Frequency of Clopidogrel Resistance in Patients of Ischemic Heart Disease

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ABSTRACT:

Clopidogrel and Aspirin are widely used antiplatelet agents in the prevention and treatment of ischemic heart disease (IHD). Many patients have been noticed with recurrence of major ischemic events, due to resistance of these drugs. Different platelet function tests can be used to evaluate the degree of achieved platelet inhibition in patients treated with clopidogrel. The objective of this study was to determine frequency of clopidogrel resistance in patients of ischemic heart disease. Seventy one patients of IHD were selected from out-patient department of Punjab Institute of Cardiology Lahore. Platelet aggregation studies were performed on Diamed Impact R. Clopidogrel response assay was performed with DiaAdin(ADP 110µmol/L). Chi-square test was applied to measure statistical significance. Resistance to Clopidogrel was observed in 17% (12 out of 71). Clopidogrel resistance was significantly associated with female gender (p=0.046). In our study no statistically significant association was observed between clopidogrel resistance and risk factors like diabetes mellitus, family history ischemic heart disease, hypertension and smoking. We concluded that resistance to Clopidogrel therapy is seen in significant number of patients and female patients are at high risk of developing the resistance to clopidogrel therapy. These patients can be identified by performing platelet aggregation studies on Impact R.

INTRODUCTION

Ischemic heart disease is a major public health problem and a leading cause of death in the world. Its risk increases with age, smoking, hypercholesterolemia, diabetes and hypertension [1]. Currently, cardiovascular diseases accounts for 16.7 million deaths worldwide each year and strategies for improving prevention and management of thrombotic conditions are required [2]. The most commonly used antiplatelet drugs are Acetylsalicylic acid (Aspirin) and thienopyridines (clopidogrel).

Clopidogrel is a prodrug, which needs to be metabolized in the liver to active metabolites. Clopidogrel inhibits the ADP receptor P2Y12, and thereby inhibiting the ADP mediated platelet activation. In vivo transformation of clopidogrel to active metabolites is an important and critical step for the drug effect. This metabolisation is dependent on the hepatic cytochrome P450 isoenzymes like CYP2C19, CYP1A2, CYP2B6, CYP2C9 and CYP3A4 [3]. One of the key enzymes in clopidogrel metabolism is CYP2C19, which is involved in both stages of clopidogrel biotransformation [4,5].

A number of studies have shown that aspirin or clopidogrel resistance is associated with increased risk of recurrent cardiovascular events [6,7]. The term resistance is used to indicate failure of an agent to prevent the clinical condition for which it is used or failure of the agent to achieve the biochemical (pharmacokinetic and/or pharmacodynamic) effect [8]. Different platelet function tests have been used to measure the degree of platelet inhibition in patients treated with clopidogrel. Light transmission aggregometry with ADP as an agonist, is the most used method, but the test is time consuming and not practical for routine use.

New point of care devices like PFA100, VerifyNow and The cone and plate(let) analyser are being utilised for the determination of antiplatelet therapy effectiveness. The PFA-100® measures the fall in flow rate as platelets within a collagen coated membrane. The disadvantages of PFA 100 are: This is inflexible, VWF dependent, Haematocrit dependent and insensitive to clopidogrel [9].
VerifyNow is a fully automated platelet aggregometer which can be used to monitor the therapy of aspirin, clopidogrel, and GpIIbIIIa inhibitors, but this assay is expensive and cartridges can only be used for single purpose [9]. The cone and plate(let) analyser, originally developed by Varon and Savion, monitors platelet adhesion and aggregation on a plate coated with collagen or extracellular matrix (ECM) under high shear conditions of 1800 s⁻¹. In the commercial version of the device, the Impact® (DiaMed), a plastic plate is utilized instead of a collagen or an ECM-coated surface.

In this study, we used Diamed Impact R to determine the frequency of resistance of clopidogrel in patients of IHD. Diamed Impact R is the instrument which provides the physiological condition for platelet adhesion and aggregation. Platelet adhesion and aggregation on the polystyrene surface is evaluated using an image analysis system. The results are 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.

**PATIENTS AND METHODS**

**Study Population**

It was a descriptive cross-sectional study conducted at the department of Haematology, University of Health Sciences Lahore in collaboration with Punjab Institute of Cardiology, Lahore Pakistan. The study was approved by the ethical committee of University of Health Sciences Lahore. Patients were enrolled after informed written consent from April 2011 to March 2012. Diagnosed patients of IHD who were more than 21 years of age and on Clopidogrel 75 mg for at least more than 07 days were included in study and patients who have Family or personal history of bleeding disorders, platelet count <150 or >450 × 10⁹/L, Hemoglobin <8 g/dl, Major surgical procedure within one week before enrollment and Administration of unfractionated or low molecular weight heparin within 24 hours before enrollment were excluded from study.

**Laboratory Techniques**

5ml of venous blood was collected using aseptic methods. Citrated whole blood (3ml) was used for study platelets (platelet adhesion and aggregation) using Impact R (Diamed, Israel). DiaAdin (ADP 110µmol/L) was used as platelet agonist. 2ml blood (in EDTA) was used to determine Haemoglobin, Haematocrit and platelet count using Sysmex XI-1800 (manufact). Resistance to Clopidogrel therapy was assessed by Impact R on the basis of software generated results: Surface covered (SC) > 2.8% was considered as response to clopidogrel and SC < 2.8% was considered as no response (or Resistance) to Clopidogrel. Resistance to clopidogrel therapy in a patient is illustrated in Figure 1.

**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.

**RESULTS**

Out of 71 patients 37 patients had IHD without PCI (Percutaneous Coronary Intervention) or CABG (Coronary Arterial Bypass Grafting). Nineteen patients had IHD with Angioplasty and 15 patients were treated with CABG. 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 ± 39 (Tukey’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 (Table 2).
Table 1: Demographic Data of the Subjects included in the Study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency</th>
<th>Percentage%</th>
<th>Resistant</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>52</td>
<td>73</td>
<td>6</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
<td>27</td>
<td>7</td>
<td>0.046</td>
</tr>
<tr>
<td>DM</td>
<td>10</td>
<td>14</td>
<td>2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>HTN</td>
<td>36</td>
<td>50</td>
<td>3</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Family H/o IHD</td>
<td>40</td>
<td>56</td>
<td>4</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Rec. ischemia</td>
<td>21</td>
<td>30</td>
<td>3</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Smokers</td>
<td>21</td>
<td>30</td>
<td>4</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

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

Table 2: Demographic Data of the Subjects included in the Study

<table>
<thead>
<tr>
<th>Laboratory Parameters</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb of the patients (g/dl)</td>
<td>13.38±1.42 (10.0–16.0)</td>
</tr>
<tr>
<td>Hct. of the patients (%)</td>
<td>40.05±3.20 (35 – 49 )</td>
</tr>
<tr>
<td>Platelets count of the patient (109/L)</td>
<td>252.0±70.9 (150 – 400)</td>
</tr>
</tbody>
</table>

*Hb: Haemoglobin *Hct: Hematocrit *SD:Standard Deviation

Clopidogrel Response Assay

Out of 71 patients 12 (17%) were found resistant to clopidogrel. The frequency of resistance to clopidogrel is shown in Figure 2. Samples of resistant patients were retested, without DiaAdin to assess any baseline platelet functional aggregation defect. No case was found to have any platelet functional defect.

There was significant association (P value of 0.046) between resistance to Clopidogrel and patients with female gender. 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 Figure 3. Significant association of Clopidogrel resistance and other risk factors like hypertension, diabetes mellitus, smoking and family history of ischemic heart disease was not observed (p value of >0.05) (Table 1).
DISCUSSION

Antiplatelet therapy is effective in primary and secondary prevention of atherothrombotic events in patients of ischemic heart disease\textsuperscript{[11]}. The concept of clopidogrel resistance has emerged in the medical literature to reflect the failure to inhibit platelet function in vitro, although its existence and definition remain to be established. It has been proposed that the term resistance encompasses patients for whom clopidogrel does not achieve its pharmacological effect, and failure of therapy reflects patients who have recurrent events on therapy\textsuperscript{[12]}. Despite intensified antiplatelet treatment, up to 4.7\% of the patients undergoing coronary stenting develop thrombotic stent occlusion, suggesting incomplete platelet inhibition due to clopidogrel resistance\textsuperscript{[13]}.

The prevalence of clopidogrel non-response in patients is estimated 4\% to 30\%\textsuperscript{[14]}. The reported rates vary between studies because of the technique used to measure the extent of platelet aggregation and the presence of factors contributing to platelet reactivity\textsuperscript{[15]}. In our study non responders to clopidogrel were 12 (17\%) and all patients were on standard dose of 75mg OD. Clopidogrel assay was measured with DiaAdin (ADP110 µmol/L) on Diamed Impact R. In another study 16\% of Coronary arterial disease (CAD) patients were classified as non-responders and the electrical impedance aggregometry was performed in diluted whole blood in the presence of 5 and 20 µmol/L ADP\textsuperscript{[16]}.

In a study by Mobley et al. (2004) the prevalence of clopidogrel resistance was estimated 30\%. These patients were on 75 mg daily maintenance dose and the method used was Optical aggregometry 1 µmol/L ADP. A study conducted in India, 2.54\% patients were found to be clopidogrel resistant, 12.7\% were clopidogrel semi-responders and 84.7\% were clopidogrel responders and the optical aggregometry was used to define clopidogrel resistance, semi-responders and responders\textsuperscript{[17]}. Estimated frequency of clopidogrel resistance in different studies is summarised in Table 3.

In our study no significant association was observed between clopidogrel resistance and risk factors like diabetes mellitus, family H/O ischemic heart disease, hypertension and smoking. However resistance to clopidogrel therapy was noted more in female patients with a statistically significant P value (P = 0.046). In a study by Boris et al. (2006) it was reported that female are predisposed to clopidogrel resistance (P = 0.0002) This marked sex-related difference in clopidogrel responsiveness needs to be confirmed in a larger number of patients matched for other potential confounders. Similarly various risk factors like age, smoking, diabetes, hypertension, obesity, cholesterol, did not show any statistically significant difference among the groups in a study by Kumar et al. (2007).

Class IIB recommendations from the American College of Cardiology (ACC)/American Heart Association (AHA) have stated that platelet aggregation studies are warranted in patients undergoing PCI who are at risk of sub-acute stent thrombosis, with the option of increasing their maintenance dose of clopidogrel from 75 to 150mg/day in order to suppress platelet reactivity below 50\%\textsuperscript{[18]}. Recent studies have evaluated the effect of modifying therapy on clinical outcome for patients deemed non-responsive as measured by platelet function tests.

Clopidogrel response can also vary because of inter-individual differences in drug absorption, resulting in lower levels of the active metabolite. Medications such as statins and certain proton pump inhibitors have been proposed to affect the metabolism of clopidogrel by the CYP isozyme 3A4, although data is controversial\textsuperscript{[19,20]}. Individuals with low baseline metabolic activity of the CYP3A4 enzyme have also been shown to have poor clopidogrel responsiveness and this aspect should be evaluated on large randomised trials\textsuperscript{[21]}.

It is concluded that resistance to clopidogrel therapy is seen in significant proportion of patients. These patients can be identified by performing platelet aggregation studies on Diamed Impact R and female patients are at high risk for developing the resistance to clopidogrel therapy.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Estimated Resistance</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Müller et al.</td>
<td>2003</td>
<td>4.7%</td>
<td>Optical aggregometry</td>
</tr>
<tr>
<td>Jaremo et al.</td>
<td>2003</td>
<td>4%</td>
<td>Optical Aggregometry</td>
</tr>
<tr>
<td>Mobley et al.</td>
<td>2004</td>
<td>30%</td>
<td>Optical Aggregometry</td>
</tr>
<tr>
<td>Boris et al.</td>
<td>2006</td>
<td>16%</td>
<td>Optical Aggregometry</td>
</tr>
<tr>
<td>Kumar et al.</td>
<td>2007</td>
<td>12.7%</td>
<td>Optical aggregometry</td>
</tr>
<tr>
<td>Boris et al.</td>
<td>2009</td>
<td>10%</td>
<td>Impedence aggregometry</td>
</tr>
<tr>
<td>Our study</td>
<td>2011</td>
<td>17%</td>
<td>Impact R</td>
</tr>
</tbody>
</table>

Table 3: Clopidogrel resistance in different studies
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