Original Research Article

Prevalence of aspirin resistance in coronary artery disease among Indian patients

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ABSTRACT

Background: Aspirin is one of the most widely consumed drugs in this world. The first report of a possible antithrombotic effect of aspirin appeared in 1953 in the Mississippi Valley Medical Journal. Objective of the study was to determine the prevalence of aspirin resistance among Indian patients with coronary artery disease.

Methods: Patients were prospectively enrolled from all stable cardiac patients presenting to the outpatient wing of Department of Medicine of a tertiary care centre in South Kerala. Duration of study was one year. All patients who were more than 21years old and who had taken 150mg of aspirin for the previous seven days were eligible for enrolment.

Results: Aspirin resistance was found in 9.3% of patients. 17.3% of patients were aspirin semi responder.

Conclusions: There was statistically significant correlation of aspirin resistance with presence of diabetes mellitus, systemic hypertension and dyslipidemia. Cigarette smoking did not show any significant association with aspirin resistance.

Keywords: Aspirin, Aspirin resistance, Coronary artery disease, Dyslipidemia, Platelet aggregation, Platelet rich plasma

INTRODUCTION

Aspirin is one of the most widely consumed drugs in this world. The first report of a possible antithrombotic effect of aspirin appeared in 1953 in the Mississippi Valley Medical Journal.[1] This was followed by the discovery by numerous investigators that aspirin could significantly decrease platelet function. In 1971, the mechanism by which aspirin inhibited platelet function began to unfold[2]. It was subsequently shown that aspirin irreversibly acetylated serine-529 close to the active site of the fatty acid cyclooxygenase.[3] In the case of the anucleate platelet, the enzyme is rendered inactive for its lifetime. Samuelsson and co-workers demonstrated that thromboxane A2 along with prostaglandin H2 were the arachidonic acid metabolites responsible for platelet activation.[4] Despite the demonstrated benefit of aspirin in secondary prevention and its possible beneficial effects in selected individuals for primary prevention, there remains a large segment of the population at risk that does not benefit from aspirin.[5] There are several reports of aspirin resistance. This study aims to find out the prevalence of

aspirin resistance among Indian population and to find out any predictors for aspirin resistance.

METHODS

Seventy-five patients were prospectively enrolled from all the stable cardiac patients presented to the outpatient wing of Department of Medicine for a study period of one year. All patients who were >21 years old and who has taken 150mg of aspirin for the previous seven days were eligible for enrolment.

Exclusion criteria

- Ingestion of other antiplatelet drugs
- Ingestion of other NSAIDs
- Administration of heparin or low molecular weight heparin within 24 hours
- Family or personal history of bleeding disorders
- Platelet count <1.5 lakhs/mm^3 or >4.5lakhs/mm^3
- History of myeloproliferative syndromes
- History of heparin induced thrombocytopenia
- Patients with chronic liver or kidney diseases. Informed consent was obtained from all participants before enrolment.

Blood samples were drawn one to 24 hours after administration of last dose of aspirin. 10 ml of whole blood, anticoagulated with 3.8% sodium citrate were collected for platelet analysis and one tube of blood anticoagulated with EDTA was collected for the haemoglobin and platelet count analysis. The platelet aggregation samples were kept at room temperature and processed within one hour. Aggregation was performed using ADP at 10µM and arachidonic acid at 0.5mg/ml with PACKS-4 platelet aggregometer. Aspirin resistance was defined as a mean aggregation of >70% with ADP and >20% with arachidonic acid. Aspirin semi responders were defined as those meeting one but not both criteria.

Platelet function can be measured optically since the density of plasma decreases with platelet aggregation. Platelet rich plasma (PRP) is prepared according to the procedure supplied with the reagents, and optically measured. The initial absorbance is caused by light scattered by the floating platelets in the solution. This absorbance is nearly proportional to the number of platelets. Platelet poor plasma (PPP) made from the same sample simulates 100% aggregation. Absorbance caused by factors other than platelets is determined by measuring the absorbance of PPP. The aggregation capacity of the platelets is determined by the amount of aggregation induced when a known amount of reagent is added to the PRP.

The absorbance of the unreacted PRP mixed with the aggregation reagent represents 0% aggregation, and the absorbance of the PPP control represents 100% aggregation (no floating platelets). As platelets aggregate, the number of floating platelets decreases, reducing the light absorbed by the PRP. Various parameters related to the aggregation curve, or the maximum aggregation rate, are used as data is prepared and used to set 100% activity.

The CPU of PACKS-4 accesses test information, patient data and other user entered information stored in the CPU memory to control instrument operation. An incubator block surrounds the incubation wells and the optical chambers, pre-warming samples and reagents and maintaining the agglutination/aggregation reaction at 37°C. The samples and reagents in each optical chamber are mixed at a constant speed with a magic stirrer. The optical section of each of the four channels consists of filtered light from the lamp, delivered by fibre optics, and a photo detector. The sample inside the cuvette is flooded with collimated light (at 650mm) emitted by the source lamp. Platelets suspended in the mixture absorb light at 650mm, so that the amount of light reaching the photo detector is proportional to the number of platelets in solution. The photo detector converts the light intensity into an analog signal, which is digitized and sent to the CPU for processing.

For agglutination tests, the lid switch is pressed to start a three-minute incubation. For aggregation tests, measurements are initiated when reagent is added to the cuvette in the optical chamber. For agglutination tests, measurements start when sample plasma is added to the cuvette. The measurement begins when both switches are activated as reagent is delivered into the cuvette. Optical measurements are made continuously until the test ends. The optics analog signals are converted to digital signals. These are used to calibrate optical reference levels, establish optical scale before data acquisition and establish optical curves during data acquisition. The channels are calibrated, setting 0% and 100% activity levels for each samples.

Continuous optical measurements are made after reagent is added to patient or control samples. The raw analog is digitized and converted into percentage activity for each sample, and the results are displayed. Measurement data can be printed out at the end of measurement period. Up to four optical chambers can be used for simultaneous measurements. Since PACKS-4 is microcomputer controlled, no manual adjustments are required after measurements are started.

The baseline results were analysed using the percentage method. The comparisons between the groups were done using Chi-square test and Fisher's exact test.

RESULTS

Of the 75 patients enrolled in the study 48 were males and 27 were females. Medium age of patients was 62 years (range 32 – 80years). Majority of the patients were in the age group 55-65 years. 9.3% patients were found to have aspirin resistance. 17.3% patients were aspirin semi

responders. 54.7% patients were smokers. Cigarette smoking did not show any significant association with aspirin resistance (p value- 0.3446). 26.7% of the study population had diabetes and aspirin resistance was seen in 50% of diabetic patients in the study. 73.3% of the study population was not having diabetes. Aspirin resistance was found only in 18.2% of the non-diabetic patients in the study (Table 1). This result is found to be statistically significant (p value- 0.0139). 29.3% of the study population had hypertension and aspirin resistance was seen in 45.5% of hypertensive patients in the study. 70.7% of the study population did not have hypertension and aspirin resistance was found only in 18.9% of this group of patients (Table 2).

Table 1: Comparison between diabetic and non-diabetic patients.

Category	Total	Aspirin resistant	P value
Diabetic	20	10	
Non-diabetic	55	10	0.0139

This result is found to be statistically significant (p value- 0.037). 26.7% of the study population had dyslipidemia and aspirin resistance was seen in 50% of the dyslipidemic patients in the study. 73.3% of the study population did not have dyslipidemia and aspirin resistance was found only in 18.2% of this group of patients (Table 3).

Table 2: Comparison between hypertensive and non-hypertensive patients.

Category	Total	Aspirin resistant	P value
Hypertensive	22	10	
Non-hypertensive	53	10	0.037

This result is also found to be statistically significant (p value- 0.0139).Thus we found in present study that there was statistically significant correlation of aspirin resistance with the presence of risk factors like diabetes mellitus, systemic hypertension and dyslipidemia in our study population.

Table 3: Comparison between Dyslipidemic and Non-dyslipidemic patients.

Category	Total	Aspirin resistant	P value
Dyslipidemic	20	10	
Non-dyslipidemic	55	10	0.0139

DISCUSSION

Among patients with cardiac disease, our study showed that nine percentage are aspirin resistant and an additional 17% are aspirin semi responders. That means 26% of the

patients who take aspirin for its antiplatelet effect do not benefit from its use.

Considering the standard guideline use of aspirin and our dependence on it to decrease adverse events by its antiplatelet action, this prevalence is particularly noteworthy and of great potential clinical importance. Improved outcomes with aspirin treatment may not be realised in all patients. Initial evidence that some patients may be resistant to aspirin came from a study by Mehta et al who showed that 30% of patients with coronary artery disease had minimal inhibition of platelet aggregation after a single 650mg dose of aspirin.[6] Subsequent studies attempted to estimate the prevalence of aspirin resistance in patients with cerebrovascular disease, peripheral arterial disease and ischemic heart disease.[7-9]

Aspirin resistant patients were found in the Warfarin-Aspirin Re-infarction Study (WARIS)-II and among healthy volunteers.[10,11] The overall range of estimated prevalence of aspirin resistance in these studies varied from eight percentage to 45% and this wide range clearly depends, at least to some extent, on the variable definition of the entity. There will probably never be universal agreement on what can be termed aspirin resistance, because of the multiple parameters used to assess platelet function.

Comorbid conditions like diabetes mellitus, hypertension and dyslipidemia may contribute to aspirin resistance. Previous studies have demonstrated that smoking is associated with aspirin resistance[7]. However, history of smoking did not show any significant relation with aspirin resistance in our study. Inadequate sample size may be the reason for this disparity. Recent data have shown that cigarette smoking accentuates the formation of a platelet thrombus in a way that is not inhibited by aspirin.[12,13] Therefore, although more clinical trials are needed to elucidate relationship between aspirin and the effects of smoking on platelet aggregation, smoking induced, aspirin insensitive platelet aggregation may be considered one of the mechanisms of aspirin resistance. However, in the first trial designed to determine the prevalence of aspirin resistance, there were significantly more current smokers in the aspirin sensitive group than in aspirin resistant patients, and there were no significant differences between aspirin resistant and aspirin sensitive patients, as measured by the PFA-100 test.[14] But larger studies are needed to reach at a conclusion regarding the clinical predictors of aspirin resistance.

The limitations of present study were the small sample size and that the aspirin resistance was measured only once in present study as aspirin resistance is not absolute overtime.

CONCLUSION

There was statistically significant correlation of aspirin resistance with presence of diabetes mellitus, systemic

hypertension and dyslipidemia. Cigarette smoking did not show any significant association with aspirin resistance.

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