitudinally. Therefore, results of the present study are associative rather than causative. Smoking status was attributed to patients on the basis of smoking history, while estimation of urinary cotinine levels would have been more definitive. Sample size was unequal in smokers and nonsmokers' group due to random consecutive sampling. Light transmission aggregometry is one of the various methods to assess platelet functions but it is not the most advanced technique. It requires multiple components as part of equipment, expert technical handling to prepare PRP and PPP and relatively longer time for results finalization, but it was employed on the basis that it was the only method available in our setup.

CONCLUSION

Smoking is associated with enhanced antiplatelet action of Clopidogrel in ischemic heart disease patients, decreasing prevalence of Clopidogrel resistance among smokers. Further studies could focus on Pathophysiological and pharmacogenetic basis of the enhanced antiplatelet action of Clopidogrel secondary to smoking.

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Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

UN & MN: Conception, study design, drafting the manuscript, approval of the final version to be published.

WAK & SB: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

AN: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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