FREQUENCY OF ASPIRIN RESISTANCE IN PATIENTS WITH CARDIOVASCULAR DISEASES

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ABSTRACT

Objectives: *To find out the frequency of aspirin resistance, as measured by the inhibition of platelet aggregation, using Whole Blood Aggregometery, in patients with cardiovascular diseases presenting to out patient department of a tertiary care hospital.*

Methodology: *This study was conducted in the outpatient department of Cardiology, Lady Reading Hospital Peshawar, from October 2007 to January 2008. A total of 105 normal individuals not taking aspirin and 136 patients taking aspirin for cardiovascular diseases were randomly included. Blood was taken for measuring platelet aggregation using whole blood aggregometer. Result of each individual was noted on a proforma . Patients who were on any other ante-platelet like clopidogrel; or on warfarin and heparin were excluded from the study. Chi –square and independent t-test were used to find significant differences between different groups and variables.*

Results: *Platelet aggregability in 105 normal subjects, not taking aspirin was 9.28±3.23ohms. So cutoff for aspirin non responsiveness was taken as 9.28-3.23=6.0 ohms. Mean aggregability of 136 cardiovascular patient, taking aspirin was 5.81± 5.47 ohms. Mean age was 52.66± 10 years.Male were 80(58.8%). Patients having aggregability ⩾ 6 ohms were 47.1%(n=64). Mean aggregability of male patients was 5.66± 5.45 ohms. Mean aggregability of female patients was 6.03 ± 5.54 ohms(p=0.69). When age was correlated with aggregability, both have a weak negative correlation (Pearson correlation coefficient= -.109 (p=0.205). Mean age of patients having aggregability ⩽ 5 was 53.73± 9.73 and aggregability ⩾ 6 was 51.46 ± 10.36 (p=0.19).*

Conclusion: *Aspirin resistance as defined by inhibition of platelet aggregation measured with Whole Blood Aggregometry, is a common problem. Gender and age has no significant affect on platelet aggregability.*

Key Words: *Aspirin resistance, Whole Blood Aggregometery, Platelet aggregability*

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INTRODUCTION

Platelet adhesion, activation and aggregation is the main underlying mechanism of thromboembolic vascular events; like myocardial infarction and stroke. Inhibiting platelets is the primary strategy for preventing vascular events. Aspirin is the most widely used anti platelet drug world wide[1].

Its use reduces the risk of major vascular events by approximately 25% in high risk cardiovascular patiens[2].

Aspirin inhibits platelet aggregation by acetylation of platelet cyclo-oxygenase (COX) enzyme, thus blocking the transformation of arachidonic acid (AA) into thromboxane (TX)A_2[3].

Aspirin's antiplatelet effect may not be uniform in all patients because many patients taking aspirin still suffer from vascular events. The term aspirin resistance is used to describe clinical and laboratory (biochemical) phenomena.

Clinical aspirin resistance include patients who, despite aspirin use, experience vascular events[4,5].

Biochemical aspirin resistance can be defined as incomplete suppression of platelet

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aggregation, as assessed by various platelet function tests[6-8].

Previous studies have estimated that 0.4-83.3% of patients were aspirin resistant[7].

In our country very limited work has been done on this subject so far[9].

This study was initiated to evaluate frequency of aspirin resistance in our local patients with cardiovascular disease.

METHODOLOGY

This study was conducted in the outpatients department (OPD) of Cardiology, Post-Graduate Medical Institute, Lady Reading Hospital (PGMI/LRH) Peshawar, from October 2007 to January 2008. A total of 105 normal individuals not taking aspirin and 136 patients, taking aspirin for cardiovascular diseases, were randomly included in the study. Patients who were included were aged 18 years or above and taking aspirin in doses 75 to 325 mg for atleast 10 days duration. Patients who were on any other ante-platelet like clopidogrel; or on warfarin and heparin were excluded from the study.

We measured the inhibition of platelet aggregation (using WBA aggregometer) in these patients. Result of each individual along with age,gender and diagnosis were noted on a specially designed proforma prepared in accordance with the objective of the study.

The machine used for platelet aggregation was chronolog whole-blood platelet aggregometer (WBA). Other supplies needed for performing whole-blood aggregation were reagents, cuvettes, stir bars, micropipettes, tips etc.

Whole blood (0.5 ml) was diluted with an equivalent volume of isotonic saline and incubated for 5 minutes. The impedance of each sample was monitored at sequential 1-minute intervals until a stable baseline established. The agonist ADP (20 µmol/L) was then added to the sample and aggregation was monitored for 6 minutes. With time platelets aggregated over electrodes and impedance increases. The final increase in impedance (in ohms) over this period was displayed as a numeric readout.

In addition, a graphical printout (i.e. chart tracing) of each electrical impedance aggregometry was also obtained. Each reading was noted on the patient's proforma. Normal individuals were used as control group. Mean aggregability (in ohms) of the normal individuals minus 1 standard deviation was taken as Cutoff value for aspirin resistance in cardiovascular patients.

All the information collected was analyzed using SPSS version 11. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as mean± SD. Categorical variables were compared using chi square test. Continuous variables were compared using independent sample t-test. Pvalue less than 0.05 was considered significant.

RESULTS

When Platelet aggregability was performed in the control group (105 normal subjects, not taking aspirin)it was found to be 9.28±3.23ohms (Figure 1).

Mean age of the 136 cardiovascular patients, taking aspirin was 52.66±10.00 years. Male were 80(58.8%). Female were 56(41.2%). Most (82.4%) were suffering from IHD. Mean aggregability of all these patients was found to be 5.81±5.47 ohms (Figure 2).

Patients having aggregability ≥6 ohms (aspirin resistance) were 47.1 %(n=64). Patients having aggregability,< 6 ohms (aspirin sensitive) were 52.9 %(n=72) {Figure 3}.

Mean aggregability of all the male patients was 5.66±5.45 ohms. Mean aggregability of all the female patients was 6.03±5.54 ohms (p=0.69) {Figure 4}.

Sub group analysis of aspirin sensitive and resistant patients, on the basis of gender showed that among total 72 patients having aggregability <6 ohms, male were 43 (59.7%) female were 29 (40.3%).

Among total 64 patients having aggregability ≥6 ohms male were 37 (57.8%) female were 27 (42.2%) (p=0.821) {Figure 5}.

When age was correlated with aggregability, both have a weak negative correlation. Pearson correlation coefficient= -.109 (p=0.205) {Figure 6}. Mean age of patients having aggregability < 6 ohms was 53.73±9.73 years. Mean age of Patients having aggregability ≥6 ohms was 51.46 ± 10.36 years (p=0.19).

As the cut off value for aspirin resistance was taken as the mean aggregability of normal individuals minus one standard deviation, so it was calculated as 9.28-3.23=6.05 ohms.

DISCUSSION

The issue of aspirin resistance has been emphasized in medical literature for more than a decade[10-13].

Very limited work has been done in Pakistan to test platelet aggregability in patients

Figure 1: Platelet Aggregability of control
(normal individuals not on aspirin)

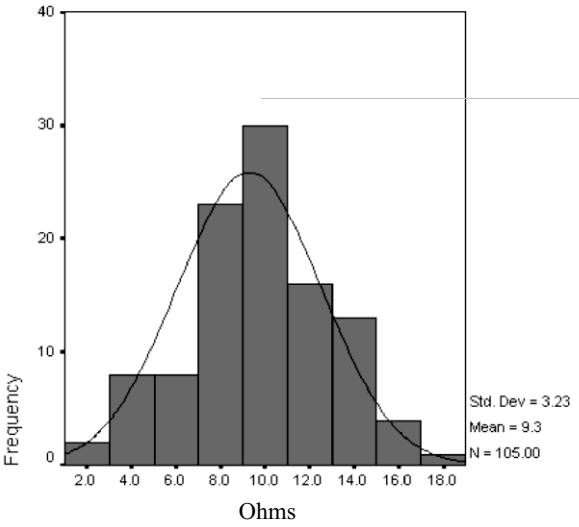


Figure 2: Platelet Aggregability
(all patient taking aspirin)

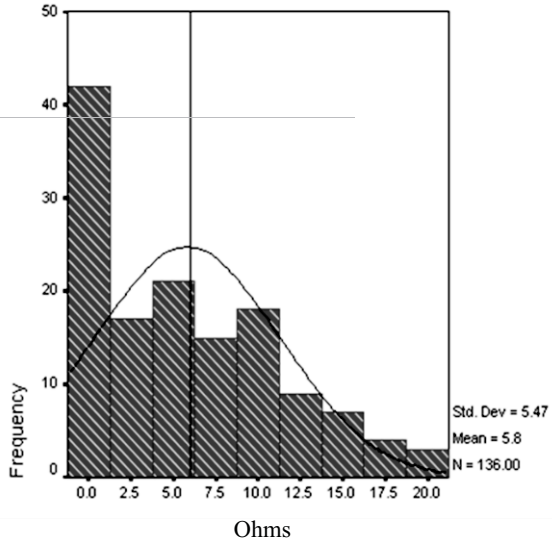


Figure 3: Aspirin resistance

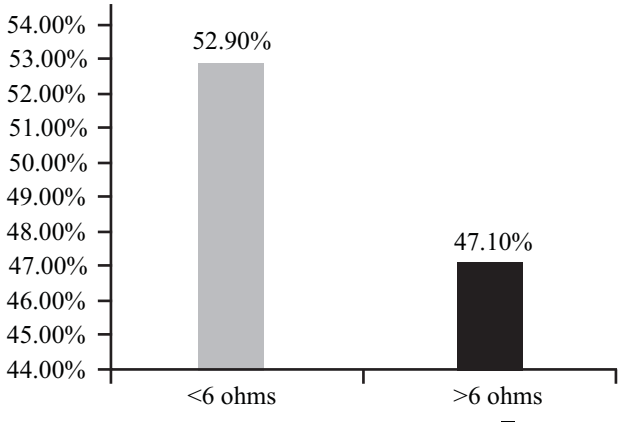


Figure 4: Gender Effect on platelet aggregability

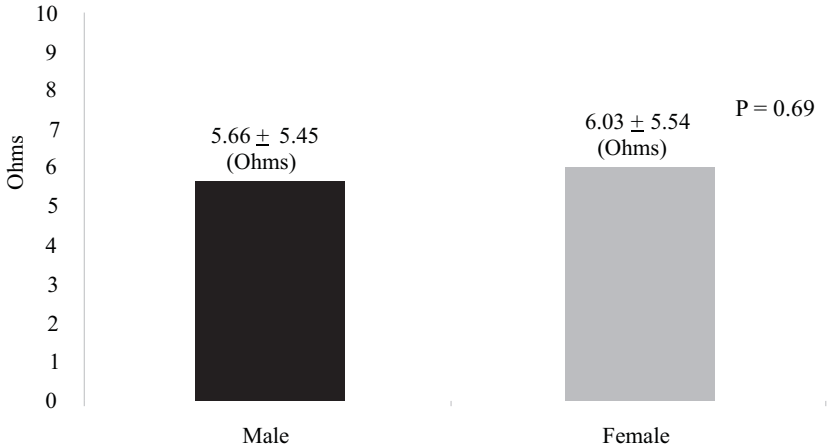


Figure 5: Gender effect on platelet aggregability

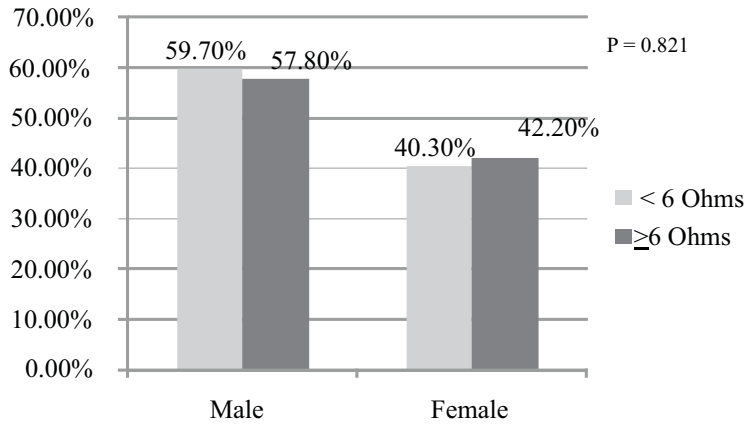
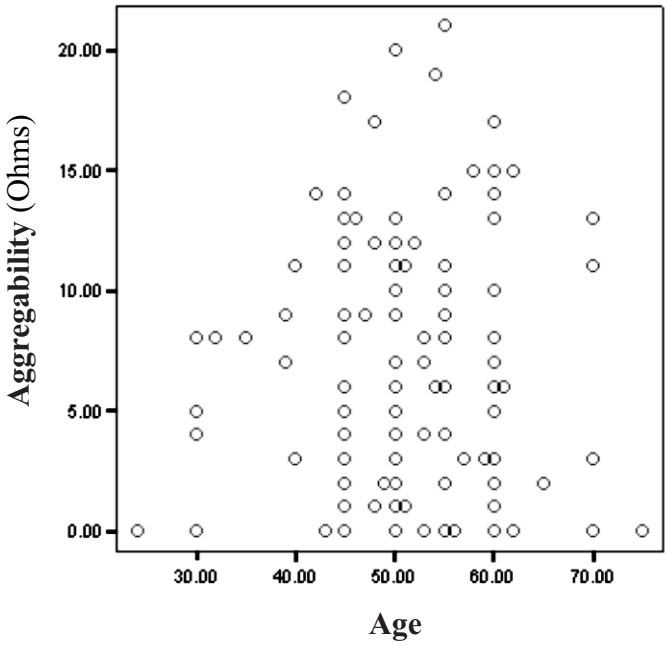


Figure 6: Effect of Age on platelet aggregability



Pearson's Correlation Co-efficientr= -0.109

taking aspirin[9].

Prevalence of aspirin resistance reported by previous studies ranged from 0.3 to 80%[7].

Our study showed 47.8% of patients with cardiovascular disease to be aspirin resistant.

Marielorkipandze et al reported 18% prevalence of aspirin resistance. They used whole blood aggregometry and took 3 ohms as cutoff value for defining aspirin resistance[14].

Harrison P et al, using PFA 100 device, reported 22% prevalence of aspirin resistance in patients taking aspirin after TIA or stroke[15].

Sane DC et al found that 57% of patients with congestive Heart failure were aspirin resistance[16].

Anderson K at al found 35% aspirin resistance in post MI patients, using PFA 100 point of service assay[17].

With the ultra point of care rapid platelet function assay ASA, the prevalence of aspirin resistance was 19% to 23%[18,19].

The reason for this high range of prevalence among different studies, may be differences in studied populations, lack of formal definition of aspirin resistance, selecting different cutoff values for defining aspirin responders and non- responders and use of different diagnostic methodologies by different researches[7].

Light transmission aggregometry has been in use for last 40years and is considered the historical gold standard for testing platelet function[20].

Its use is very time consuming as it involves preparation of platelet rich plasma, needs manipulation by a skilled technician and its standardization is often poor limiting its use to dedicated laboratories[20-25].

We used whole blood aggregometry (WBA) using chronolog aggregometer, because it is easy to use, gives faster results, does not require centrifugation and evaluates platelets in a physiologic milieu in presence of red and white blood cells, which are known to modulate platelet functions[26].

WBA measures the electrical impedance, in ohms, between two electrodes immersed in whole blood as the platelets are aggregated on them[21,22,27].

Previous studies, which used WBA for platelet function, used different cutoff values ranging from 3ohms to 18ohms for defining aspirin resistance[16,28].

We evaluated 105 normal subjects, not taking aspirin and used their mean minus 1 SD agregability as cutoff value for aspirin resistance.

This principle has been used previously by other researchers[28,29].

Literature review shows that which ever test is used and whatever criteria is applied , still a good percentage of patients will turn out to be aspirin resistant.

Considering the common use of aspirin, this prevalence is of particular importance.

On the other hand studies like ours, in which platelet functions are tested in vitro, can find only biochemical resistance.

To see whether this biochemical resistance translates in to clinical resistance the patients need to be followed up in prospective studies for occurrence of any clinical events.

Work has been done in this regard and studies have suggested that risk of major cardiovascular events increases in patients who are non responder to aspirin[30,31].

Our study did not show any significant difference between the platelet aggregabililty of male and female patients (5.66$\pm$5.45 ohms vs 6.03$\pm$5.54 ohms) with p=0.69.

Previous studies showed female patients to have high platelet aggregability despite aspirin use[29,32].

In our study there is a trend towards lower platelet aggregability as the age increases, but this negative correlation is weak (r=-.109) and insignificant (p= 0.205).

Previous studies reported contradicting results about the association of age and platelet aggregability.

Ivandic BTet al showed that age has no association with platelet aggregability[29].

Gum PA at al reported that aspirin semi responders were significantly older[32].

Different mechanisms have been implicated to cause aspirin resistance including poor compliance, inadequate dosing, interindividual variability in response to aspirin, genetic polymorphism in platelet receptors, extra platelet sources of thromboxane A2[33](e.g. monocytes/macrophages) and concomitant NSAIDs use[34].

In addition platelet aggregability responses can vary with mental stress, age , gender , race, diet and hematocrit level and a person may have different responses on repeated determinations[35].

CONCLUSION

Aspirin resistance as defined by inhibition of platelet aggregation measured with Whole Blood Aggregometry, is a common problem. Gender and age has no significant affect on platelet aggregability.

REFERENCE

1. Jack DB. One hundred years of aspirin. Lancet 1997;350:437-9.
2. Antithrombotic Trialists Collaboration. Collaborative metaanalysis of randomized trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 2002;324:71-86.
3. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin like drugs. Nat New Biol 1971;231:232-5.
4. Helgason CM, Bolin KM, Hoff JA, Winkler SR, Mangat A, Tortorice KL, et al. Development of aspirin resistance in persons with previous ischemic stroke. Stroke

1994;25:2331-6.

5. Vejar M, Fragasso G, Hackett D, Lipkin DP, Maseri A, Born CV, et al. Dissociation of platelet activation and spontaneous myocardial ischemia in unstable angina. Thromb Heamost 1990;63:163-8.
6. Hankey GJ, Eikelboom JW. Aspirin resistance. Lancet 2006;367:606-17.
7. Lordkipanidze M, Pharand C, Palisaitis DA, Diodati JG. Aspirin resistance: truth or dare. Pharmacol Ther 2006;112:733-43.
8. Michelson AD, Cattaneo M, Eikelboom JW, Gurbel P, Kottke-Marchant K, Kunicki TJ, et al. Aspirin resistance: position paper of the working group on aspirin resistance. J Thromb Heamost 2005;3:1309-11.
9. Naveed A, Ayesha J, Ayesha K, Waqas A, Mumtaz A, Habib R. Frequency of Aspirin resistance in patients with coronary artery disease in Pakistan. Pak J Pharm Sci 2009;22:230-3.
10. Cattaneo M. Aspirin and clopidogrel: efficacy, safety and the issue of drug resistance. Arterioscler Thromb Vasc Biol 2002;24:1980-7.
11. Patrono C. Aspirin resistance: definition, mechanisms and clinical read outs. J Thromb Haemost 2003;1:1710-13.
12. Cambell CL, Steinhubl SR. Variability in response to aspirin: do we understand the clinical relevance? J Thromb Heamost 2005; 3: 665-669.
13. Undas A, Brummel-Ziedins KE, Mann KG. Antithrombotic properties of aspirin and resistance to aspirin: beyond strictly anti platelet actions. Blood 2007;109:2285-92.
14. Lordkipanidze M, Pharand C, Schampaert E, Turgeon J, Palisaitis DA, Diodati JG. A comparison of six major platelet function tests to determine the prevalence of aspirin resistance in patient with stable coronary artery disease. Eur Heart J 2007;28:1702-8.
15. Harrison P, Segal H, Blasbory K, Furtado C, Silver L, Rothwel PM. Screening for aspirin responsiveness after transient ischemic attack and stroke. Stroke 2005;36:1001-5.
16. Sane DC, McKee SA, Malinin A, Serebrauny VL. Frequency of aspirin resistance in patients with congestive heart failure treated with antecedent aspirin. Am J Cardiol 2002;90:893-5.
17. Anderson K, Hurlen M, Arnesen H, Selijiflot I. Aspirin nonresponsiveness as measured By PFA-100 in patients with CAD. Thromb Res 2002;108:37-42.
18. Wang JC, Aucoin Barry D, Manuelian D, Monbouquette R, Reisman M, Gray W, et al. Incidence of aspirin non responsiveness using the ultegra rapid platelet function assay ASA. Am J Cardiol 2003;92:1492-4.
19. Chen WH, Lee PY, Ng W, Tse HF, Lau CP. Aspirin resistance is associated with high incidence of myonecrosis after non urgent percutaneous coronary intervention despite clopidogrel pre treatment. J Am Coll Cardiol 2004;43:1122-6.
20. Michelson AD. Platelet function testing in cardiovascular diseases. Circulation 2004; 110:e489-93.
21. Harrison P. Advance in platelet counting. Br J Heamatol 2000;111:733-44.
22. Nicholson N, Panzer-Knodle S, Haas N, Taite B, Szalony J, Page J, et al. Assessment of platelet function assays. Am Heart J 1998;135: 170-8.
23. Gum PA, Kottke-Marchant K, Weish PA, White J, Topol EJ. A prospective, blinded determination of natural history of aspirin resistance among stable patients with cardiovascular disease. J Am Coll Cardiol 2003; 41:961-5.
24. Harrison OP, Frelingeh AL, Furman MI, Michelson AD. Measuring anteplatelet drug effect in the laboratory. Thromb Res 2007;120:323-36.
25. Shantsila E, Watson T, Lip GYH. Laboratory investigations of platelets. In: Gresele P, Fuster V, Lopez JA, editors. Platelets in haematologic and cardiovascular disorders: a clinical handbook. New York: Cambridge University Press; 2008. p. 124-46.
26. Dyszkienicz-Korpantyt AM, Frenkal EP, Sarode R. Approach to the assessment of platelet function: comparison between optical based platelet rich plasma and impedance-based whole blood platelet aggregation methods. Clin Appl Thromb Hemost 2005;11:25-35.
27. Rand ML, Leung R, Packham MA. Platelet function assays. Transfus Apher Sci 2003; 28:307-17.
28. Ivandic BT, Giannitsis E, Schlick P, Staritz P, Katus HA, Hohlfeld T. Determination of aspirin responsiveness by use of whole blood platelet aggregometry. Clin Chem 2007; 53:614-9.

29. Ivandic BT, Schlick P, Steritz P, Kurz Kerstin, Katus HA, Giannitsis E. Determination of clopidogrel resistance by whole blood platelet aggregabiltiy and inhibitors of the p2y12 receptor. Clin Chem 2006;52:383-8.

30. Crescente M, Di-Castelnuovo A, Lacoviello L, de Gaetano G, Cerletti C. PFA-100 closure time to predict cardiovascular events in aspirin treated cardiovascular patients: a meta analysis of 19 studies comprising 3003 patients. Thromb Heamost 2008;99:1129-31.

31. Eikelboom JW, Hankey GJ. Aspirin resistance: a new independent predictor of vascular events? J Am Coll Cardiol 2003;41:966-8.

32. Gum PA, Kottke-Marchant K, Poggio ED, Gurm H, Welsh PA, Brooks L, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. Am J Cardiol 2001;88:230-5.

33. Cipollone F, Ciabattoni G, Patrignani P, Pasquale M, Di Gregorio D, Bucciarelli T, et al. Oxidant stress and aspirin insensitive thromboxane biosynthesis in severe unstable angina. Circulation 2000;102:107-13.

34. Catella-Lauson F, Reilly MP, Kapoor SC, Cucchiara AJ, DeMarco S, Tournier B, et al. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. N Eng J Med 2001;345: 1809-17.

35. George JN, Shattil SJ. The clinical importance of acquired abnormalities of platelet function. N Eng J Med 1991;324:27-39.

CONTRIBUTORS

MF conceived the idea and planned the study. JA, IS, S & H, did the data collection and analyzed the study. MA, FA, AG & MH contributed significantly in the write up of research that resulted in the submitted manuscript.