

Frequency of aspirin non responsiveness in patients of ischemic heart disease

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Abstract: Aspirin is widely used as an antiplatelet agent. Many patients have been noticed with recurrence of major ischemic events in spite of antiplatelet therapy. The objective of this study was to determine frequency of aspirin non-responsiveness /resistance in patients of ischemic heart disease. Seventy one patients of IHD were selected from out-patient department of Punjab Institute of Cardiology Lahore. Whole Blood Platelet aggregation studies were performed on Diamed Impact R. Aspirin response assay was performed with DiaChidon (Arachidonic Acid 16mmol/L). Non responders to aspirin were assessed on the basis of software generated results: Surface covered (SC) $\geq 2.5\%$ was considered as response to aspirin and SC $< 2.5\%$ was considered as no response (or resistance) to Aspirin. Chi-square test was applied to measure statistical significance. Non-response to Aspirin was observed in 11% (8 out of 71). There was significant association ($p=0.045$) between resistance to aspirin and Diabetes mellitus. Treatment resistance was also significantly associated with female gender ($p=0.015$). We concluded that non response to Aspirin is seen in significant number of patients of IHD. Diabetes mellitus and female gender are strong risk factors of developing failure to aspirin therapy.

Keywords: Ischemic heart disease, platelet aggregation, aspirin, non response.

INTRODUCTION

Ischemic Heart Disease is the most frequently encountered cause of death in the world (Bonow *et al.*, 2002). Currently, cardiovascular diseases accounts for 16.7 million deaths worldwide each year (Frans *et al.*, 2007). The most commonly used antiplatelet drugs are Acetylsalicylic acid (Aspirin) and thienopyridines (Clopidogrel). Aspirin inhibits COX-1 and prevents the conversion of arachidonic Acid to the unstable prostaglandin (PG) intermediate PGH₂, that is converted to Thromboxane A₂ (TXA₂) and TXA₂ acts as a potent vasoconstrictor and platelet agonist (weber *et al.*, 1999). In many clinical trials, the beneficial effects of aspirin have been shown in prevention and treatment of IHD (Tantry *et al.*, 2009). However, some patients have a resistance to aspirin. Aspirin resistance is the failure of inhibition of cyclooxygenase -1 and ability of platelet to aggregate after stimulation by various agonists (Poulsen *et al.*, 2007). Frequency of aspirin resistance has been estimated as 5 to 45% of population depending on the method of determining the resistance (Gum *et al.*, 2003). Several factors may contribute to aspirin resistance which include drug noncompliance and presence of certain conditions e.g. acute coronary syndrome and congestive heart failure in which there is increased platelet activity (Serebruany *et al.*, 2003). Different platelet function tests are used to measure platelet inhibition. In this study we

have used Diamed Impact® (The cone and plate (let) analyzer). This instrument monitors platelet adhesion and aggregation under high shear conditions of 1800 s⁻¹ and provides the physiological condition for platelet adhesion and aggregation. The platelet adhesion and aggregation on the polystyrene surface were evaluated using an image analysis system. The results were expressed as the percentage of surface covered (%SC) by platelets and the average particle size (AS; micron m²). Normal value of % SC is > 7.5 and AS is > 25 micron m² for physiological platelet function. Surface covered (SC) $\geq 2.5\%$ was considered as response to aspirin and SC $< 2.5\%$ was considered as no response (or resistance) to aspirin.

MATERIALS AND METHODS

Study population

It was a descriptive cross sectional study. This study was approved by ethical committee of University of health sciences Lahore. Patients were enrolled after informed written consent. Diagnosed patients of IHD who were more than 21 years of age and were on aspirin 150mg once daily, for at least 07 days were included in the study. Patients with history of bleeding disorders, Patients who were taking aspirin irregularly, Platelet count $< 150 \times 10^9$ /L, Hemoglobin < 8 g/dl, Haematocrit of $< 32\%$, major surgical procedure within one week and patients who were taking un-fractionated or low molecular weight heparin were excluded from the study population.

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Laboratory techniques

5ml of venous blood was collected using aseptic methods. Two ml blood (in EDTA) was used to determine Haemoglobin, Haematocrit and platelet count using Sysmex XI-1800i. Citrated whole blood (3ml) was used for platelet studies (platelet adhesion and aggregation) using Impact R (Diamed, Israel). Diachidon (Arachidonic Acid 16mmol/L) was used as platelet agonist. Resistance to Aspirin therapy was assessed by Impact R on the basis of software generated results: Surface covered (SC) $\geq 2.5\%$ was considered as response to aspirin and SC $< 2.5\%$ was considered as no response (or resistance) to Aspirin. Results generated as non response / resistance to aspirin therapy in a patient is illustrated in fig. 2.

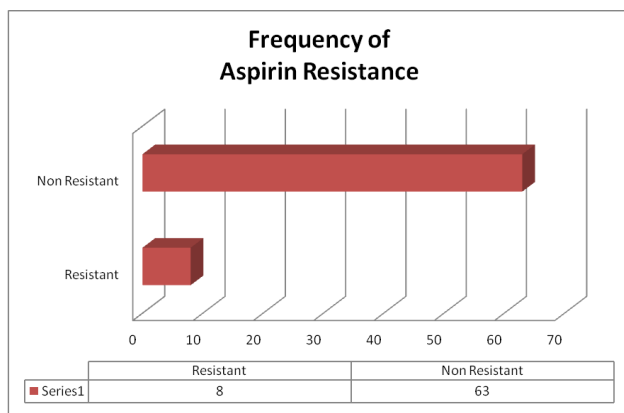


Fig.1: Frequency of aspirin resistance/non responsiveness

Table 1: Demographic data of the seventy one subjects included in the study

| Variables | Frequency | Percentage% |
|----------------|-----------|-------------|
| Male | 52 | 73 |
| Female | 19 | 27 |
| DM | 10 | 14 |
| HTN | 36 | 50 |
| Family H/o IHD | 40 | 56 |
| Rec. ischemia | 21 | 30 |
| Smokers | 21 | 30 |

*DM: Diabetes Mellitus *HTN: Hypertension *IHD: Ischemic Heart Disease *Rec: Recurrent

Data collection

Data about patient’s demographic features (age, sex, and address), clinical diagnosis, duration of illness, drug intake history, history of recurrent ischemic events and relevant clinical history was obtained on a specially designed proforma. The data about platelet aggregation studies was entered after performing the laboratory tests in the specified columns.

STATISTICS ANALYSIS

All data was entered and analyzed with the help of SPSS version 18.0. The quantitative variables were expressed as mean \pm SD. Chi-square test was applied to measure

the statistical significance of frequency of drug resistance between groups e.g. Diabetics vs. Non-diabetics, Males vs. Females. A p value of less than 0.05 was considered for statistical significance.

RESULTS

Mean Age in the study population in years was 52.85+ 1.14 (95%CI 50.56-55.13) Median duration of illness in the study population was 24+ 3 (Tukeys’s Hinges: 9-48) months. Demographic Characteristics of study population are shown in table 1. Hemoglobin, Hematocrit and platelet count were measured for each subject, before performing the platelet aggregation studies to fulfill exclusion criteria for Hb, HCT% and platelet count and results are listed in table 2.

Table 2: Haemoglobin, Haematocrit and Platelet Count of Subjects

| Laboratory Parameters | Mean \pm SD (Range) |
|---|-----------------------|
| Hb of the patients (g/dl) | 13.38 \pm 1.42 |
| | (10.0–16.0) |
| Hct. of the patients (%) | 40.05 \pm 3.20 |
| | (35 – 49) |
| Platelets count of the patient ($10^9/L$) | 252.0 \pm 70.9 |
| | (150 – 400) |

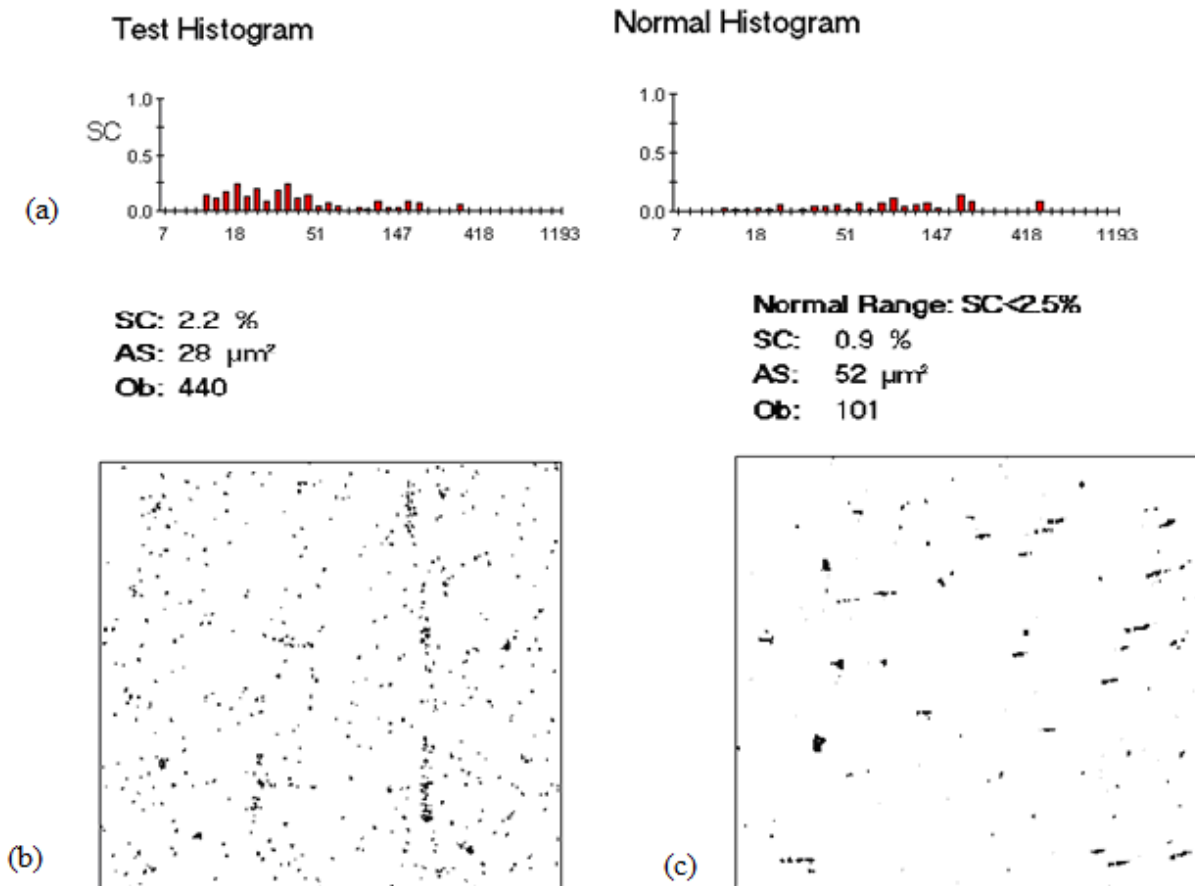
Hb: Haemoglobin Hct: Hematocrit SD: Standard Deviation

Aspirin response assay

Out of 71 patients 8 (11%) were found to be non - responsive to aspirin (SC was < 2.5) shown in fig. 1. Patients who were found to be non- responsive, their samples were tested again without Diachidon to assess any baseline platelet aggregation defect and no case was found with such platelet functional defects. There was significant association (P value of 0.045) between resistance to aspirin and patients with Diabetes mellitus and aspirin resistance was observed significantly high in female patients (P value of 0.015). It was also observed that variance in % SC (surface covered) was higher in female patients as compare to male patients. Variance of %SC of male and female patients is shown in fig. 3.

DISCUSSION

Antiplatelet therapy is effective in prevention of atherothrombotic events in patients of ischemic heart disease (Gum *et al.*, 2003). The issue of resistance to beneficial effects of aspirin has been raised in literature years ago. The prevalence of aspirin resistance depends on the method used and parameters measured (Armen *et al.*, 2007). Hoven *et al* (2007) has emphasized that aspirin resistance is lowest with optical aggregometry using arachidonic acid (6%; 95% CI 0% to 12%) and highest with PFA-100 analyzer (26%; 95% CI, 21% to 31%) (Hovens *et al.*, 2007). In our study the resistance to



*SC: Surface Covered; *AS: Average Size; *Ob: Object Number Illustration of a test report of aspirin resistance response of a patient (Processed on Diamed Impact-R, Software Version: 1.28). The test results report include: (a) a particle size distribution histogram; (b) the percentage of surface coverage (SC); the average size (AS) of the stained Objects; the number of objects (Ob) per picture; and a representative picture of the tested sample. (c) For comparison, the results of a representative normal sample and the normal range are presented on the right side.

Fig. 2: Example of test report of aspirin resistance case.

aspirin was estimated 11% (8 out of 71) and all subjects included were on standard dose of aspirin i.e. 150mg OD (Prescribing dose) In this study aspirin assay was performed on point of care device Diamed Impact R. One study by Naved Akhtar *et al.* (2009) has reported the frequency of aspirin resistance about 12%. In another study aspirin was assayed with the Verify Now System with a 12.1% of resistance to 81 mg/day and 5.3% resistance to 325mg/day (Deepak *et al.*, 2003). In this study we have noted a statistically significant association (P value of <0.045) between diabetic patients and resistance to aspirin. There is limited data on the prevalence of aspirin resistance in diabetes mellitus. Due to complex pathophysiology of diabetes mellitus and association with other disorders like, hyperlipidemia, hyperglycemia, accelerated atherogenesis, micro- and macro-vasculopathy, there is increased platelet activation and aggregation, and it is found that the prevalence of aspirin resistance is to be increased (Evangelista *et al.*,

2005; Coccheri *et al.*, 2007). In HOPE (Heart Outcomes Prevention Evaluation) trial, 32.6% patients had diabetes mellitus, the prevalence of aspirin resistance was reported 21.5% and the method used was the PFA- 100 analyzer with collagen/epinephrine cartridges (Fateh *et al.*, 2005). We have noted high resistance to aspirin therapy in female Patients. In this study out of 19 female patients 5 were resistant to aspirin (26%) while out of 63 male patients only 3 (5%) were resistant to aspirin (P =0.015). One study conducted by Chen *et al.*(2004) showed that aspirin resistance is significantly more prevalent in female (44.8%) than in male patients (19.7%). Similar results were reported by Gum *et al.* (2001) higher number of female patients were either aspirin resistant or aspirin semi-responders (34.4% vs 17.3%, p=0.001). The exact cause of high aspirin resistance in female patients is unknown; however the possible mechanism may be, higher platelet activity in female as compare with male (Chen *et al.*, 2004)

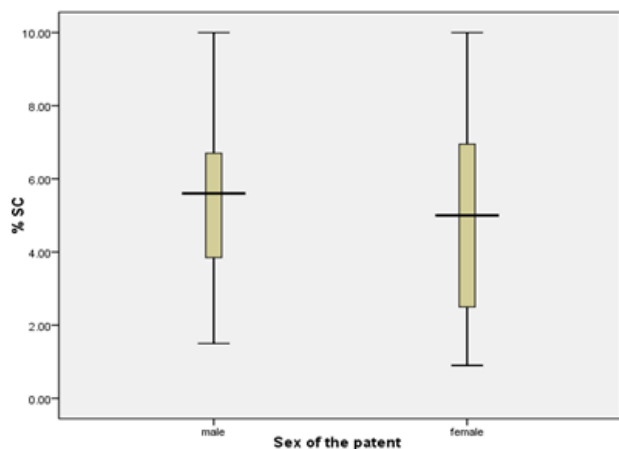


Fig. 3: Comparison of % SC (Surface Covered) of aspirin response assay between male and female patients.

CONCLUSIONS

Our study was a descriptive study with its inherent limitations. The exact prevalence may be different from the one which is observed in the present study due to small sample size. In our study dose of aspirin was uniform and the compliance was based on patient history. As the phenomenon of aspirin resistance is multifactorial and it was not possible to exclude such factors in our study.

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