

## Profile and Prevalence of Aspirin Resistance in Indian Patients with Coronary Artery Disease

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**Background:** Aspirin resistance is considered to be an enigma and the data available on aspirin resistance is scarce. This study was initiated to prospectively evaluate the prevalence of aspirin resistance in patients with stable coronary artery disease by using an established method of optical platelet aggregation.

**Methods and Results:** We studied 50 patients who were on 150 mg of aspirin for the previous 7 days. Fasting blood samples were assessed using optical platelet aggregation (Chronolog Corp, USA). The mean platelet aggregation with 10  $\mu$ m of adenosine diphosphate in our patient group was  $49.42 \pm 23.29\%$  and with 0.5 mg/ml of arachidonic acid it was  $13.58 \pm 21.40\%$ . Aspirin resistance was defined as a mean aggregation of  $\geq 70\%$  with 10  $\mu$ m of adenosine diphosphate and a mean aggregation of  $\geq 20\%$  with 0.5 mg/ml of arachidonic acid. Aspirin semi responders were defined as those meeting only one of the criteria. Based on these criteria, 2.08% patients were found to be aspirin-resistant, 39.58% were aspirin semi responders and 58.33% were aspirin responders. Females tended to be more aspirin semi responsive ( $p = 0.08$ ). All other parameters tested, namely, age, smoking, diabetes mellitus, hypertension, obesity, lipids, hemoglobin, platelet count, ejection fraction and drug intake did not show any statistically significant difference among the groups. Thus, in our group 41.66% patients showed inadequate response to aspirin.

**Conclusions:** This study shows that aspirin resistance and aspirin semi responsiveness do occur in the Indian patients and there are no reliable clinical predictors for this condition. The diagnosis therefore relies primarily on laboratory tests. (*Indian Heart J 2005; 57: 658-661*)

**Key Words:** Aspirin resistance, Platelet aggregation, Coronary artery disease

Since the time of introduction of aspirin in 1897 and the elucidation of the mechanism of its benefit, aspirin has become a cornerstone in the treatment of coronary artery disease (CAD).<sup>1</sup> The beneficial role of aspirin in the secondary prevention of vascular events is now well established.<sup>2,3</sup> The Antithrombotic Trialist Collaboration's meta-analysis with approximately 100,000 subjects treated with aspirin showed a 25% reduction in death, myocardial infarction (MI) and stroke in the high risk patients.<sup>4,5</sup> However, it has been recently shown that its effect may not be uniform in all patients. Various laboratory parameters assessing its efficacy, like bleeding time, platelet reactivity, thromboxane A<sub>2</sub> (TXA<sub>2</sub>) production and measurement of platelet aggregation have confirmed the lack of its uniform effect on the platelets, among patients who manifest breakthrough events with thrombotic and embolic complications despite being on therapeutic doses. It has been suggested that one out of every eight high-risk

individuals may experience an event in the next 2 years despite aspirin therapy.<sup>6</sup> Based on this fact, the concept of aspirin resistance has emerged. Few studies have estimated that 5% to 45% of patients with vascular disease are aspirin-resistant.<sup>7-10</sup> This variability in incidence is due to non-standardization of method and definition of aspirin resistance used in these studies.

The present study was initiated to prospectively evaluate the prevalence of aspirin resistance in patients with stable CAD as there is scarce data on this subject from the Indian sub-continent. The test was carried out by using an established traditional method of optical platelet aggregation which, although tedious to accomplish, is considered the gold standard. Optical platelet aggregation utilizes a modified spectrophotometer in which platelet-rich plasma is assessed using optical density changes, which detect photoelectrically, as platelets begin to aggregate. Adenosine diphosphate (ADP), when added to platelet-rich plasma, promotes the release of additional endogenous ADP, causing irreversible aggregation. The addition of arachidonic acid (AA) to platelet-rich plasma produces TXA<sub>2</sub>, which enhances platelet aggregation.<sup>10</sup>

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### Methods

The present study was done on a group of 50 patients attending our outpatients department as follow-up patients of CAD. The inclusion criteria for the study were patients with a documented history of previous MI, or with angiographically proven coronary disease. All patients who were  $\geq 21$  years old and who had taken 150 mg of aspirin for the previous 7 days were eligible for enrolment. We relied on statement of our patient group to ascertain that adequate dose of aspirin had been taken. Age, sex, occupation, clinical history, previous history of diabetes, smoking, hypertension, family history, hospitalization for ischemic heart disease were noted. A thorough clinical examination and 12-lead electrocardiogram (ECG) was done. Fasting blood samples were taken for biochemical tests, which were conducted at the Central Drug Research Institute, Lucknow. An informed consent was obtained from each patient and the study protocol conformed to the standard ethical guidelines. Exclusion criteria included use of clopidogrel, dipyridamole, non-steroidal anti-inflammatory drugs (NSAIDs), administration of heparin/other antithrombotics in last 48 hours, family or personal history of bleeding disorders, platelet count  $< 150 \times 10^3/\mu\text{l}$  or  $> 450 \times 10^3/\mu\text{l}$ , hemoglobin  $< 8$  gm/dl, history of blood dyscrasias, heparin-induced thrombocytopenia, major surgical procedure within one week before enrolment or malignant paraproteinemias.

Optical platelet aggregation utilizes a modified spectrophotometer in which platelet-rich plasma is incubated, stirred, and evaluated as aggregating agents are added. It assesses optical density changes which are detected photoelectrically as platelets begin to aggregate.<sup>11</sup> Whole blood (9 ml) was drawn within 24 hours of administration of the last dose of aspirin and tests were done within 4 hours of sampling. The sample was added to 1 ml of 3.8% trisodium citrate (pH=6.5) solution and centrifuged for 20 min at 20°C. The platelet-rich plasma was removed, and followed by centrifugation of remaining specimen to obtain platelet-deficient plasma. Platelet count was adjusted to  $2 \times 10^8/\text{ml}$  with the spectrophotometer (absorption of 0.650 at 6.30 mm refers to the  $2 \times 10^8/\mu\text{l}$ ). Platelet-rich plasma (400  $\mu\text{l}$ ) was taken in the aggregometer (whole blood Lumi Aggregometer-Chronolog Corp, USA). After 5 min of incubation, ADP (10  $\mu\text{m}$ ) and AA (0.5 mg/ml) was added to get the respective aggregation. Aggregation was monitored for 7-10 min. The inhibition of aggregation was compared with the control value. Another sample of 2 ml blood anticoagulated with EDTA was collected for hemoglobin and platelet count analysis.

Aspirin resistance was defined as a mean aggregation of  $\geq 70\%$  with 10  $\mu\text{m}$  ADP and a mean aggregation of  $\geq 20\%$  with 0.5 mg/ml AA. Aspirin semi responders were

defined as those meeting only one of the criteria.<sup>10</sup> This is a stringent definition, as other studies have defined aspirin resistance by merely a lack of aggregation inhibition by ADP. Laboratory standards were established by screening 35 age- and sex-matched healthy controls without any history or family history suggestive of CAD and they were not on any drugs for the past 7 days.

**Statistical analysis:** All analysis were done on SPSS 11.5 software. The analysis of continuous variables was done using the paired *t* test with equal variance. The chi-square test and Fisher's Exact test was calculated to see the association between variables. All variables were expressed as mean  $\pm$  SD.

### Results

Fifty patients having stable CAD underwent platelet aggregation studies. Two patients were excluded from the statistical analysis due to hemolysis of the sample. Amongst the remaining 48 patients there were 18.75% females, 18.75% smokers, 12.25% diabetics and 33.3% obese patients. There were 52% post-MI patients, 39% with chronic stable angina, 4.2% post-coronary artery bypass grafting (CABG) and 4.2% with ischemic cardiomyopathy. In the patient group ( $n = 48$ ), with 10  $\mu\text{m}$  of ADP the mean platelet aggregation was found to be  $49.42 \pm 23.29\%$  and with 0.5 mg/ml of AA it was  $13.58 \pm 21.40\%$  while in the controls ( $n=35$ ) with 10  $\mu\text{m}$  of ADP the mean platelet aggregation was found to be  $54.53 \pm 16.81\%$  and with 0.5 mg/ml of AA it was found to be  $56.00 \pm 21.93\%$ .

Out of 48 patients, 1 (2.08%) was found to be aspirin-resistant, 19 (39.58%) were aspirin semi responders and 28 (58.33%) were aspirin responders. The mean age in the aspirin semi responder group ( $n=19$ ) was  $56.69 \pm 10.14$  years and aspirin responder group ( $n=28$ ) was  $53.36 \pm 8.35$  years ( $p = 0.2$ ). There were 6 (31.57%) females in the semi responder group and 3 (10.7%) females in the responder group ( $p = 0.08$ ). All other parameters tested like smoking, diabetes, hypertension, obesity, lipid fractions, hemoglobin, platelet count, ejection fraction and drug intake did not show any statistically significant difference among the two groups (Table 1).

Thus in our group, 41.66% patients showed inadequate response to aspirin i.e. 2.08% aspirin resistant plus 39.58% aspirin semi responders. Among females there was a trend to be more aspirin semi responsive (31.67% v. 10.7%,  $p = 0.08$ ).

### Discussion

The concept of aspirin resistance is receiving increasing attention in recent literature. Till now the data available on aspirin resistance is scarce, and to the best of our

**Table 1. Characteristics of patients as per sensitivity to aspirin**

Variable	Aspirin-resistant (n=1; 2.08%)	Aspirin semi responder (n=19; 39.58%)	Aspirin-sensitive (n=28; 58.33%)	p value*
Age (years)	72	56.59±10.14	53.36±8.85	0.20
Females	0	6 (31.57%)	3 (10.7%)	0.08
Smokers	0	2 (10.52%)	7 (25%)	0.20
Diabetes	0	3 (15.78%)	9 (32.14%)	0.18
Hypertension	0	10 (52.63%)	11 (39.28%)	0.37
Obesity	0	5 (26.31%)	11 (39.28%)	0.36
Hemoglobin (gm%)	14	13.75±1.22	13.45±1.18	0.40
Platelet count (×10 <sup>5</sup> )	2.75	2.44±0.30	3.09±3.72	0.35
Ejection fraction (%)	45	57.89±7.51	55.89±8.06	0.40
Total cholesterol	162	165.95±29.58	181.82±42.85	0.25
LDL-cholesterol	117	98.66±22.35	114.97±37.38	0.12
HDL-cholesterol	28	37.66±10.26	34.82±8.25	0.30
Triglycerides	83	131.26±60.74	157.39±79.06	0.25
Beta-blockers	1	17	23	0.40
Calcium blockers	0	3	8	0.26
Statins	1	10	16	0.76
Fibrates	0	1	1	0.65
Nicorandil	0	2	1	0.36
Trimetazidine	0	2	1	0.36

\*p values between aspirin semi responders plus resistant (41.66%) versus aspirin sensitive (58.33%)

LDL: low-density lipoprotein; HDL: high-density lipoprotein

knowledge, no study has been reported on this subject from the Indian subcontinent. Data using varying methodologies have given inconsistent results with aspirin resistance reported in 5% to 50% patients depending on the type of test used.<sup>7-10</sup> In fact a study by Tantry et al.<sup>12</sup> showed that only one out of 143 patients studied was aspirin-resistant. The present study enrolled patients taking 150 mg aspirin daily because this is the most commonly used dosage in clinical practice in our country. Among these patients of stable CAD, 2.08% were aspirin-resistant and an additional 39.58% were aspirin semi responders, thus a cumulative inadequate response of 41.66% was noted.

In a study by Gum et al.<sup>10</sup> aspirin resistance was found in 5.5% while 23.3% were semi responders thus giving an inadequate response of 28.8%. The patients were taking 325 mg aspirin and those who showed inadequate response were more likely to be females (34.4% v. 17.3% p = 0.001) and less likely to be smokers (0% v. 8.3% p = 0.004). There was a trend toward increased age of patients showing inadequate response (65.7 v. 61.3 years, p = 0.06). They used the same methodology of measuring aspirin resistance which was carried out in our work. Hung et al.<sup>13</sup> have shown that smoking was significantly associated with aspirin resistance (p < 0.05). A recent study has also shown that aspirin resistance was significantly more in men (p = 0.02) and those using tobacco (p = 0.03).<sup>14</sup> In our study there was no difference related to age (p = 0.2). There was only a trend for females to be semi responders (31.67% v. 10.7%, p = 0.08). We could not find any statistically significant difference related to smoking among the groups (p = 0.2). All other parameters tested including diabetes,

hypertension, obesity, lipid fractions, hemoglobin concentration, platelet count, ejection fraction and concomitant drug intake did not show any statistically significant difference among the groups.

Thus it was shown that inadequate response to aspirin is prevalent in Indian patients and there are no predictors for this condition. The diagnosis is primarily laboratory-based, and this incomplete therapeutic response may be of clinical importance. Although, much is currently known about aspirin's effect on platelets, the mechanism by which some platelets are resistant has not been ascertained. The proposed mechanisms for the aspirin resistance can be broadly classified into extrinsic and intrinsic factors.<sup>15</sup> The extrinsic factors like smoking have been shown to accentuate platelet thrombosis.<sup>12,13</sup> However, some studies have also refuted this claim by showing that aspirin resistance was less likely among smokers.<sup>10</sup> Other factors such as use of NSAIDs, which act through the same pathway, may compete with aspirin.<sup>16</sup> There is also a suggestion of a dose response curve and up to 8% of patients appear to be resistant even at 1300 mg daily doses.<sup>7</sup> There are proposed intrinsic mechanisms also leading to failure of adequate suppression of TXA<sub>2</sub>. Recent evidence has established that platelets may contain Cox-2 mRNA<sup>17</sup> and this could provide an alternate pathway for the platelets to act. Besides platelets, the nucleated cells are also rich sources of TXA<sub>2</sub> and single nucleotide polymorphism of Cox-1 has been reported, which imparts resistance.<sup>18</sup>

A few long-term studies have suggested the clinical importance of aspirin resistance. In a cohort of stroke patients, 30% of subjects were found to be aspirin non-

responders and after a follow-up of 2 years, major clinical vascular end points were higher in this group compared to responders ( $p < 0.0001$ ).<sup>9</sup> In yet another study, aspirin non-responder status was seen in 34% of patients with recurrent cerebrovascular ischemic events, despite regular use of aspirin for more than 5 years.<sup>19</sup> A subgroup analysis from the HOPE trial,<sup>20</sup> reported higher adverse outcomes at a follow-up of 5 years in patients showing aspirin resistance. Gum et al.<sup>10</sup> showed that among stable patients with CAD over a mean follow-up period of  $679 \pm 185$  days, aspirin resistance was associated with an increased risk of composite end points of death, MI or cerebrovascular accident ( $p = 0.03$ ) and the multivariate analysis showed aspirin resistance to be a significantly independent predictor of long-term major adverse outcome.

**Limitations of the study:** It has been suggested by some researchers that aspirin resistance may not be absolute over time, that measurement of aspirin resistance should be done more than once as a single measure may overestimate its prevalence.<sup>21</sup> Use of aspirin needs to be confirmed by serum salicylate levels to assess the adequacy of the dose used but in the present study we relied on the history of drug intake given by our patient group to ascertain that adequate dose of aspirin had been taken prior to assessment of resistance. In our study, the sample size was small and we measured aspirin resistance only once which may have a bearing on the final results.

**Conclusions:** Aspirin resistance and aspirin semi responsiveness occurs frequently in the Indian patients and there are no reliable clinical predictors for this condition. The diagnosis relies primarily on a laboratory assessment of platelet functions and we may be overestimating its risk by using different methods and definitions.

Further, we need to formulate a policy on aspirin usage and ascertain whether all patients taking aspirin need to be investigated, whether all patients with so-called aspirin resistance be put on clopidogrel and lastly whether there is a serious issue of clopidogrel resistance at hand as well. We also foresee further advancements in the diagnostic tests for aspirin resistance like PFA-100 and estimation of 17 hydroxy TXA<sub>2</sub> which are user-friendly. Once these are commercially available, the true picture of aspirin resistance may come to light.

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