

## FREQUENCY OF DUAL ANTI PLATELET RESISTANCE IN PATIENTS WITH CORONARY ARTERY DISEASE

Mohammad Faheem, Ibrahim Shah, Jabar Ali, Muhammad Adil, Zahur Ahmad, Mohammad Hafizullah

Department of Cardiology, Postgraduate Medical Institute, Lady Reading Hospital, Peshawar- Pakistan

### ABSTRACT

**Objectives:** To find the frequency of dual antiplatelet resistance in patients with coronary artery disease.

**Material and Methods:** This study was conducted in the Department of Cardiology, Lady Reading Hospital, Peshawar, from January 2007 to February 2008. A total of 105 normal individuals not taking any anti platelet drug and 108 patients taking dual anti platelet drugs for coronary artery diseases were included. Blood was taken for measuring platelet aggregation using whole blood aggregometer and results were recorded on a proforma. Patients who were on warfarin and heparin were excluded from the study. Chi-square and independent t-test were used to find significant differences between different groups and variables.

**Results:** Platelet aggregability in 105 normal subjects, not taking aspirin was  $9.28 \pm 3.23$  ohms. So cutoff for dual anti platelet non responsiveness was taken as  $9.28 - 3.23 = 6.0$  ohms. Patients who were taking dual antiplatelets were 108. Their mean age was  $51.94 \pm 8.36$  years. Male were 64 (59.3%) and female were 44 (40.7%). Mean aggregability of all these patients was found to be  $3.91 \pm 3.50$  ohms. Patients having aggregability of  $>6$  ohms (dual antiplatelets resistance) were 29.6% (n=32), while patients having aggregability of  $<6$  ohms (dual antiplatelets sensitive) were 70.4% (n=76). Mean aggregability of sensitive patients was  $2.06 \pm 1.76$  ohms, while mean aggregability of resistant patients was  $8.31 \pm 2.55$  ohms. Mean aggregability of all the male patients was  $4.00 \pm 3.38$  ohms, while mean aggregability of female patients was  $3.79 \pm 3.70$  ohms. ( $p=0.769$ ). When age was correlated with aggregability, both have a weak positive correlation  $r = .121$  ( $p=0.213$ ).

**Conclusion:** Dual anti platelet resistance as defined by inhibition of platelet aggregation measured with Whole Blood Aggregometry, is a common problem. Gender and age has no significant affect on platelet aggregability.

**Key Words:** Dual anti platelet resistance, Whole Blood Aggregometry, platelet aggregability.

### INTRODUCTION

Inhibiting platelet activation and aggregation is the primary strategy for preventing ischemic events in patients with atherothrombotic diseases, including acute coronary syndromes. This aim is commonly achieved by giving aspirin which remains the cornerstone for antiplatelet therapy across the spectrum of ischemic heart disease and has shown to reduce risk of major vascular events by about one quarter.<sup>1</sup> Aspirin alone is a relatively weak antiplatelet agent because it inhibits only one of many pathways i.e thromboxane (TX)<sub>2</sub> mediated pathway to platelet activation. Despite aspirin use many patients still suffer vascular events. Inadequate inhibition of platelets has been shown by different platelet function tests in patients regularly using aspirin.<sup>2</sup> This lead to the emergence of other antiplatelet drugs

including clopidogrel which acts via blocking P2y<sub>12</sub>ADP platelet receptors. By blockade of an adjunctive signaling pathway, the addition of clopidogrel to aspirin leads to synergistic platelet inhibitory effects. Thus in addition to aspirin, use of other anti platelet came into practice in high risk patients especially those with Acute Coronary Syndrome (ACS) and those undergoing elective or emergent Percutaneous Coronary Intervention (PCI). Different platelet function tests showed that combination of antiplatelets was more effective in inhibition of platelets than aspirin alone.<sup>3</sup> This combination significantly reduced the risk of cardiovascular events.<sup>4</sup>

But unfortunately despite dual anti platelet strategy, many patients still suffer vascular events. Aspirin and clopidogrel resistance are emerging clinical entities with potentially severe consequences such as myocardial infarction, stroke or death. The results of dual antiplatelet failure are especially catastrophic after PCI and stenting when patients come with stent thrombosis. Platelet function tests show inadequate inhibition of platelets in these patients. Antiplatelet failure or resistance can be caused by a

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#### Address for Correspondence:

**Dr. Mohammad Faheem**

Senior Registrar

Department of Cardiology, Post Graduate Medical Institute, Lady Reading Hospital, Peshawar - Pakistan  
Cell: 0333-9202002

E-mail: drfaheem@live.com

variety of mechanisms including noncompliance,<sup>5</sup> poor absorption, suboptimal dose, drug interactions,<sup>6</sup> co morbidities, smoking and most importantly genetic polymorphism.<sup>7</sup>

The prevalence of dual anti platelet resistance has been reported to be 4% to 30%.<sup>8</sup> Most of these studies were performed on western population. In our country, very little work has been done in this regard. Few researchers have worked on prevalence of aspirin resistance.<sup>9</sup> Other studies have worked on different aspects of clopidogrel.<sup>10,11</sup> We could not find any local study on the prevalence of dual anti platelet resistance. Patients with Coronary Artery Disease (CAD) are increasing day by day in our country and many of these patients undergo PCI, requiring dual anti platelets. The aim of this study was to evaluate frequency of dual antiplatelet resistance in our local patients with coronary artery disease.

## MATERIAL AND METHODS

This study was conducted in the Department of Cardiology, PGMI, Lady Reading Hospital, Peshawar from January 2007 to February 2008. Patients of both genders, aged more than 20 years and taking combination of aspirin (75-325mg) and clopidogrel 75mg for at least 10 days for coronary artery disease were included. Patients taking any anticoagulant like heparin or warfarin or having history of bleeding disorder or deranged Red Blood Counts and White Blood Counts (RBC, WBC) or Platelets were excluded from the study.

The machine used for platelets aggregation was chronolog whole-blood platelet aggregometer (WBA). Other supplies needed for performing whole-blood aggregation were reagents, cuvettes, stir bars, micropipettes, tips etc.

Whole blood (0.5 ml) was diluted with an equivalent volume of isotonic saline and incubated for 5 minutes. The impedance of each sample was monitored at sequential 1-minute intervals until a stable baseline established. The agonist Adenosine Dehydrogenase Phosphate (ADP) (20  $\mu$ mol/L) was then added to the sample and aggregation was monitored for 6 minutes. With time, platelets aggregate over electrodes and impedance increases. The final increase in impedance (in ohms) over this period was displayed as a numeric readout. Each reading was noted on the patient's proforma.

After informed written consent to participate in the study, data was analyzed using SPSS version 14. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as mean  $\pm$  SD. Groups were compared using independent sample t-test. P-value less than 0.05 was considered significant.

## RESULTS

When Platelet aggregability was performed in 105 normal subjects, not taking antiplatelets, it was found to be  $9.28 \pm 3.23$  ohms. As the cut off value for antiplatelet resistance was taken as the mean aggregability of normal individuals minus one standard deviation, so it was calculated as  $9.28 - 3.23 = 6.05$  ohms. Total number of patients on dual anti platelets were 108. Among them, 46 (42.59%) patients were post ST elevation myocardial infarctions, 24 (22.22%) were post non ST elevation myocardial infarction and 38 (35.18%) were post PCI patients. Mean age of these patients was  $51.94 \pm 8.36$  years. Male were 64 (59.3%). Female were 44 (40.7%). Mean aggregability of all these patients was found to be  $3.91 \pm 3.50$  ohms. Patients having aggregability  $> 6$  ohms (dual antiplatelets resistance) were 29.6% (n=32). Patients having aggregability  $< 6$  ohms (dual antiplatelets sensitive) were 70.4% (n=76). Mean aggregability of sensitive patients was  $2.06 \pm 1.76$  ohms, while mean aggregability of resistant patients was  $8.31 \pm 2.55$  ohms. Mean aggregability of all the male patients was  $4.00 \pm 3.38$  ohms, while mean aggregability of all the female patients was  $3.79 \pm 3.70$  ohms. (p=0.769). When age was correlated with aggregability, both had a weak positive correlation. Pearson correlation coefficient = 0.121 (p=0.213) Fig-1.

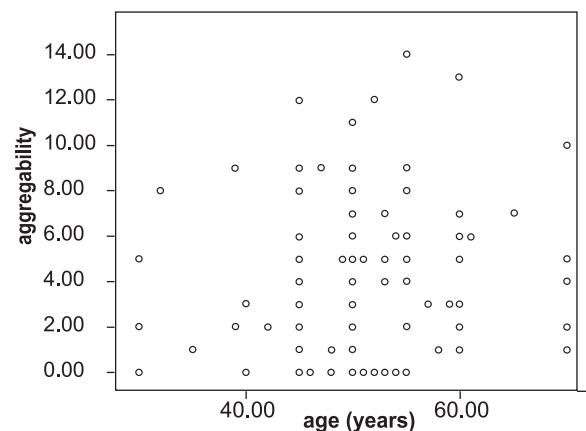


Fig. 1: Correlation of age with platelet aggregability.

## DISCUSSION

Antiplatelet drugs hold a key role in the management of patients with coronary artery disease. Current guidelines recommend use of dual antiplatelet drugs in patients with ACS treated conservatively or invasively with PCI and stenting.<sup>12</sup> Its prolong use is especially recommended after deployment of Drug Eluting Stenting. The antiplatelet effect of aspirin as well as clopidogrel had not been uniform in all patients. Many researchers have addressed this issue during last few years. They have shown that platelet functions were not completely inhibited in a significant number of patients using dual antiplatelets.

Prevalence of dual anti platelet resistance or non responsiveness reported by previous studies ranged from 4% to 30%.<sup>8</sup> Our study showed 29.6% of patients with coronary artery disease to be dual antiplatelets resistant.

Guha S et al. reported 32.5% prevalence of dual antiplatelets resistance in patients with recurrent ACS.<sup>13</sup> Grdinic A et al. found dual antiplatelets resistance in 12% patients. These patients were more often smokers and were having elevated inflammatory markers, a higher platelet count, LDL, and CRP and a lower HDL.<sup>14</sup> Woo KS et al using different assays found prevalence of aspirin and clopidogrel resistances ranging from 0% to 22.7% and from 9.1% to 48.5%, respectively, depending on the assay used.<sup>15</sup> Bouman HJ et al found that patients with a history of early Stent Thrombosis exhibit a poor response to clopidogrel and aspirin.<sup>16</sup> Awidi A et al found 26% patients to be nonresponsive, and 16% patients were partially responsive to aspirin.<sup>17</sup> Fong J et al reported 9.3% patients with ischemic stroke were biochemical nonresponders to dual antiplatelets.<sup>18</sup> In a study on patients undergoing PCI, Moerenhout CM Et al found 7% patients to be unresponsive to clopidogrel as assessed by the point-of-care test PFA-100C/ADP.<sup>19</sup> Djukanovic N et al found that after 7 days of aspirin + thienopyridine treatment, insufficient antiplatelet response was observed in 28% of the patients.<sup>20</sup> This wide range of prevalence among different studies arises from the lack of a "gold-standard" definition to assess antiplatelet drug response and differences in assays, agonist concentrations, and cut-off values and differences in studied populations.<sup>21</sup> Regardless of the variability in the prevalence of aspirin or clopidogrel resistance, several studies have indicated a clear relation between this resistance and cardiovascular events.<sup>22</sup>

Previous studies, which used WBA for platelet function, used different cutoff values ranging from 3 ohms to 18ohms for defining resistance.<sup>23,24</sup> In a study by Müller I et al. non-responsiveness (resistance) was defined by an inhibition of platelet aggregation that was less than 10% when compared to baseline values. Semi-responders were identified by an inhibition of 10 to 29%.<sup>25</sup> We evaluated 105 normal subjects, not taking antiplatelets and used their mean minus 1 SD aggregability as cutoff value for dual antiplatelet resistance. This principle has been used previously by other researchers.<sup>24,26</sup>

Light transmission aggregometry has been considered the gold standard for testing platelet function.<sup>27</sup> Its use is very time consuming as it involves preparation of platelet rich plasma by a skilled technician and its standardization is often poor limiting its use to dedicated laboratories.<sup>28</sup> We used whole blood aggregometry (WBA) using chronolog

aggregometer, because it is easy to use, gives faster results, does not require centrifugation and evaluates platelets in a physiologic milieu.

Antiplatelet resistance or hyporesponsiveness also may be due to age or gender issues. Our study did not show any significant difference between the platelet aggregability of male and female patients (4.00±3.38 ohms Vs 3.79±3.70 ohms. (p=0.769) Previous studies showed female patients to have high platelet aggregability.<sup>26, 29</sup> However, this has been disputed in other studies.<sup>30</sup>

In our study there is a trend towards higher platelet aggregability as the age increases, but this correlation is weak (r= .121) and insignificant (p=0.213). Previous studies reported contradicting results about the association of age and platelet aggregability. Some studies have shown that advanced age may be an important factor.<sup>31</sup> Gum PA et al reported that aspirin semi responders were significantly older.<sup>29</sup> Ivandic BT et al showed that age has no association with platelet aggregability.<sup>26</sup> As there is a significant prevalence of variable response to aspirin and clopidogrel, it is imperative to understand this variable response or hyporesponsiveness. A clear definition of this response should be established and based on this, one may be able to categorize patients as responders, hyporesponders, nonresponders, or resistant and thus manage their therapeutic regimen accordingly.

The routine measurement of platelet reactivity and adjusting dual anti platelet therapy accordingly has been evaluated in different trials. In the GRAVITAS Trial among patients with high on-treatment platelet reactivity after PCI with drug-eluting stents, the use of high-dose clopidogrel compared with standard-dose clopidogrel did not reduce the incidence of death from cardiovascular causes, nonfatal MI, or stent thrombosis.<sup>32</sup> The ARCTIC study is ongoing and will answer same question.<sup>33</sup>

Wang XD et al found that modifying clopidogrel maintenance doses according to platelet reactivity monitoring decreases the rate of MACE after PCI without increasing bleeding in patients with clopidogrel resistance during 1-year follow-up.<sup>34</sup> In the future, routine measurement of platelet function in patients with cardiovascular disease may become the standard of care. Personalized antithrombotic treatment strategies may be determined by ex-vivo measurements of platelet function.

At present, different strategies are proposed for managing resistant patients. They can be treated by increasing the anti platelet dose.<sup>35</sup> Potent new antiplatelet agents, such as prasugrel and ticagrelor can also be used in combination with aspirin (dual

antiplatelet regimens)<sup>36</sup> or with aspirin and clopidogrel (triple antiplatelet therapy) to treat these patients.<sup>37</sup>

## CONCLUSION

Dual anti platelet resistance as defined by inhibition of platelet aggregation measured with Whole Blood aggregometry, is a common problem. Gender and age has no significant affect on platelet aggregability.

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