Anti platelet effect of Aspirin in Ischemic Stroke : A Hospital Based Study

ABSTRACT

Introduction: Aspirin is widely used for the treatment of stroke. Therefore resistance to aspirin can lead to a significant increase in the burden of stroke. Platelet aggregation studies can evaluate platelet function, and this may help to detect anti platelet resistance.

Methods: This is a hospital based study of the antiplatelet effect of aspirin in ischemic stroke, during a duration of one year.

All first time ischemic stroke patients >18 years age were included. Platelet aggregometry test was done by LTA (Light transmission optical aggregometer), after starting the patients on oral aspirin.

Results: Total of 113 ischemic stroke patients were included for antiplatelet effect of aspirin study. Aspirin resistance was found in 18.58% patients. Patients with aspirin resistance had higher mortality, and less improvement on follow up, as compared to aspirin sensitive patients. They had more incidence of smoking, alcohol abuse, diabetes mellitus and dyslipidemia, as compared to aspirin sensitive group.

Conclusion: Aspirin resistance can lead to less functional improvement and more mortality than aspirin sensitive patients. However, further study for drug interactions, adequate risk factor control, genetic profile of the population is needed, to come to a definite conclusion.

Key Words: Ischemic stroke, aspirin resistance, aspirin sensitive

Key message: Aspirin is the main treatment for ischemic stroke. Aspirin resistance can lead to recurrent ischemic stroke. Therefore studies to test for aspirin resistance is needed to prevent ischemic stroke related mortality and morbidity.

Running title: Antiplatelet effect of Aspirin in Ischemic Stroke

Introduction

Ischemic stroke accounts for approximately 80% of the new or recurrent strokes.[1] Anti platelet agents are used for the prevention of atherothrombotic events , as they inhibit the formation of intraarterial platelet aggregates. While there are a number of anti platelet medications used for preventing ischemic stroke, aspirin remains the most widely used, due to its low cost, generally good safety profile, and long term experience with the medication. Large meta analysis have shown that the relative risk reduction of aspirin for stroke in patients with a prior stroke or transient ischemic attack was 20% to 25% ,[2] the absolute risk reduction varies considerably depending on the patient's risk.

However there are concerns about the efficacy of aspirin, as several patients manifests recurrent stroke, despite regular intake of aspirin. The occurrence of thrombotic events despite the use of anti platelet drugs has led to the concept of anti platelet resistance. Several studies have found aspirin resistance in 20% to 30% of the patients.[3]

Platelet aggregation studies can evaluate platelet function, and this may help to detect anti platelet resistance. The results can guide anti platelet therapy which may translate into decrease in recurrent stroke.

Historically, Light transmittance aggregometry (LTA), was considered the gold standard platelet function assay.[4] Therefore inspite of several limitations,[5] it is still the most widely used method to study platelet aggregation.

We conducted a prospective observational, hospital based study for a duration of one year, for aspirin resistance in patients with first time ischemic stroke. The objectives were to detect the incidence of aspirin resistance in ischemic stroke patients; and to study the demographic pattern and stroke outcome of aspirin resistant and aspirin sensitive patients.

Materials and Methods:

We obtained ethics approval from institute ethics committee. Written informed consent was obtained from all the participants or their guardians.

The study was carried out in the department of Neurology, and Pathology, in a teaching hospital, in North East India. Patients were enrolled from the emergency department and from outpatient department according to the inclusion criteria.

The following inclusion and exclusion criteria were used:

Inclusion criteria: All first time stroke patients more than 18 years who were diagnosed to have ischemic stroke on neuroimaging.

Exclusion criteria: Patients less than 18 years age, history of recent head injury, past history of stroke, any hemorrhagic stroke, patients planned for thrombolysis or already

taking oral anti coagulants or anti platelet drugs or history of coagulopathy, patients with history of hypersensitivity to aspirin, patients taking NSAID's (non steroidal anti inflammatory drugs), patients with low platelet count ($<100*10^3$ /microL) and patients refusing informed consent.

Ischemic stroke was diagnosed clinically (we used the WHO definition of stroke), and by neuroimaging (non contrast computerized tomography or magnetic resonance imaging). All enrolled patients were evaluated for demographic profile, symptoms of the index event, medication history, vascular risk factor history, and clinical examination as part of routine clinical evaluation. The degree of disability on admission was assessed by mRS (modified Rankin Scale). Vascular risk factors evaluated included history of hypertension, diabetes, dyslipidemia, coronary artery disease, congestive heart failure, current or previous smoking, and moderate or heavy alcohol consumption (2 or more alcohol drinks per day). Hypertension was defined as systolic blood pressure $\geq$ 140mmHg or diastolic blood pressure $\geq$ 90 mmHg, any anti hypertensive drug use, or self reported hypertension history. Diabetes was defined as fasting glucose $\geq$ 126 mg/dl, random blood glucose $\geq$ 200 mg/dl with symptoms of hyperglycemia, any use of hypoglycemic agents or self reported history of diabetes. Dyslipidemia was defined as serum low density lipoprotein cholesterol (LDL) $\geq$ 130 mg/dl, serum triglyceride $\geq$ 150 mg/dl, high density lipoprotein cholesterol (HDL) $\leq$ 40 mg/dl, any use of lipid lowering drugs, or self reported history of dyslipidemia. History was confirmed from care givers in patients unable to provide reliable information.

All enrolled patients were started on similar preparation of oral aspirin 150mg once daily on day 1 of admission. Care was taken to ensure compliance.

Blood sample for platelet aggregation study was collected on the fifth day of tablet aspirin intake. Venous blood sample was collected in (EDTA) ethylenediaminetetraacetic acid vacutainer from each patient. Platelet aggregation test was done within 2- 3 hours after sampling.

Testing for anti platelet activity of aspirin was done by using LTA (Light transmission optical aggregometer Bio Data Corporation, PAP 8, V2.0 Optics) .This works on the principle that when citrated platelet rich plasma (PRP) is continuously stirred in a platelet aggregometer and a light beam is passed through the suspension, platelet aggregation in response to an added chemical stimulus can be monitored by changes in light transmittance. Transmission of light is detected by the photocell and recorded as a function of time. 5ml of whole blood was collected in 3.2 % tri sodium citrate in a 9:1 ratio(4.5ml blood: 0.5ml TSC). Preparation of Platelet rich plasma (PRP) & Platelet poor plasma (PPP) was carried out. 250uL of PPP was added in a cuvette which acted as a blank. Then 225uL of PRP is added in another cuvette. A magnetic stir bar was added to the cuvette containing PRP. PRP was incubated at 37˚C by keeping it in stir well. PPP was placed and set as BLANK in theMenu, then PRP was placed and set as START test in the Menu. 5µL of Epinephrine was added the procedure was started in the Menu. Aggregation pattern which is plotted as a XY axis graph against light transmission and time was observed, and this is printed out and interpreted (Figure 1). The same procedure was done for ADP, Collagen and Arachidonic acid.

The aggregation pattern is plotted for Epinephrine, ADP(adenosine diphosphate), Arachidonic acid, and Collagen. Decreased or absent aggregation response to the above chemical indicates normal anti platelet response of aspirin. Failure to show decrease or

absent aggregation response is suggestive aspirin resistance. The tests were performed after proper calibration of the LTA.

Aspirin resistance was defined as a mean platelet aggregation of $\geq$ 20% with 5 microL Arachidonic acid, and a mean aggregation of $\geq$ 70% with 10 microL ADP. Aspirin sensitive is defined as a mean platelet aggregation of $\leq$ 20% with 5microL Arachidonic acid, and a mean aggregation of $\leq$ 70 % with 10microL ADP.

Analysis was done for aspirin resistance and sensitivity with respect to demographic and risk factor profile, and functional outcome.

The patients were followed up during hospital stay, 30 day and 90 day after ictus, by the mRS (modified Rankin Scale) for functional outcome. Improvement was defined as any decrease in mRS score from baseline on admission. During this duration the patients were followed up for recurrent stroke.

Statistical analysis: The adjusted sample size was calculated to be 110. Continuous variables are presented as mean $\pm$ standard deviation, and categorical variables are presented as absolute values. In all analysis a $p < 0.05$ was considered statistically significant.

Results:

We prospectively studied 113 patients enrolled with a diagnosis of first time ischemic stroke (after exclusion of 17 patients due to lack of follow up) . The mean age was 62.16, with a male predominance of 1.89:1. Hypertension was the most common risk factor (71.68%), followed by smoking (33.62%), with others like diabetes mellitus,

dyslipidemia ,and alcohol (17%-20%). Cardioembolic stroke was excluded, as they had to be started on oral anticoagulants, except patients with Dilated cardiomyopathy with ejection fraction >20% were included. Aspirin was started and blood test for assessing anti platelet function was done according to protocol.

On antiplatelet effect study, aspirin resistance was found in 21 patients (18.58%). A mean age of 59.52, with a male predominance of 3.2:1 was found. The most common risk factor in the aspirin resistance group was hypertension (66.66%), followed by smoking (57.14%), dyslipidemia (23.80%), diabetes mellitus (23.80%), and alcohol (23.80%).On investigation ECG abnormalities was seen in 33.33%, and Carotid Doppler was abnormal in 28.57%. Polycythemia was seen in 3 patients.

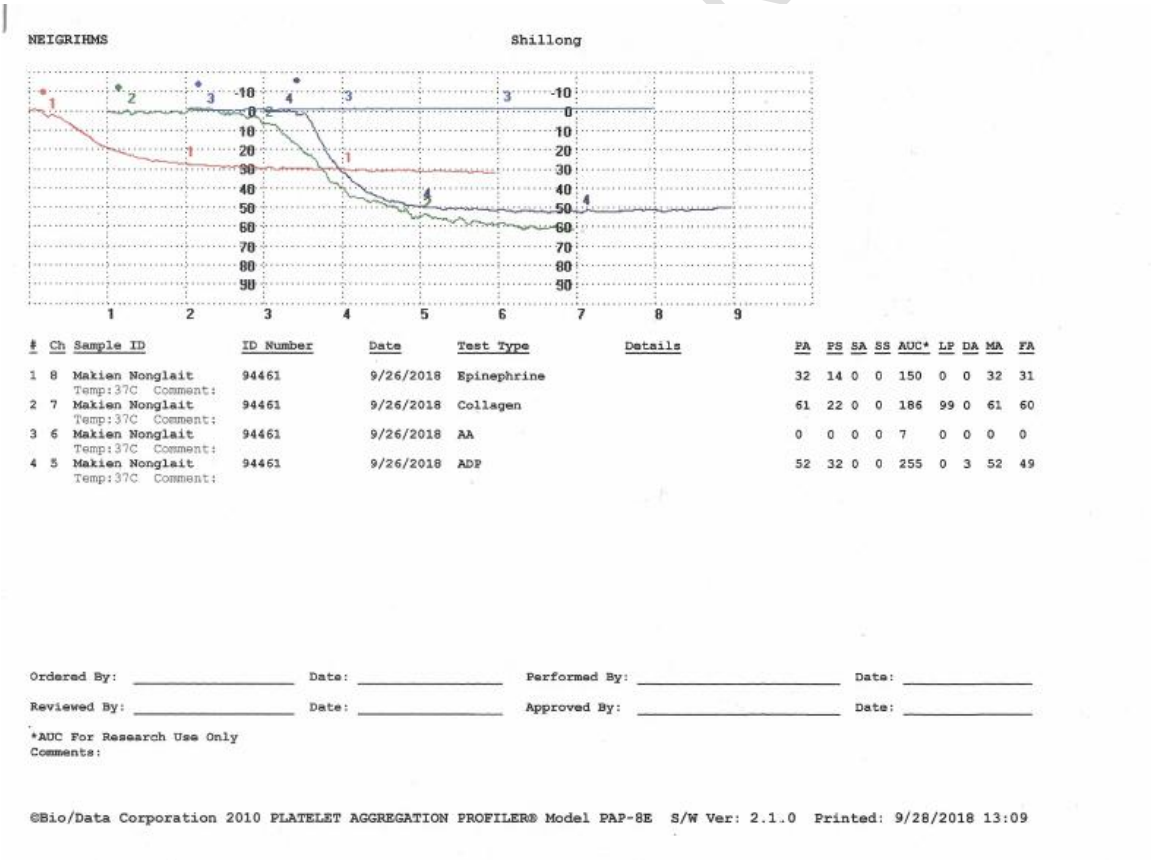


Figure 1. Platelet aggregation curve of Aspirin sensitive patient

Table: 1 Comparison of Demographic and Clinical characteristics of Aspirin sensitive and Aspirin resistant Ischemic stroke

	Aspirin sensitive (n=92)	Aspirin resistant (n=21)	*p* value
Age (mean)	62.75±15.54	59.53±16.55	0.199856
Gender (M:F)	1.7:1	3.2:1	
Risk factors(%)			
Hypertension	72.82	66.66	0.287947
Diabetes	17.39	23.80	0.257118
Tobacco	28.26	57.14	0.331819
Alcohol	18.47	23.80	0.333157
Dyslipidemia	16.30	23.80	0.398
ECG abnormal	43.47	33.33	0.158
Carotid doppler abnormal	36.95	28.57	0.14
Follow up mRS			
30 days	63 improved (68.47%)	12 improved (57.14%)	
90 days	51 improved (55.43%)	7 improved (33.33 %)	
Mortality	(13.04%)	(14.28%)	
Hospital	11	2	0.223362
30 days	1	0	
90 days	0	1	

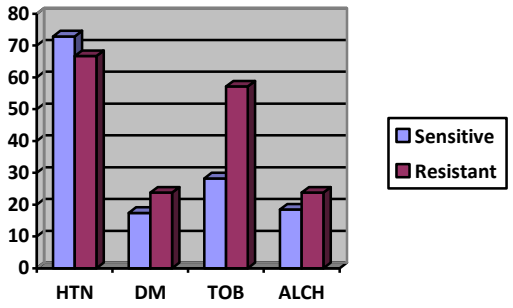
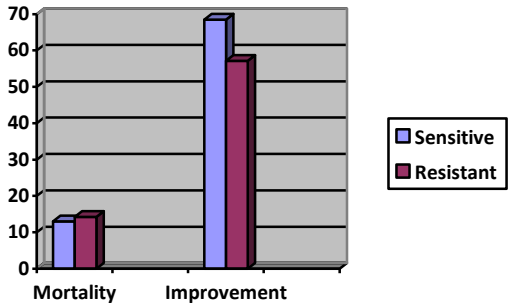


Figure 2. Comparison of risk factors in aspirin sensitive and resistant patients

Figure 3. Comparison of mortality, and mRS Score in aspirin sensitive and resistant



Discussion:

Aspirin is widely used for the treatment of stroke. Therefore resistance to aspirin can lead to a significant increase in the burden of stroke. Though aspirin resistance remains a poorly defined term, clinically it can be defined as, the failure of the drug to prevent an ischemic event despite regular intake of appropriate dose.[6] Platelet function test can be done by various methods, of which LTA (Light transmittance aggregometry) is considered the "gold standard".

Aspirin resistance was found in 18.58%, which is less as compared to previous studies which shows aspirin non responders in nearly 30% patients.[7] However, some studies shows similar aspirin resistance rates of about 16%. [8] The heterogeneity in the results may be because platelet resistance studies are highly technique dependent.

A gender difference in the efficacy of aspirin had been seen in some clinical trials, involving stroke. However, the difference in efficacy had been claimed to be due to an artifact . [9]Our study shows more incidence of aspirin resistance in males.

Previous studies have shown a higher prevalence of aspirin resistance in lacunar stroke, [10]similar finding was also seen in our study.

Aspirin resistance was associated more with diabetes mellitus, smoking, alcohol, and dyslipidemia. Platelet function is influenced by multiple factors like smoking,[11] glucose control in diabetics,[12]serum cholesterol,[13] and triglycerides. [14]These factors can at times confound test results. Therefore the specificity of the above associations as due to aspirin resistance, cannot be ascertained.

The mortality was higher in aspirin resistant patients, as well as they had less improvement on follow up. Several studies have shown higher end points of death in aspirin resistant patients. [15]

Though the above results show more adverse outcome of ischemic stroke, in aspirin resistant patients, a definite clinical –laboratory association of aspirin resistance and poor outcome cannot be definitely made. This is because of two reasons: [16] 1. Stroke is an etiologically heterogeneous disorder. The extent to which platelets contribute to stroke pathophysiology, varies according to the underlying etiology. Therefore, recurrent stroke or mortality may be related to non platelet related factors.2. Platelet may continue to aggregate and cause stroke, by recruitment of compensatory pathways, not blocked by the anti platelet agent.

The study has few limitations. No data was collected regarding recurrence of stroke during follow up. Due to this a definite association between laboratory aspirin resistance and clinical aspirin resistance could not be ascertained.

Even though aspirin resistance is a reality, there are no specific guidelines for its management. Higher dose may be associated with more potential side effects.

Conclusion:

This is one of the very few studies done in India,[17] and the only study from North East India, on the anti platelet response to aspirin, as measured by platelet aggregometry test. The study shows that there is evidence of less recovery, and increase in mortality in patients showing resistance to aspirin, on platelet aggregation study, as compared to those patients with normal anti platelet response, with no statistically significant differences. Further studies are needed to be done regarding racial variation in aspirin response, standardization of anti platelet tests, standardizing laboratory and clinical definition of aspirin resistance.

Till further studies throw more light on the subject, aspirin remains the most appropriate choice as the first line anti platelet for ischemic stroke.

Reference:

1.Foulkes MA,Wolf PA, Price TR, Mohr JP, Hier DB. The Stroke Data Bank: design, methods, and baseline characteristics. Stroke.1988;19: 547-554

2. Antithrombotic Trialists Collaboration. Collaborative meta analysis of randomized trials of anti platelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ.2002;324:71-86

3. Hegalson CM,Bolin KM, Hoff JA, Winkler SR, Mangat A, Tortorice KL, Brace LD. Development of aspirin resistance in persons with previous ischemic stroke. Stroke.1994;25: 2331-2336

4.Michelson AD, Frelinger AL 3rd, Furman MI. Current options in platelet function testing. Am J Cardiol.2006;98(10A): 4N-10N

5. Cattaneo M, Hayward CP, Moffat KA, Pugliano MT, Liu Y, Michelson AD. Results of a worldwide survey on the assessment of platelet survey by light transmission aggregometry: a report from the platelet physiology sun committee of the SSC of the ISTH.J Thromb Haemost 2009;7: 1029.Epub 2009

6.Weber AA, Przytulski B, Schanz A, Hohlfeld T, Schror K. Towards a definition of aspirin resistance : a typological approach. Platelets 2002; 13: 37-40

7.Grotemeyer KH, Scharafinski HW, Husstedt IW. Two year follow up of aspirin responders and aspirin non responders. A pilot study including 180 post stroke patients. Thromb Res 1993 ; 71: 397-403

8.Grau A, Reiners S, Med C, Lichy C, Buggle F, Ruf A. Platelet function under aspirin, clopidogrel, and both after ischemic stroke. Stroke, 2003; 34: 849-855

9. Kelton JG, Powers P, Julian J, Boland V, Carter CJ, Gent M, Hirsh J. Sex related differences in platelet aggregation: Influence of hematocrit. Blood 1980; 56: 38-41

10.Englyst NA, Horsefield G, Kwan J, Byrne CD. Aspirin resistance is more common in lacunar strokes than embolic strokes and is related to stroke severity. J Cereb Blood Flow Metab 2008; 28(6): 1196-1203

11. Gremmel T, Steiner S, Seidinger G, Koppensteiner R, Panzer S, Kopp CW. Smoking promotes clopidogrel mediated platelet inhibition in patietns receiving dual anti platelet therapy. Thromb Res. 2009; 124(5): 588-591

12. Ertugrul DT, Tutal E, Yildiz M, et al. Aspirin resistance is associated with glycemic control , the dose of aspirin, and obesity in Type 2 diabetes mellitus. J Clin Endocrinol Metab. 2010; 95(6): 2897-2901

13. Hovens MM, Snoep JD, Groeneveld Y, Tamsma JT, Eikenboom JC, Huisman MV. High levels of low density lipoprotein cholesterol and triglycerides and suboptimal glycemic control predict diminished ex vivo aspirin responsiveness in patients with Type 2 diabetes. J Thromb Haemost. 2007; 5(7): 1562-1564

14. Karepov V, Tolpina G, Kuliczkowski W, Serebruany V. Plasma triglycerides as predictors of platelet responsiveness to aspirin in patients after first ischemic stroke. Cerebrovasc Dis.2008;26(3): 272-276

15. Gum P, Kotke-Marchant K, Welsh P, White J, Topol E. A prospective blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. J Am Coll Cardiol.2003;41: 961065

16. Breet NJ, van Werkum JW, Bouman HJ, et al. Comparison of platelet function tests in predicting clinical outcome in patients undergoing coronary stent implantation. JAMA.2010;303(8): 754-762

17. Samir Patel, Vandana Arya, Amrita Saral, Manorama Bhargava, C.S.Agarwal. Aspirin and Clopidogrel resistance in Indian Patients with Ischemic Stroke and its Association s with Gene Polymorphism: A Pilot Study. Annals of Indian Academy of Neurology.2019;22(2): 147-152

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