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Antiplatelet Effect of Aspirin in Ischemic Stroke: A Hospital-based Study

Masaraf Hussain [a≡*], Yookarin Khonglah [b≡], S. R. Sharma [aφ], Baia Synmon [a≡] and Yasmeen Hynniewta [a†]

[a] Department of Neurology, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences (NEIGRIHMS), Shillong 793018, India.
[b] Department of Pathology, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences (NEIGRIHMS), Shillong 793018, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Aspirin is widely used for the treatment of stroke. Therefore aspirin resistance 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 of age were included. Platelet aggregometry test was done by LTA (Light transmission optical aggregometer), after starting the patients on oral aspirin.
Results: A total of 113 ischemic stroke patients were included for the antiplatelet effect of the aspirin study. Aspirin resistance was found in 18.58% of patients. Patients with aspirin resistance

[≡] *Associate Professor;*
[φ] *Professor;*
[†] *Senior Resident*
**Corresponding author: E-mail: masarafhussain@yahoo.co.in, masarafhussain74@gmail.com;*

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 the aspirin-sensitive group. The results reveal that there is a non-statistically significant trend in both mortality and prognosis between the two study groups compared: aspirin-resistant versus aspirin-sensitive patients.
Conclusion: Aspirin resistance can lead to loss of functional improvement and more mortality than aspirin-sensitive patients. However, further study for drug interactions, adequate risk factor control, the genetic profile of the population is needed, to come to a definite conclusion.

Keywords: 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 are needed to prevent ischemic stroke-related mortality and morbidity.

1. INTRODUCTION

Ischemic stroke accounts for approximately 80% of the new or recurrent strokes [1]. Antiplatelet agents are used for the prevention of atherothrombotic events, as they inhibit the formation of intraarterial platelet aggregates. While there are several antiplatelet 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-analyses 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 manifest recurrent stroke, despite regular intake of aspirin. The occurrence of thrombotic events despite the use of antiplatelet 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 antiplatelet therapy which may translate into a decrease in recurrent stroke.

Historically, Light transmittance aggregometry (LTA), was considered the gold standard platelet function assay [4]. Therefore despite 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.

2. MATERIALS AND METHODS

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 the 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 an ischemic stroke on neuroimaging.

Exclusion criteria: Patients less than 18 years age, history of recent head injury, past history of stroke, and hemorrhagic stroke, patients planned for thrombolysis or already taking oral anticoagulants or antiplatelet drugs or history of coagulopathy, patients with a history of hypersensitivity to aspirin, patients taking NSAID's (nonsteroidal anti-inflammatory drugs), patients with low platelet count ($<100*10^3$/microL) and patients refusing informed consent. Daily use of aspirin is not advisable for patients with a history of hypersensitivity to aspirin, patients with a history of coagulopathy, and patients with low platelet count.

Ischemic stroke was diagnosed clinically (we used the WHO definition of stroke), and by neuroimaging (noncontrast 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 antihypertensive 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 by caregivers 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. The dosing and administration of aspirin were according to accepted guidelines formulated by national and international stroke organizations. Care was taken to ensure compliance.

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

Testing for the antiplatelet 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 decreased 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 sensitivity 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 concerning demographic and risk factor profile, and functional outcome.

The patients were followed up during hospital stay, 30 days and 90 days 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 a recurrent stroke.

Statistical analysis: The adjusted sample size was calculated by keeping the power at 80%, and two sided alpha error 0.05%. Minimum110 patients were required to observe resistance

patterns as per the previous assumption. Continuous variables are presented as mean ±standard deviation, and categorical variables are presented as absolute values. The *p* value was calculated by chi- square test. In all analyses a $p < 0.05$ was considered statistically significant.

3. 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 a 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%). 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 were seen in 33.33%, and Carotid Doppler was abnormal in 28.57%.Polycythemia was seen in 3 patients.

Aspirin resistance was detected highest in lacunar stroke (45%) followed by large vessel stroke (20%), and stroke of undetermined cause (35%).

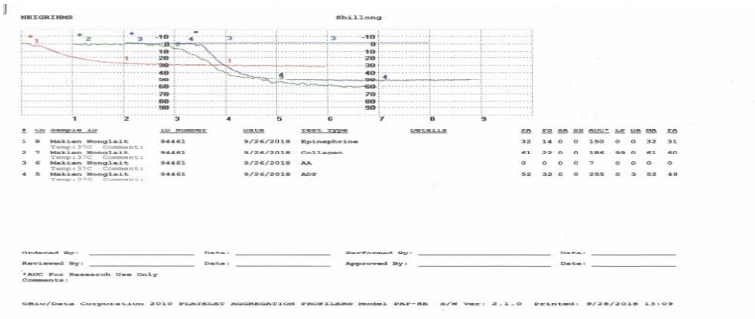

Fig. 1. Platelet aggregation curve of Aspirin sensitive patient

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	

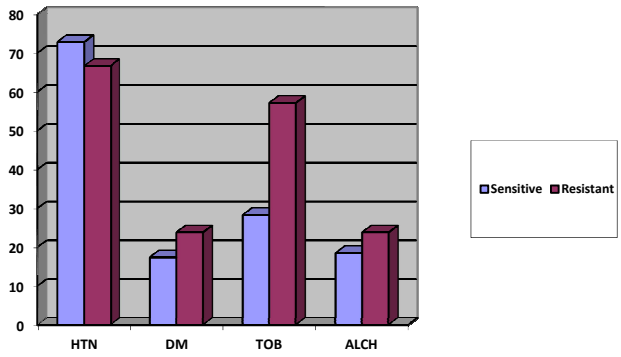

Fig. 2. Comparison of risk factors in aspirin sensitive and resistant patients

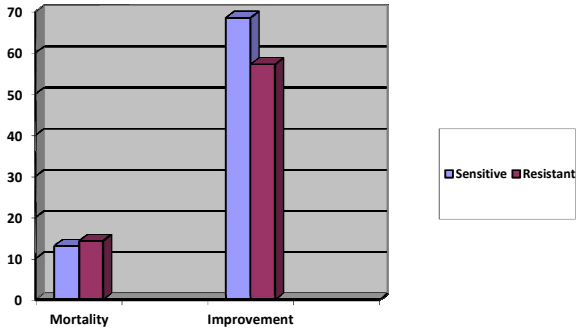

Fig. 3. Comparison of mortality, and mRS Score in aspirin sensitive and resistant

The results reveal that there is a non-statistically significant trend in both mortality and prognosis between the two study groups compared: aspirin-resistant versus aspirin-sensitive patients.

A total of 113 patients were studied, 92 were aspirin sensitive and 21 were resistant. The aspirin sensitive group had a mean age of 62.75_+ 15.54 and a gender ratio (M:F) of 1.7:1. The risk factors were hypertension (72.82%), Diabetes (17.39%), tobacco consumption (28.26%), alcohol consumption (18.47%), dyslipidemia (16.30%), abnormality in ECG (43.47%), and abnormality in carotid Doppler (36.95%). On follow up m RS the 30 days and 90 days improvements were 68.47% and 55.43% respectively. The total mortality was 13.04%.The aspirin resistant group had a mean age of 59.53+_ 16.55 and a gender ratio (M:F) of 32.:1. The risk factors were hypertension (66.66%), diabetes (23.80%), tobacco consumption (57.14%), alcohol consumption (23.80%), abnormality in ECG (33.35%), and abnormality in carotid Doppler (28.57%). On follow up m RS the 30 days and 90 days improvements were 57.14% and 33.33% respectively. The total mortality was 14.28%.

4. DISCUSSION

Aspirin is widely used for the treatment of stroke. Therefore aspirin resistance 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 show aspirin non responders in nearly 30% of patients [7]. However, some studies show 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. Lacunar strokes are associated with neuropsychological abnormalities, and mild neuropsychological disturbance (57.5%) is present in acute lacunar infarcts. This may be a predictor of subcortical vascular dementia in the medium to long term [11].

Aspirin resistance was associated more with diabetes mellitus, smoking, alcohol, and dyslipidemia. Platelet function is influenced by multiple factors like smoking [12], glucose control in diabetics [13], serum cholesterol [14] and triglycerides [15]. 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 endpoints of death in aspirin resistant patients [16].

The in hospital mortality in both the aspirin resistance and sensitive group was primarily Cerebrovascular with post stroke infection as a secondary cause. The 30 days and 90 days mortality in both groups were primarily Cerebrovascular.

Though the above results show the more adverse outcome of ischemic stroke, in aspirin resistant patients, a definite clinical –laboratory association of aspirin resistance and the poor outcome cannot be made. This is because of two reasons:[17] 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. Hematological disorders which account for about 1.3% of all strokes, can present with stroke or may appear in due course. Therefore any evidence of such correctable factor may influence negatively the action of aspirin, and lead to resistance to aspirin. [18]

The study has a 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. The study also does not study the dose and time dependent antiplatelet effects of aspirin.

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

5. CONCLUSION

This is one of the very few studies done in India,[19] 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 an increase in mortality in patients showing aspirin resistance,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, dose and time-dependent anti platelet effects of aspirin, 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.

CONSENT

Written informed consent was obtained from all the participants or their guardians. Treatment of stroke and dosing and administration of aspirin

was according to the guidelines set by national and international stroke organizations.

ETHICAL APPROVAL

We obtained ethics approval from the institute ethics committee (NEIGR/IEC/2013/58).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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