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CORRELATION OF CLINICAL AND LABORATORY ASPIRIN RESISTANCE:

A PILOT STUDY

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ABSTRACT

Aspirin resistance may be biochemical or clinical. Data related to the presence of aspirin resistance in the Indian population is scarce. We conducted a cross sectional study to address the issue of clinical aspirin non responsiveness and to assess the association between inhibition of platelet aggregation, clinical risk factors and occurrence of vascular events. We studied platelet aggregation by optical aggregometry in 20 patients on aspirin. No patient was found to be aspirin-resistant on the basis of previously defined criteria. This led us to relook at the current cut offs for resistance, and an analysis of 60 normal patients showed lower cut off values suggesting ethnic variability. The data was reanalyzed using these cutoffs. An association between poor clinical aspirin response, older age, male sex, smoking and dyslipidemia was found, suggesting a trend, though not significant. 25% of patients had vascular events on aspirin suggesting clinical aspirin resistance. A lower cut off value for aspirin resistance in normal Indians may be needed to detect true prevalence of this entity. In patients with multiple atherothrombotic risk factors lab detection of resistance may be useful in identifying patients with high risk for recurrent vascular events. This may help to modify antiplatelet therapy to prevent vascular events.

Key words: aspirin, platelet function, clinical correlation, risk factors

INTRODUCTION:

Aspirin is an effective antiplatelet agent for preventing the complications consequent to atherothrombosis. Various clinical trials and meta analysis have demonstrated a 23% reduction of vascular events in the aspirin treated groups ^{1,2}. However aspirin fails to prevent more than four-fifths (81%) of recurrent serious vascular events among high-risk patients, and one in eight high-risk patients (12.9%) experiences a recurrent vascular event in the next two years despite taking aspirin. ³ This shows that the antiplatelet effect of aspirin is not uniform in all patients and studies estimate that 8% to 45% of the population is aspirin resistant ⁴⁻⁸. Biochemical aspirin resistance refers to the inability of aspirin to inhibit the production of platelet thromboxane or inhibit tests of platelet function (platelet aggregation) that are dependent on thromboxane production.

Various factors such as genetic predisposition, nonadherence, and variable response to different doses, co-morbid conditions and drug interactions are responsible for aspirin resistance. A few studies have suggested an association of aspirin resistance with the other cardiovascular risk factors such as diabetes mellitus, hypertension, smoking and atherosclerosis ⁸⁻¹⁰

The relation between laboratory evidence of aspirin resistance and clinical aspirin resistance have been studied .There is some evidence to show that aspirin resistant patients detected by aggregation studies are at increased risk for vascular events.¹¹

Data related to the presence of aspirin resistance in the Indian population is scarce. A prevalence of 2% was reported by Sadiq et al.¹² We conducted a cross sectional study to address the issue of aspirin non responsiveness using aggregometry which is the traditional method for in vitro assessment of platelet function. In this study we have also

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tried to assess the association between inhibition of platelet aggregation, clinical risk factors and occurrence of vascular events in patients on prophylactic aspirin.

METHODS:

Study setting and design:

This study was a cross sectional study conducted at Kasturba hospital, Manipal between August 2006 and February 2007 after clearance by the institutional ethics committee. Males and females of Indian ethnicity in the age group above 20 years of age who were on aspirin alone as antiplatelet drug for primary prophylaxis or secondary prophylaxis after Ischemic heart disease (IHD), Cerebrovascular accident (CVA) or Peripheral vascular disease (PVD) were included. Only those fully compliant with therapy participated in this study. All patients were on aspirin (any dose) for a period of \geq 7 days at the time of lab testing.

Patients who were on other antiplatelet drugs such as clopidogrel, ticlopidine, dipyridamole or non-steroidal anti-inflammatory drugs or anticoagulants were excluded. Those who had a family or personal history of bleeding disorders, or those with abnormal lab parameters like platelet count $<150.000/\mu\text{l}$ or $>450.000/\mu\text{l}$ or haemoglobin <8 g/dl and history of myeloproliferative disorders were also not selected.

All patients were included in the study after an informed consent. Patient records were reviewed and the demographic characteristics such as age, gender, indication for aspirin and dose of aspirin were collected using a pre-designed proforma. The presence of atherothrombotic risk factors such as age, male sex, diabetes, hypertension, current smoking and dyslipidemia was determined by chart review. The occurrence of recurrent vascular events was also noted.

Platelet function analysis

Platelet function was assessed by optical aggregometry using adenosine 5'-diphosphate (ADP) and arachidonic acid (AA) as agonists. Four samples of whole blood were collected in 3.8% sodium citrate for platelet aggregation testing. Room-temperature blood samples were processed within 1 h of blood collection. Whole-blood specimens were centrifuged for 15 min at 100 g to obtain platelet-rich plasma. Platelet-poor plasma was obtained on the remaining specimen by recentrifugation at 2,400 g for 20 min. A platelet count was measured on the platelet-rich plasma and was adjusted to between $200 \times 10^3/\mu\text{l}$ and $300 \times 10^3/\mu\text{l}$ with platelet-poor plasma. The baseline optical density was set with platelet poor plasma. Aggregation was performed using adenosine 5'-diphosphate (ADP) (Chrono-par) at 10 μM and arachidonic acid (AA) at 0.5 mg/ml with a Chronolog 400 optical aggregometer (Chronolog corporation, PA, USA)

For optical platelet aggregation, optical density changes were detected photoelectrically as platelets began to aggregate. *Adenosine diphosphate* promotes the release of endogenous ADP and thromboxane A2 when added to platelet-rich plasma, causing irreversible aggregation. *Arachidonic acid* is used to evaluate the degree of inhibition of platelet aggregation by aspirin. The addition of AA to platelet-rich plasma enhances platelet aggregation by producing thromboxane A2. These tests are abnormal in patients using aspirin, having aspirin-like release defects, and Glanzmann's thrombasthenia. The results were reported as percentage aggregation.

Definitions and statistical analysis

In order to establish the range of normal for platelet aggregation, screening of 60 normal samples was done and the normal range of ADP induced aggregation was determined to be 33-89 % (Mean \pm 2SD, Mean =61.25% SD= 13.97%).

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For purpose of the study, patients whose platelet aggregation tests were done were stratified according to the percentage aggregation into one of three groups i.e.

- A. ADP induced aggregation >60 % (poor response to aspirin)
- B. ADP induced aggregation 30-60 % (moderate response to aspirin)
- C. ADP induced aggregation <30 % (good response to aspirin)

The groups were then compared with respect to the occurrence of risk factors such as age, gender, diabetes mellitus, hypertension, dyslipidemia and smoking, all of which may have some effect on the response to aspirin. The occurrence of vascular events, in spite of being on aspirin (clinical aspirin resistance) in various groups was also analyzed, to find out correlation if any between biochemical and clinical aspirin resistance.

Statistical analyses were performed with the SPSS 10.0 for Windows statistical software. Non-parametric data were analyzed by the Mann-Whitney test and data correlation was determined using Spearman's rank-correlation coefficient. A two-tailed p value of ≤ 0.05 was considered statistically significant. Comparisons of categorical data between groups were made using Chi Square test.

RESULTS:

Of the twenty patients studied, 14 (70%) were male and mean age was 62 ± 14 years. One patient studied was on aspirin for primary prophylaxis and in the remaining it was for secondary prophylaxis (Table I).

Aspirin yielded a 0 to 67 % reduction in the aggregation tests with ADP in the study population. Variability in platelet response to aspirin was seen and patients were stratified into three tertiles according to the aspirin response (Table II).

As expected there was an inverse relationship between aspirin dosage and ADP induced aggregation with the dose of 150 mg causing the maximum amount of inhibition of platelet aggregation (Fig.1)

The three groups did not differ significantly ($p>0.05$) with respect to the risk factors. However patients with a poor response to aspirin (ADP aggregation $> 60\%$) had a higher proportion of smokers .It was also seen that the response to aspirin was less in the elderly, making them more prone to atherothrombotic events (Table III).

Patients who developed recurrent vascular events (25%) were older (75 vs 52) and had a higher percentage of smokers (60% vs 27%) as compared to those who did not develop clinical aspirin resistance. There was no significant difference in the ADP induced aggregation between the two groups (Table IV)

Table I. Indications of aspirin in the patients studied

Primary prophylaxis n (%)	1 (5)
Secondary prophylaxis	
IHD n (%)	12 (60)
CVA n (%)	5 (25)
PVD n (%)	4 (20)

Table II. Aspirin responsiveness in patients studied.

ADP induced aggregation	
$>60\%$ (Poor response to aspirin) n (%)	7 (35)
30-60% (Moderate response to aspirin) n (%)	6 (30)
$<30\%$ (Good response to aspirin) n (%)	7 (35)

Fig. 1 Relation of aggregation to dose of aspirin

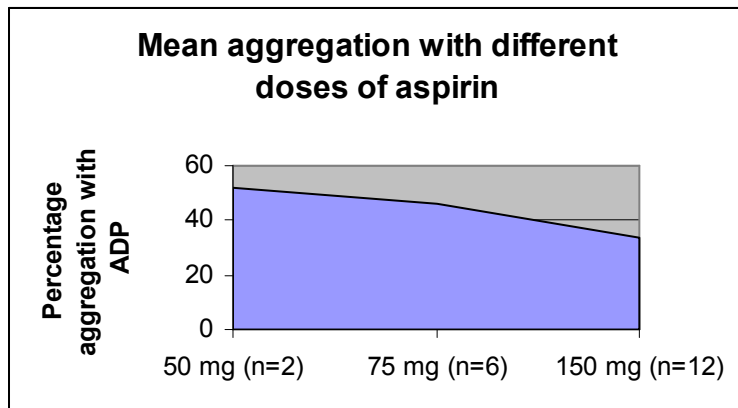


Table III. Demographics and clinical characteristics of the patients in the different groups according to ADP induced aggregation.

	Total study population (n=20)	1. <30% (n=7)	2. 30-60% (n=6)	3. >60% (n=7)
Age, y	62 ± 14	58 ± 16	65 ± 14	64 ± 13
Male n (%)	14 (70)	5 (71)	4 (67)	5 (71)
Smoking n (%)	7 (35)	2 (29)	2 (33)	3 (43)
DM n (%)	14 (70)	6 (86)	5 (83)	3 (43)
HTN n (%)	16 (80)	6 (86)	4 (67)	6 (86)
Dyslipidemia. n (%)	11 (55)	4 (57)	4 (67)	3 (43)

Table IV. Characteristics of patients who developed recurrent vascular events despite aspirin therapy (Clinical aspirin resistance)

	With events	Without events
No of patients	5 (25)	15 (75)
Age (mean±SD)	75±7	58±14
ADP induced aggregation (%, mean±SD)	34.4±29.5	40.6±21.1
Poor response to aspirin (ADP aggregation > 60%) n (%)	2 (40)	3 (60)
Males n (%)	3 (60)	11 (73)
Smokers n (%)	3 (60)	4 (27)
Diabetes n (%)	2 (40)	12 (80)
Hypertension n (%)	4 (80)	12 (80)
Dyslipidemia n (%)	2 (40)	9 (60)

DISCUSSION

Aspirin responsiveness

This study shows high degrees of inter-individual variability in response to aspirin as assessed by ADP induced aggregation (turbidometric). This interindividual variability in ex vivo responsiveness has also been reported by other investigators^{5,13,14} The antiplatelet effect of aspirin thus shows a continuous distribution similar to blood pressure and cholesterol levels.

None of the patients in this study were found to be aspirin resistant by using previously used criteria which define aspirin resistance as a mean aggregation of $\geq 70\%$ with 10 μM ADP and a mean aggregation of $\geq 20\%$ with 0.5 mg/ml AA. Two patients (10%) were found to be semi responders on the basis of presence of one of the above

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criteria (mean aggregation of $\geq 20\%$ with 0.5 mg/ml AA). These criteria are based on different populations and the low prevalence of aspirin resistance on the basis of these criteria may be more apparent than real.

The normal aggregation pattern in Indians is not known. To the best of our knowledge there is only one published report addressing this issue in the Indian population by Sadiq et al who reported a prevalence of aspirin resistance of 2% on the basis of the above mentioned criteria.¹² Analysis of aggregation patterns in 60 normal individuals in our population revealed the mean ADP induced aggregation to be $\sim 61\%$ suggesting that ADP induced aggregation is lower in our population. Thus lower cut offs for definition of aspirin resistance may be more appropriate in our population. However larger studies of normal aggregation patterns are required to settle this issue.

Association with risk factors

Patients were stratified into three groups on the basis of ADP induced aggregation with $>60\%$ aggregation classified as a poor response (group A), 30-60% as Group B, and $<30\%$ classified as Group C, with a good response to aspirin. There was no significant difference between the three groups in relation to demographic features and atherosclerotic risk factors. However, patients with a poor response to aspirin were of an older age group as compared to the patients who showed a good response to aspirin. This feature is in agreement with the finding by Gum et al who reported a trend towards increased age in patients with aspirin resistance or semi responders.¹⁰

In our study patients with a poor response to aspirin were more likely to be smokers as compared to the responders. The prothrombotic effects of smoking has been postulated to be related to catecholamine release and heightened aggregation.¹⁵ This could lead to a less inhibition of aggregation by conventional aspirin dosage and smokers could possibly benefit from a higher dose of aspirin.

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The three patient groups did not vary significantly with respect to the frequency of dyslipidemic patients. Platelet responsiveness to aspirin has been reported to be reduced in patients with dyslipidemia.¹⁶ Friend et al found that in a cohort of 56 patients with stable coronary artery disease and aspirin 325 mg/day, 14 patients with poor responsiveness to aspirin had significantly higher mean concentrations of total cholesterol than the 42 patients with good responsiveness using aggregometry tests (6.2 vs 4.8 mmol/l, p=0.004). Nine of the 13 patients with total cholesterol concentration > 6.2 mmol/l were aspirin resistant¹⁵.

The mean ADP induced aggregation was higher in males as compared to females in our study. This is in contrast to Sadiq et al¹² and Gum et al⁸ who have reported a higher degree of aspirin non responsiveness in females. The higher aggregation in males in our study is probably related to the confounding factor of smoking which was seen only in the male population

Hypertension is another factor that has been associated with aspirin resistance. The clinical benefit with aspirin has been found to be greater at lower systolic blood pressure.¹⁷ This was reflected in our study by the marginally higher mean ADP induced aggregation seen in hypertensives as compared to non hypertensives.

The ADP induced aggregation was higher in non diabetics when compared to diabetic patients. Platelets play a major role in the pathogenesis of atherothrombotic complications in diabetic patients. Increased platelet activity is critically involved in the increased thrombogenic potential among diabetic patients.¹⁸ The finding of a higher aggregation in non diabetics in our study is in contrast to the findings of others⁹. This pattern however may be due to the higher age group and a higher proportion of smokers seen amongst the non diabetics in our study.

Relationship of laboratory aspirin resistance to vascular events on aspirin

Five patients had developed an event while receiving aspirin. Patients who developed recurrent vascular events (25%) were older (75 vs 52) and had a higher percentage of smokers (60% vs 27%) as compared to those who did not develop clinical aspirin resistance. Two of these patients had a poor response to aspirin by aggregometry (>60%) and only one qualified for the definition of aspirin resistance. There was no significant difference in the mean ADP induced aggregation between the two groups. These findings suggest the increased risk for vascular event with increasing age and smoking.

There are limitations of this study, the most important being the small sample size. This may not allow us to draw major conclusions, and larger numbers will be necessary to conclude on the trends seen in this pilot study.

CONCLUSIONS AND RECOMMENDATIONS:

The platelet response to aspirin shows a continuous distribution. There were no aspirin resistant patients among the study population as defined by standard criteria. 10% were semi responders. However, the cut off values to define aspirin resistance may be lower in our population, as evidenced by the lower aggregation values even in normal individuals.

There was no significant association between risk factors for atherothrombosis and aspirin response, however the elderly and smokers had a poorer response to aspirin and may require larger doses or additional antiplatelet drugs.

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But platelet aggregation is one event in the complex process of atherothrombosis and measurement of aggregation has its limitations. Given the seriousness and consequences of atherothrombotic events and the low cost, safety and efficacy of aspirin in the prevention of the same it is important to address the issue of aspirin resistance. Platelet function testing in the form of tests of aggregation may have an adjunctive role in addition to tests of conventional risk factors in helping the clinician in predicting, and more importantly preventing a vascular event.

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