Frequency, Gene Defect, Factors Associated with Aspirin Resistance with Clinical Outcome Among Patients with Recurrent Ischemic Stroke on Aspirin Admitted in a Referral Neurology Hospital of Bangladesh

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Background

- The prevalence of stroke in Bangladesh is 1.14% [1]
- Among those 80-85 % are ischemic strokes [1]
- Incidence of recurrent stroke in the world ranges from 7%-20% at 1 year to 16%-35% at 5 years [2]
- The cumulative recurrence rate of ischemic stroke in India is 5.4% at 1 year, and 11.3% at 5 years [3]
- Among different factors, antiplatelet resistance is one of the neglected causes of ischemic stroke
- The rate of antiplatelet resistance in patients with stroke or transient ischemic attack (TIA) is highly variable, ranging from 3 to 85% for aspirin and 28 to 44% for clopidogrel [3]

Background

- Antiplatelet (Aspirin or Clopidogrel) resistance is associated with more recurrence and increased clinical severity, large infarct size & mortality of ischemic stroke [4]
- Many genes like ITGB3, GPIBA, TBXA2R, ITGA2, PLA2G7, HMOX1, PTGS1, PTGS2, ADRA2A, ABCB1 and PEAR1 are associated with aspirin resistance [4,5]
- Integrin (ITGB3) gene defect is an important factor for platelet dysfunction
- The polymorphism ITGB3 at position 1565 in exon encoding glycoprotein IIIa leads to its diallelic polymorphism (PlA1/A2) that modulates platelet dysfunction, results in enhanced thrombin formation and an impaired antithrombotic action of Aspirin [5,6]

References

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Rationale

- In Bangladesh, Aspirin is widely prescribed antiplatelet for secondary prevention of atherothrombotic stroke, myocardial infarction, and peripheral vascular diseases
- So, Inadequate Aspirin responsiveness correlates with an increased risk of recurrent ischemic vascular events in patients with stroke and acute coronary syndrome
- There is a possibility of tailoring aspirin therapy based on platelet function might act as an effective strategy for reducing recurrent ischemic stroke

Objectives

As far as we know our research work is the 1st study till now conducted to observe and determine the following issues

- Frequency of Aspirin resistance
- Gene defect & polymorphism (ITGB3)
- Factors associated with aspirin resistance and
- Clinical outcomes at 90 days

Methods

- **Study Design:** Prospective Cohort
- **Study Site:** National Institute of Neurosciences & Hospital, and Department of Molecular Biology & Biomolecular Science, Dhaka University
- **Study duration:** January to December 2022
- **IRB registration number:** NINS-119-0122
- **Participant:** All recurrent ischemic stroke patients (aged > 18 years) on Aspirin were admitted to NINS
- **Sample size:** 105
- **Sampling technique:** purposive

Exclusion criteria

- Who did not give informed consent
- Patients who were non-compliant with Aspirin for the last 1 month before an ischemic event
- Patients with recurrent ischemic strokes with cardiac arrhythmia
- Patients with acute myocardial infarction
- Patients with structural cardiac abnormalities like a valvular defect or septal defect
- Patients who had any thrombophilic conditions, covid-19 were excluded
- Patients who were severely ill with respiratory failure, multiple organ failure, sepsis
- Patients who were taking COX inhibitors or like ibuprofen, naproxen, etoricoxib

Measurement of Outcomes

- The severity of stroke assessed by the National Institute of Health Stroke Scale
- Functional disability was assessed by the Modified Rankin Scale at 3 months
- Infarct size was assessed by the ASPECT score

Study Procedure

DNA Extraction

- DNA was extracted from the collected blood sample as per the protocol of the DNA extraction kit (QIAGEN DNA extraction kit).

Polymerase Chain Reaction

- Total PCR reaction volume was 10 µL, containing 5 µL master mix, 0.15 µL forward primer (FP) and 0.15 µL reverse primer (RP), 2.7 µL nuclease-free water, and 2 µL DNA. The PCR mixtures were run in Veriti™ 96-well Fast Thermal Cycler using the tetra-primer amplification refractory mutation system (T-ARMSPCR) for the sample. Promega GoTaq Green Master Mix (Promega, USA) was used.

Study Procedure

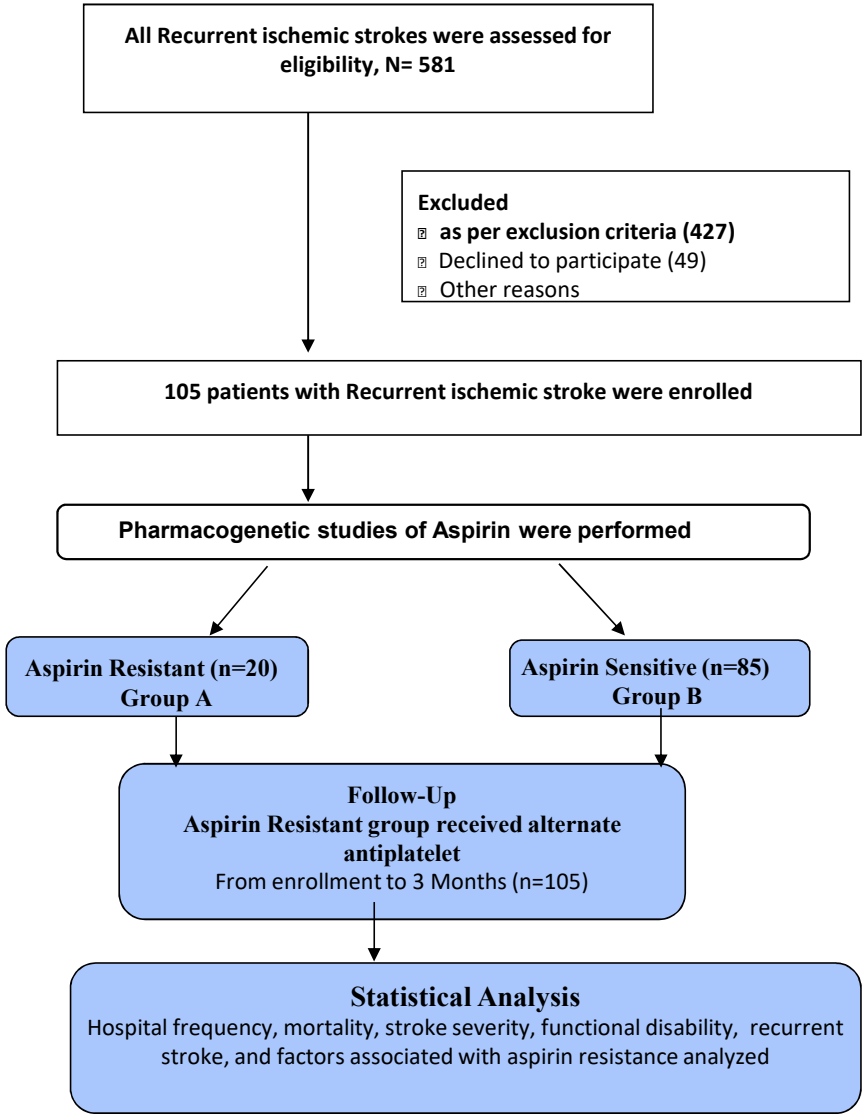
Genotype Analysis

- T-ARMS-PCR were done to identify missense rs5918 (PlA1/A1) polymorphism of the ITGB3 gene for genotyping
- At the same time, the amount of aspirin was measured from the used strip (from which the patient was taking his pill)

Statistical Analysis

- All data were analyzed in STATA 15 version.
- The continuous variable was expressed with the number, mean, and standard deviation (SD)
- Categorical values were expressed with frequency and percentage
- Comparison between groups (Group A- Aspirin resistant, Group B- Aspirin sensitive) was analyzed by Pearson chi-square test for categorical variable.
- For continuous variable independent student ‘t-test’ (normally distribute) or Mann Whitney U test for skewed data.
- For assessing factors related to Aspirin resistance, multivariate logistic regression analysis was done. Factors were expressed with an odds ratio with a 95% confidence interval

Results


All Recurrent ischemic strokes were assessed for eligibility, N= 581
Excluded
as per exclusion criteria (427)
Declined to participate (49)
Other reasons
105 patients with Recurrent ischemic stroke were enrolled
Pharmacogenetic studies of Aspirin were performed
Aspirin Resistant (n=20) Group A
Aspirin Sensitive (n=85) Group B
Follow-Up
Aspirin Resistant group received alternate antiplatelet
From enrollment to 3 Months (n=105)
Statistical Analysis
Hospital frequency, mortality, stroke severity, functional disability, recurrent stroke, and factors associated with aspirin resistance analyzed

Trait	Group A n=20 Aspirin Resistant	Group B n= 85 Aspirin Sensitive	P value < 0.05 significant
Age (years)	63.7±10.5	63.1±13.1	0.9
Sex (frequency, %) Male Female	 14 (70) 6 (30)	 55 (65) 30 (35)	 0.94
Smoking (n, %)	7 (35)	67 (78)	0.2
Alcoholic (n, %)	1 (5)	7 (8.2)	
BMI (n, %) Normal Overweight	 8 (40) 12 (60)	 43 (51) 24 (49)	 0.08
Comorbidities (n, %) HTN DM IHD CKD Dyslipidemia	 15 (75) 12 (60) 1 (5) 1 (5) 3 (15)	 63 (74) 40 (47) 8 (9.4) 4 (4.7) 23 (27.1)	 0.5 **0.04** 0.4 0.9 **0.02**
Amount of Aspirin	70 ± 6.9	71 ± 7.4	0.9
Clinical Outcome	Mean (SD)		
Number of stroke events	2.9±0.6	2.0±0.3	**0.001**
Severity of stroke	15.1±5.1	11.6±6.9	**0.03**
ASPECT score	7.1± 2.5	5.4±1.9	**0.01**
Functional disability score (mRS)	3.6±1.5	2.7±1.2	**0.02**
Mortality	6(30)	19(22.3)	**0.03**
Laboratory Profile			
Fasting glucose	13.6 ± 4.9	8.9 ± 4.2	**0.001**
HbA1C	8.8 ± 2.7	6 .3± 1.9	**0.001**
SGPT	34.8 ± 14.3	32.5 ± 19.8	0.67
LDL-C	109.3±63.1	121.5± 47.7	0.54
HDL- C	36.4±8.1	35.6±11.4	0.76
Total Cholesterol	213±77.9	209±60.7	0.84
Uric acid	6.2±1.6	5.7±1.7	0.92

Table 1: Comparison of baseline characteristics between Group A (Aspirin Resistant) and Group B (Aspirin Sensitive)

Trait	Group A n=20 Aspirin Resistant	Group B n= 85 Aspirin Sensitive	P value < 0.05 significant
Age (years)	63.7±10.5	63.1±13.1	0.71
Sex			
Male	14 (70)	55 (65)	0.94
Female	6 (30)	30 (35)	
Smoking (n, %)	7 (35)	67 (78)	0.2
Alcoholic (n, %)	1 (5)	7 (8.2)	0.7
HTN	15(75)	63(74)	0.5
DM	12 (60)	40 (47)	**.04**
IHD	1 (5)	8 (9.4)	0.4
CKD	1 (5)	4 (4.7)	0.9
Dyslipidemia	3 (15)	23 (27.1)	**.02**
Amount of Aspirin	69.5 ± 6.9	71 ± 7.4	0.9
Systolic BP	134 ± 19	148 ± 27	**.01**

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Uric acid	6.2 ± 1.6	5.7 ± 1.7	0.92
Creatinine	1.2 ± 0.5	1.1 ± 0.4	0.41

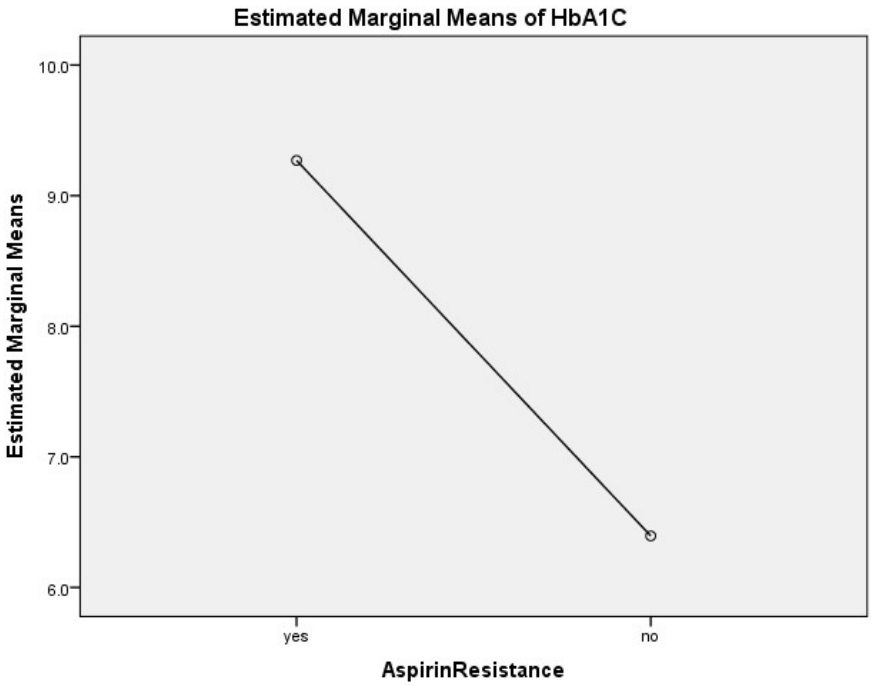
How Aspirin Resistance looks in the Thermocycler

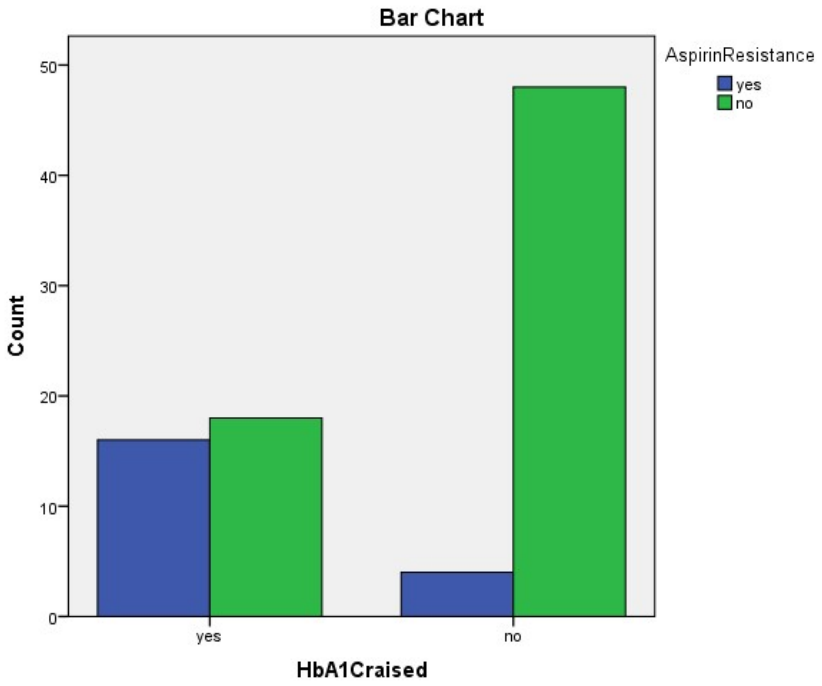
3 bands (424bp, 285bp, 180bp) means heterozygous mutant;
sample no- 43,44 2 Bands (424bp, 285bp) means normal;
sample no- 41,61,388,37,21,33,50

Table 2: Factors Associated with Aspirin Resistance

Factors	OR [95%, CI]	P value
Age	0.7 [0.5-2.9]	0.6
Sex	1.04 [0.4-3.1]	0.9
Smoker	1.4 [0.5-1.7]	0.12
Alcoholic	0.9 [0.3-2.1]	0.17
DM	1.3[0.5-3.9]	0.31
HTN	1.2[0.7-2.3]	0.28
Raised fasting blood sugar	2.9 [1.9-4.5]	0.001
Raised HbA1C	1.8 [1.3-4.3]	0.001
Raised total cholesterol	1.5[0.6 - 4.2]	0.4
Raised LDL cholesterol	0.9[0.3 - 2.5]	0.8
Raised HDL cholesterol	0.7 [0.2 - 2.7]	0.5
Raised Creatinine	0.6[0.2 - 2.1]	0.4
Raised Uric acid	1.8 [0.5-6.1]	0.3

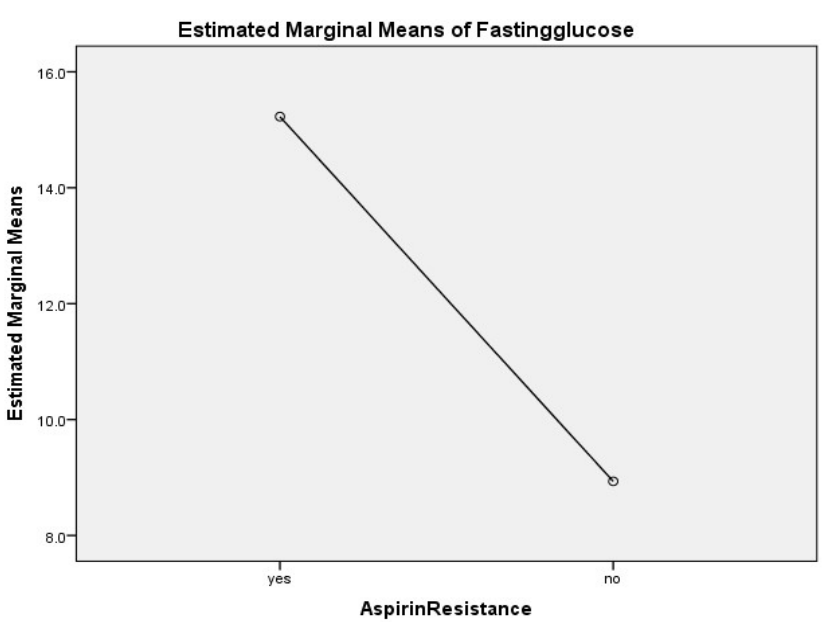
Mean Plot for HbA1C with Aspirin Resistance



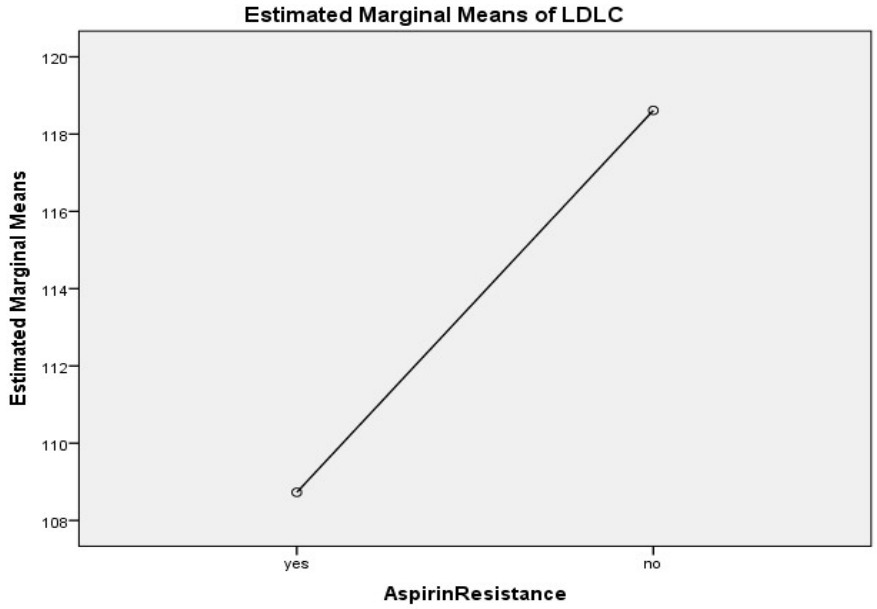


Comparison of Mean Plot

Fasting Plasma Glucose



LDL Cholesterol



Our Observations

- Hospital frequency of aspirin resistance (ITGB3) among recurrent ischemic stroke patients who were on aspirin was 20 (19.1%)
- Out of 20 patients, 11 (55%) and 9 (45%) had single and double gene defects respectively.
- The severity of a stroke, infarct size, functional disability, and mortality rate were higher in the Aspirin resistant group
- High fasting plasma sugar and HbA1c were significantly associated with aspirin resistance

Limitations

- Single center study
- Small sample size
- Not all known gene defect with polymorphism was determined

Conclusion

- As Aspirin resistance due to ITGB3 gene defect is high (19.1%), a large-scale, multicenter study is needed for further evaluation of our clinical & statistical observation
- Patient with recurrent ischemic stroke on Aspirin medication, more or less compliance to medications but the cause is unknown, please do a Pharmacogenetic study of Aspirin
- It is high time to reduce the ischemic stroke burden because it is one of the leading causes of morbidity & mortality not only in the world but also in Bangladesh


THANK
YOU