ORIGINAL ARTICLE

IMPACT OF SMOKING ON ANTI-PLATELET EFFECTS OF ASPIRIN

Mudassar Noor, Usman Nawaz*, Saleem Ahmad Khan, Akbar Waheed, Chaudhry Altaf†**

Department of Pharmacology & Therapeutics, Army Medical College, National University of Medical Science, Rawalpindi, *Pak International Medical College, Peshawar, **Pathology, Army Medical College, National University of Medical Science, Rawalpindi, †Department of Hematology, Armed Forces Institute of Pathology, National University of Medical Science, Rawalpindi, Pakistan

Background: Cigarette smoking stimulates platelet activation and aggregation and can also influence adversely the antiplatelet effects of aspirin, which is the mainstay of treatment in the primary and secondary prevention of ischemic heart disease. This study was carried out to analyze the effect of smoking on aspirin efficacy in coronary heart disease patients. **Methods:** In this cross sectional analytical study, 384 ischemic heart disease patients were enrolled. Light transmission Aggregometry (LTA) with arachidonic acid was utilized to assess the platelet function. Data was analyzed using SPSS-23. Chi-square test Odds Ratio were utilized to find out association of smoking with aspirin response status. **Results:** The study contained 272 (70.8%) male and 112 (29.2%) females with the mean age of 48.22±11.87 years. There were 199 smokers (51.82%) and 185 non-smokers (48.18%). Frequency of aspirin resistant among smokers was 41 (20.60%) whereas the frequency in non-smokers was 12 (6.50) and the difference was significant (p<0.001). The adjusted Odds Ratio was 4.44 with 95% confidence interval of 2.07–8.90. Frequency comparison of aspirin responders between males and females was non-significant both in, smokers and non-smokers (p=0.14 and 0.92 respectively). **Conclusion:** Cigarette smoking adversely affects the antiplatelet efficacy of aspirin irrespective of gender.

Keywords: Aspirin resistance, smoking, platelet aggregation

Pak J Physiol 2018;14(3):3–6

INTRODUCTION

Cardiovascular disorders (CVD) are leading cause of morbidity and mortality. It has been predicted that CVD deaths will reach 25 million per year by 2020 globally.[1] Aspirin has acquired the pivotal place in the primary and secondary prevention of ischemic heart disease (IHD) by virtue of its dramatic antiplatelet effects. In that it permanently acetylates the platelet cyclooxygenase enzyme and halts the production of thromboxane which is a potent platelet aggregator. Thus, significantly trims down the occurrence of ischemic vascular attacks and improves the long-term prognosis.[2,3] Despite its proven efficacy in the management of ischemic heart disease, every patient taking aspirin does not benefit equally and may encounter adverse ischemic cardiac events. This state of aspirin non-responsiveness is termed as aspirin resistance (AR).[4] Considerable queries remain unanswered regarding definitions, causes, detection and treatment of AR. Cigarette smoking is considered very imperative factor among the postulated mechanisms predisposing to AR due to its stimulating actions on platelets and procoagulant effects.[5]

Smoking is one of the most significant preventable risk factor for ischemic heart disease which is held responsible for almost 10% of cardiovascular disorders.[6] The balance between antithrombotic and prothrombotic factors is tainted due to disturbed functionality of fibrinogen, coagulation factors, endothelial cells and platelets caused by exposure to cigarette smoke.[7]

There are almost 4,000 diverse chemicals found in cigarette smoke, due to which it is considered the most intricate and least comprehended among all the risk factors of IHD.[8,9] Smoking contributes in the development of cardiovascular disease by multiple ways, for instance by reduction in flow mediated dilatation due to decrease in nitrous oxide, increase in triglyceride, total cholesterol, low density lipoprotein and very low-density lipoprotein concentrations.[10–12] It also escalates pro inflammatory cytokines levels[13] and disturbs coagulation system of body by platelet stimulation, coagulation cascade activation and reduction in fibrinolysis[14,15]. Cigarette smoking promotes platelet stimulation through enhanced interaction of subendothelial collagen exposure as well as tissue factor mediated proteolytic cascade which activates platelets through protease activated receptor 1 and 4. As these platelets set into motion and further employ additional platelets by releasing serotonin and adenosine diphosphate and biosynthesis of thromboxane A_2 which ultimately results into thrombus formation.[16–18]

Considerable decline has been observed in the ischemic cardiovascular events in patients who have given up the smoking. Almost 36% drop was seen in the cardiovascular mortality as compared with those who continued smoking in a meta-analysis of 20 studies.[15] The sudden rise in platelet aggregation was observed in IHD patients as well as healthy volunteers after smoking of two cigarettes as evident by declining of platelet aggregate ratio. Several studies have concluded that smoker's platelet activity is much higher than nonsmokers' platelet activity.[19,20] Current study was planned to evaluate the impact of cigarette smoking on

antiplatelet efficacy of aspirin in ischemic heart disease patients.

MATERIAL AND METHODS

This cross-sectional analytical study was conducted from Oct 2015 to December 2016 at Pharmacology Department, Army Medical College, National University of Medical Sciences, Rawalpindi, in collaboration with Hematology Department, Armed Forces Institute of Pathology, and Armed Forces Institute of Cardiology/National Institute of Heart Diseases, Rawalpindi. The study protocol was approved by Ethical Review Committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College as well as Institutional Ethical Review Board of Armed Forces Institute of Cardiology, Rawalpindi.

Sample size was calculated using WHO sample size calculator. Considering the values of alpha as 0.05, beta as 0.1, proportion of AR in smokers as 0.22 and in non-smokers as 0.13, a sample size of 373 was calculated. However, we included 384 patients in the current study. Patients from both the genders taking aspirin 75–100 mg/day for at least last 7 days, aged between 18 to 70 years, attending outpatient department or admitted were recruited by convenient sampling after written informed consent. The aspirin compliance was established on individual interview of patient. They were subsequently alienated into smoker and non-smoker groups depending upon their smoking habits, according to WHO's 10th revision of international statistical classification of diseases and related health problems criteria of harmful use.[21] The smoker was defined as having 5–15 pack years history of smoking (1 pack year=20 cigarette per day for 1 year).[22] The patients concurrently using other antiplatelet or anticoagulant drugs, having any bleeding disorder or platelet count $<150\times10^3/mm^3$ were excluded.

Blood sample of 4.5 ml was collected from all patients, 2–12 hours after the last dose of aspirin and were stored in conical plastic tubes, containing 0.5 ml of trisodium citrate for anticoagulation. The tubes were then shifted to hematology laboratory, for platelet aggregation studies to evaluate the efficacy of aspirin.

After complete blood count the samples were given a 10 minutes spin at 800 rpm to get native platelet rich plasma (PRP) and further centrifuged for 5 minutes at 4,000 rpm to extract platelet poor plasma (PPP). The amount of platelet in PRP was managed in the range of $200\times10^3/\mu l$ to $350\times10^3/\mu l$ by utilizing PPP. Platelet aggregation studies were performed on Chrono-Log Aggregometer (Chrono-log, Havertown, Pa., USA) by using Arachidonic acid (0.5 mM) as an agonist. Results were obtained in the form of graph. Platelet aggregation studies were completed within three hours of sampling.

The results were analyzed using SPSS-23. Categorical variables were presented as frequency and percentage whereas numerical variables as mean and standard deviation. Chi-square test was used to find out the association between aspirin resistance and smoking. Strength of association was measured by calculating odds ratio. Binary logistic regression was used to determine the adjusted odds ratio after controlling the effect of confounders, and $p\leq0.05$ was considered significant.

RESULTS

Our sample population contained 272 (70.8%) males and 112 (29.2%) females with mean age of 48.22±11.87 years. There were 199 (51.82%) smokers and 185 (48.18%) non-smokers. Out of these smokers, 177 (88.94%) were male and 22 (11.05%) were female. Among non-smokers, 95 (51.35%) were male and 90 (48.64%) were female.

Table-1 is a cross tabulation between asprin response and smoking status showing frequency of smokers and non-smokers for each category of aspirin response status. The table also shows crude and adjusted odds ratios.

Table-2 shows association of gender with aspirin response status in smokers and non-smokers separately. In both cases aspirin response is not associated with gender (p=0.14 and 0.92 respectively). This illustrates that smoking affects aspirin response independent of gender.

Table-1: Association of smoking with aspirin response status

	Smoker	Non-smoker
Aspirin Resistant	41 (20.6%)	12 (6.5%)
Aspirin Responder	158 (79.4%)	173 (93.5%)
***p*-value**	<0.001	
Odds Ratio (95% CI)	3.74 (1.89–7.37)	
Adjusted Odds Ratio (95% CI)	4.44 (2.07–8.90)	

Table-2: Association of gender with aspirin response status in smokers and non-smokers separately

			Aspirin responder	Aspirin resistant	*p*
Smokers	**Male**	Count	143	34	0.14
		% within Gender	80.8	19.2	
	Female	Count	15	7	
		% within Gender	68.2	31.8	
Non-smokers	**Male**	Count	89	6	0.92
		% within Gender	93.7	6.3	
	Female	Count	84	6	
		% within Gender	93.3	6.7	

DISCUSSION

The outcomes of current study suggest that tobacco smoking, irrespective of gender, adversely affects the antiplatelet efficacy of aspirin and smokers are particularly at higher risk of development of aspirin non-responsiveness. Our results have reiterated the

significance of termination of smoking especially in IHD patients. Such patients may be vigilantly reviewed as aspirin may not be effectively reducing the platelets in these individuals.

Various studies have advocated that tobacco smoking enhances the platelet stimulation and aggregation. Mirkhel and colleague utilized 'verify now' assay and indicated the firm involvement of cigarette smoking with antiplatelet therapy resistance on the basis of multivariate analysis.[23] Pamukcu *et al* also concluded that aspirin could not successfully inhibit the platelet activation caused by smoking in IHD patients. Platelet Function Analyzer (PFA) with collagen was utilized to assess platelet function in this study.[24] The instigation and progression of cardiovascular disease is significantly influenced by cigarette smoking due to variety of its actions including enhanced oxidation of pro-atherogenic cholesterol, endothelial injury and stimulation of platelet activation and aggregation leading to procoagulant state in body.[6]

However, some studies have concluded that there is no difference in hemostasis and platelet aggregation in smokers as well as in non-smokers. For instance, a study concluded almost similar platelet competency for hemostasis and their ability to respond to stimulating factors in regular smokers as well as in non-smokers.[25] In the same way, another study probed the impact of smoking on platelet function and failed to show any adverse consequence of consistent smoking on platelet function among healthy volunteers.[26]

The possible rationalization of contentious results obtained in various trials with comparable patient variables is the utilization of dissimilar method to ascertain the function of platelet. The current study may be considered superior in many aspects, most importantly the number of patients we enrolled was reasonably high and we utilized light transmission aggregometry with arachidonic acid for assessment of platelet function which is considered the gold standard method worldwide.

The most efficient way to avoid smoking induced ischemic cardiovascular events is to quit tobacco inhalation. Presently, IHD patients on antiplatelet treatment are not assessed for platelet function but selected individuals who are at greater risk of adverse ischemic attack, for instance, active smokers with recent history of stent thrombosis may be the possible contestant for platelet function assay. It has been indicated in a trial done in near past that aspirin in low doses may not effectively halt the platelet aggregation in smokers with IHD but combination of high dose aspirin (300 mg/day) and clopidogrel can efficiently inhibit platelet activity.[27] In a research conducted on coronary heart disease patients, who were prescribed aspirin for thrombotic prophylaxis indicated a well-defined alliance between platelet over activity and tobacco inhalation. The antiplatelet efficacy of aspirin was improved either by increasing the dose from 81 mg/day to 325 mg/day or by adding up clopidogrel in the treatment.[5] Yet another study performed on 259 elective coronary angioplasty patients revealed that clopidogrel successfully inhibited platelet aggregation in current smokers as compared to nonsmokers, these findings were contrary to the poor effects of low dose aspirin on platelet activity in smokers with coronary heart disease.[28]

CONCLUSION

Cigarette smoking adversely affects the antiplatelet efficacy of aspirin irrespective of gender. Low dose aspirin, commonly used for primary and secondary prevention of IHD, may not be effective in smokers with IHD and thus explicates, to some extent, the comparatively higher mortality and morbidity in such patients. It is thus essential for IHD patients to refrain from smoking and those who do not give up, may be kept on higher aspirin dose to achieve efficient antiplatelet effects.

REFERENCES

1. Zupanets I, Zaprovalna OY, Grintsov IF, Otrishko I. Differences of the antiplatelet treatment with acetylsalicylic acid. Clin Pharm 2016;20(4):11–6.
2. Godley RW, Hernandez-Vila E. Aspirin for Primary and Secondary Prevention of Cardiovascular Disease. Tex Heart Inst J 2016;43(4):318–9.
3. Schrör K. Pharmacology and cellular/molecular mechanisms of action of aspirin and non-aspirin NSAIDs in colorectal cancer. Best Pract Res Clin Gastroenterol 2011;25(4-5):473–84.
4. Cai G, Zhou W, Lu Y, Chen P, Lu Z, Fu Y. Aspirin resistance and other aspirin-related concerns. Neurol Sci 2016;37:181–9.
5. Li W, Zhang HY, Miao CL, Tang RB, Du X, Shi JH, *et al*. Cigarette smoking inhibits the anti-platelet activity of aspirin in patients with coronary heart disease. Chin Med J 2011;124:1569–72.
6. Messner B, Bernhard D. Smoking and cardiovascular disease: mechanisms of endothelial dysfunction and early atherogenesis. Arterioscler Thromb Vasc Biol 2014;34:509–15.
7. Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. J Am Coll Cardiol 2004;43(10):1731–7.
8. Burns D. Cigarettes and cigarette smoking. Clin Chest Med 1991;12(4):631–42.
9. Bernhard D, (Ed). Cigarette smoke toxicity: Linking individual chemicals to human diseases. Singapur: John Wiley & Sons; 2011.
10. Craig WY, Palomaki GE, Haddow JE. Cigarette smoking and serum lipid and lipoprotein concentrations: an analysis of published data. BMJ 1989;298:784–8.
11. Freedman DS, Srinivasan SR, Shear CL, Hunter SM, Croft JB, Webber LS, *et al*. Cigarette smoking initlation and longitudinal changes in serum lipids and lipopoproteins in early adulthood the bogalusa heart study. Am J Epidemiol 1986;124(2):207–19.
12. Nakamura K, Barzi F, Huxley R, Lam TH, Suh I, Woo J, *et al*. Does cigarette smoking exacerbate the effect of total cholesterol and high-density lipoprotein cholesterol on the risk of cardiovascular diseases? Heart 2009;95(11):909–16.
13. Barbieri SS, Zacchi E, Amadio P, Gianellini S, Mussoni L, Weksler BB, *et al*. Cytokines present in smokers' serum interact with smoke components to enhance endothelial dysfunction. Cardiovasc Res 2011;90(3):475–83.

14. Csordas A, Bernhard D. The biology behind the atherothrombotic effects of cigarette smoke. Nat Rev Cardiol 2013;10(4):219–30.
15. Barua RS, Ambrose JA. Mechanisms of coronary thrombosis in cigarette smoke exposure. Arterioscler Thromb Vasc Biol 2013;33(7):1460–7.
16. Massberg S, Gawaz M, Grüner S, Schulte V, Konrad I, Zohlnhöfer D, *et al*. A crucial role of glycoprotein VI for platelet recruitment to the injured arterial wall in vivo. J Exp Med 2003;197(1):41–9.
17. Dubois C, Panicot-Dubois L, Merrill-Skoloff G, Furie B, Furie BC. Glycoprotein VI–dependent and–independent pathways of thrombus formation in vivo. Blood 2006;107(10):3902–6.
18. Vu TK, Hung DT, Wheaton VI, Coughlin SR. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. Cell 1991;64(6):1057–68.
19. Davis JW, Davis RF. Acute effect of tobacco cigarette smoking on the platelet aggregate ratio. Am J Med Sci 1979;278(2):139–43.
20. Davis JW, Hartman CR, Shelton L, Ruttinger HA. A trial of dipyridamole and aspirin in the prevention of smoking-induced changes in platelets and endothelium in men with coronary artery disease. Am J Cardiol 1989;63(20):1450–4.
21. WHO. The ICD-10 classification of mental and behavioural disorders: diagnostic criteria for research. Geneva: World Health Organization; 1993.
22. Sandhya M, Satyanarayana U, Mohanty S, Basalingappa DR. Impact of chronic cigarette smoking on platelet aggregation and coagulation profile in apparently healthy male smokers. Int J Clin Exp Physiol 2015;2(2):128–33.
23. Mirkhel A, Peyster E, Sundeen J, Greene L, Michelson AD, Hasan A, *et al*. Frequency of aspirin resistance in a community hospital. Am J Cardiol 2006;98(5):577–9.
24. Pamukcu B, Oflaz H, Onur I, Cimen A, Nisanci Y. Effect of cigarette smoking on platelet aggregation. Clin Appl Thromb Hemost 2011;17(6):E175–80.
25. Brockmann MA, Beythien C, Magens MM, Wilckens V, Kuehnl P, Gutensohn K. Platelet hemostasis capacity in smokers: in vitro function analyses with 3.2% citrated whole blood. Thromb Res 2001;104(5):333–42.
26. Scheinichen D, Heuft HG, Renken C, Jüttner B, Jaeger K, Schürholz T, *et al*. Impact of tobacco smoking on platelet function in apheresis products in vitro. Vox Sang 2004;86(4):252–6.
27. Ikonomidis I, Lekakis J, Vamvakou G, Andreotti F, Nihoyannopoulos P. Cigarette smoking is associated with increased circulating proinflammatory and procoagulant markers in patients with chronic coronary artery disease: effects of aspirin treatment. Am Heart J 2005;149(5):832–9.
28. Bliden KP, Dichiara J, Lawal L, Singla A, Antonino MJ, Baker BA, *et al*. The association of cigarette smoking with enhanced platelet inhibition by clopidogrel. J Am Coll Cardiol 2008;52(7):531–3.

Address for Correspondence:

Dr Mudassar Noor, Department of Pharmacology & Therapeutics, Army Medical College, National University of Medical Science, Rawalpindi, Pakistan. **Cell:** +92-333-3693588
Email: smillingdr@yahoo.com

Received: 7 May 2018 Reviewed: 2 June 2018 Accepted: 5 June 2018