World Heart Journal
Volume 8, Number 1

ISSN: 1556-4002

Therapeutic Effectiveness of Clopidogrel-Induced Platelets Inhibition: An Inter-Individual Response Variability among Iraqi Patients

Hussein Ali Sahib[1,*], Bassim Irheim Mohammad[1], and Ban A. Abdul-Majid[2]

[1]College of Pharmacy, University of Al-Qadisiyah, Diwaniyah, Iraq

[2]College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background: Despite the unmistakable beneficial effect of clopidogrel on platelet aggregation, some patients still respond poorly to the drug, leading to early and adverse cardiovascular clinical events.

Patients and methods: One hundred and twenty-seven patients with cardiovascular diseases (ACS, stroke, or TIA) were enrolled as a study group. Patients were recruited at the coronary care unit (CCU) of Al-Yarmouk Teaching Hospital. Platelet assessment was performed by using light transmission aggregometry.

Results: The mean value of platelet aggregation was 61.7±17.3; the median value was 64 and the range was 10-96. The range shows a considerable amount of variability. Types of variability can be referred to as either skewness or kurtosis. Skewness is a measure of the symmetry of a distribution. The data in Table 1 show that skewness was negative (-0.450), suggesting a deviation from the normal distribution. Kurtosis, a measure of the peakedness or flatness of a distribution, was also negative (-0.130), suggesting a flat peak. Twenty four percent of the patients enrolled in this study were hypo-responders.

Conclusions: Among the patients enrolled in this study, there was significant ($p<0.05$) inter-individual variability with negative skewness and kurtosis (-0.450, -0.130, respectively).

Keywords: thrombocyte, aggregation, variations, platelet dysfunction.

Introduction

Clopidogrel belongs to the thienopyridine family, along with prasugrel and ticlopidine, and is a potent antiplatelet agent [1]. Its absorption in the gut is mediated by a ABCB1/MDR1 protein transporter. Neither food nor antacid affect its absorption [2]. Clopidogrel is an inactive pro-drug which, to become an active metabolite, should be oxidized by the

* Corresponding author: Hussein Ali Sahib. College of Pharmacy, University of Al-Qadisiyah, Diwaniyah, Iraq. Email: hu.za815@gmail.com, mobile 009647801525905.

hepatic cytochrome P450 system involving CYP2C19 [3]. Two consecutive cytochrome P450-dependent oxidative steps are necessary to convert clopidogrel to its active form. The first step leads to the generation of 2-oxo-clopidogrel. After that, the 2-oxo-clopidogrel metabolite is further broken down to clopidogrel (active form), representing about 15% of the clopidogrel metabolism. The remaining 85% of administered clopidogrel is metabolized into an inactive metabolite in the blood by esterases and therefore does not participate in the pharmacologic effect of clopidogrel [4]. Once activated, clopidogrel exerts its effect on platelets by irreversibly blocking a specific ADP platelet receptor (P2RY12), preventing binding of ADP to its target and consequently preventing activation of fibrinogen receptor GPIIbIIIa so that platelet aggregation could be inhibited [5]. Clopidogrel has been used to avoid thrombotic events with different manifestations including peripheral vascular disease (PVD), cerebrovascular disease, and coronary artery disease [6].

Patients with unstable angina, stroke, and transient ischemic attack or myocardial infarction, treated medically or with percutaneous intervention, are all candidates for therapy with clopidogrel. Despite the unambiguous clinical benefit achieved from the adjunct of clopidogrel in ACS/PCI patients, a considerable number of patients continue to have cardiovascular events. This has been so in part because some patients respond poorly to platelet aggregation following the administration of clopidogrel. These patients, despite clopidogrel treatment, persist in enhanced platelet reactivity (high on treatment platelet reactivity HTPR), which plays a pivotal role in the development of atherothrombotic complications [7]. During the first year after ACS, about 8% to 10% of patients experienced recurrent cardiovascular events (CVs) [8, 9]. In addition, another 1% to 3% of patients undergoing PCI had a subacute stent thrombosis after PCI with probability of catastrophic consequences, involving a high risk of early mortality [10]. Thus, the aim of this study was to evaluate the inter-individual variability to clopidogrel response among Iraqi patients treated with clopidogrel employing 75mg maintenance dose or 300 mg loading dose. In addition, this study determined the percentage of clopidogrel hypo-responsiveness in a sample of Iraqi patients.

Patients and Methods

This cross sectional study was conducted from 15 February 2014 to 1 April 2015 at the Department of Pharmacology, College of Medicine, Al Nahrain University, Iraq. The study enrolled 127 patients with cardiovascular diseases (ACS, stroke, or TIA). Patients were recruited from the coronary care unit (CCU) of Al-Yarmouk Teaching Hospital. Inclusion criteria involve all patients treated with clopidogrel employing 75 mg for at least 7 days or have received a loading dose of 300 mg clopidogrel.

Their questionnaire included details of demographics (age and sex), risk factors, hypertension, diabetes mellitus and smoking, family history and recurrence (more than one admission to CCU). Exclusion criteria included platelet count $\leq 75 \times 10^9$ L^{-1}. Patients who were treated with omeprazole or lansoprazol at the time of blood sampling were also excluded. Ethical approval of this study was obtained from the Research and Ethics Committee of Medical College / Al-Nahrain University. All patients included in this study were informed about the test to be carried out in this study.

Sample Collections

Each patient donated 5 ml of venous blood using a disposable 10 ml syringe with a 22 G needle. Greatest care was taken during and after sample collection to avoid unnecessary trauma and agitation to the sample. Where a suitable large-diameter vein could be fixed, blood sample was obtained by venipuncture. After discarding 0.5 ml of the sample, a volume of 4.5 ml was transferred to sample tubes that contained 0.5 ml sodium citrate (1/10 volume sodium citrate). After sample addition, tubes were mixed by gently inverting 5 times and immediately transferred to the laboratory for platelet aggregation assay.

Platelet Function Assays

Whole blood aggregation was determined using light transmission aggregometry (LTA). LTA detect the light transmission across a sample of platelet-rich plasma (PRP). PRP could be defined as turbid

suspension of cells that significantly interfered with light transmission. Addition of a platelet agonist led to the formation of platelet aggregates. This aggregation reduced the turbidity of the suspension, thereby increasing the light transmission across suspension l (100% light transmission is set with platelet-poor plasma) [11]. The dynamics of platelet aggregation (expressed as %) were therefore measured in real time as platelets aggregate. This study employed ADP as agonist. Both PRP and PPP were prepared by using a PDQ^{Tm} Platelet Function Centrifuge. A volume of 0.225 mL of the platelet-rich plasma sample was stirred at 37°C for 3 minutes in the test cuvettes. The 100% baseline was set by placing the blank into the test well. The cuvette of the PRP sample was placed into the test well. A volume of 0.025 mL ADP reagent was added directly into the platelet-rich plasma sample. The LTA was then measured as the percentage of light passing through PRP.

Definition of Clopidogrel Response

Platelet reaction to clopidogrel was considered as hypo-response if post-treatment platelet aggregation was >70% [12].

Results

The mean age of patients enrolled in the present study was 56.87±11.6 years and the range was 24-80 years. This study included 80 males and 47 females.

Table 1 shows the mean value of platelet aggregation (61.7±17.3) and median values (64). The range was 10-96. The range shows a considerable amount of variability. Types of variability can be referred to as either skewness or kurtosis. Skewness is a measure of the symmetry of a distribution. The data in Table 1 shows that skewness was negative (-0.450), suggesting a deviation from the normal distribution. Kurtosis, a measure of the peakedness or flatness of a distribution, was also negative (-0.130), suggesting a flat peak. Patients' distribution is shown in Figure 1 for comparison.

Table 1. Descriptive statistics of platelet aggregation

Group	N	Median	Mean	SD	Minimum	Maximum	Skewness	Kurtosis
Patients	127	64.00	61.70	17.30	10.00	96.00	-0.450	-0.130

Table 2. Test of normality

Test	Statistic	df	P-value	Interpretation
Shapiro-Wilk	0.980	127	0.053	Normal distribution

The test for normality showed that the patients' distribution according to platelet aggregation was normal. There was no significant difference from a normal distribution (P > 0.05), as shown in Figure 1 and Table 2.

Classification of Patients into Two Groups (Responsive and Hypo-Responsive) According to Aggregation Cutoff of 70%

Figure 2 shows the distribution of patients according to their response to clopidogrel, measured by platelet aggregation, in which patients with >70% aggregation were regarded as hypo-responders and the remaining patients were regarded as responders. This figure shows that 24% of patients were hypo-responders. There was no significant relationship between patient characteristic and responsiveness to clopidogrel. Risk factors for ACS also did not significantly affect the clopidogrel inhibition effect on platelet aggregation.

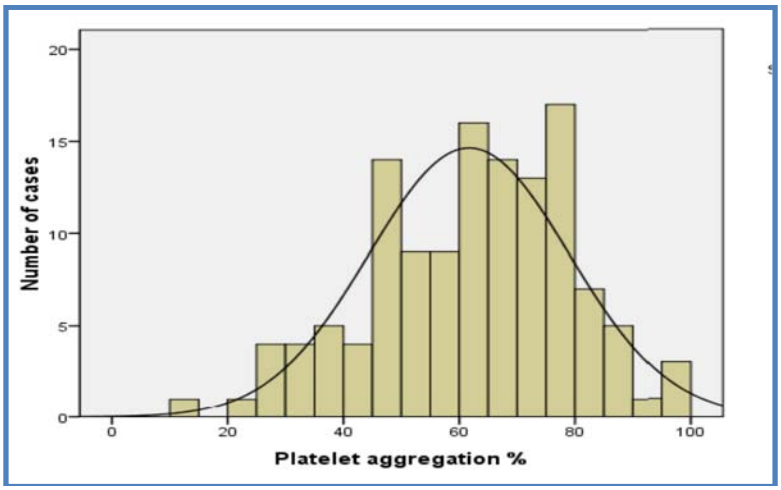

Figure 1. Distribution of patients according to platelet aggregation.

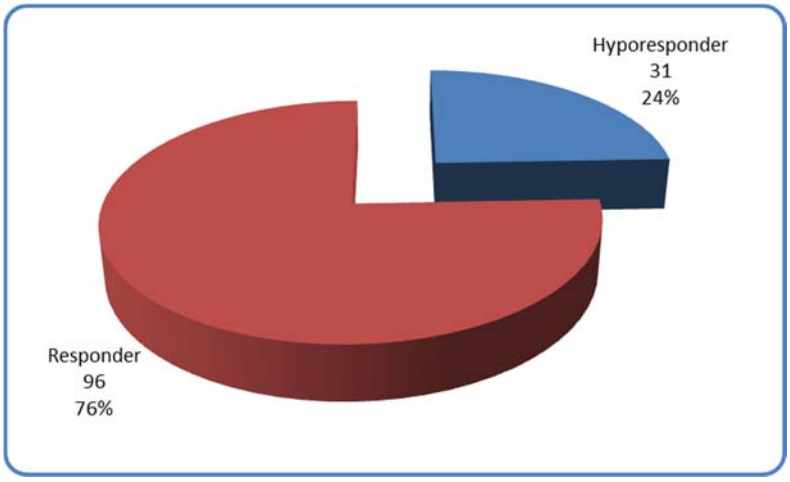

Figure 2. Pie chart representing the frequency distribution of responders and hypo-responders to clopidogrel according to platelet aggregation measurement.

Table 3. General characteristics of all patients (responders and hypo-responders)

Characteristic		Responder	Hypo-responder	P Value
Gender	Male	63 (78.75)	17 (21.25)	0.280
	Female	33 (70.21)	14 (29.79)	
Smoking	Smoker	44 (81.48)	10 (18.52)	0.184
	Nonsmoker	52 (71.23)	21 (28.77)	
Hypertension	Hypertensive	54 (76.06)	17 (23.94)	0.891
	Nonhypertensive	42 (75.00)	14 (25.00)	
Diabetes mellitus	Diabetic	35 (74.47)	12 (25.53)	0.821
	Nondiabetic	61 (76.25)	19 (23.75)	
Recurrence of ACS	Have recurrent ACS	21 (72.41)	8 (27.59)	0.650
	Do nothave recurrent ACS	75 (76.53)	23 (23.74)	
Family history of CHD	Negative family history of CHD	71 (75.53)	23 (24.47)	0.979
	Positive family history of CHD	25 (75.76)	8 (24.24)	

Discussion

By using skewness and kurtosis tests, the present results have demonstrated that there is inter-individual variability in the response to clopidogrel employing either a 75 mg maintenance dose or a 300 mg loading dose. Despite adequate antiplatelet treatment, up to 4.7% of the patients undergoing coronary stent placement develop thrombotic stent occlusion, suggesting inadequate platelet inhibition due to clopidogrel resistance [13]. The results from this study have shown that 24% of patients involved in this study were hypo-responders.

The variability in individual responsiveness to clopidogrel has been recognized by almost all those who have tested clopidogrel efficacy by ADP-stimulated aggregometry [13]. Hence, clopidogrel resistance has recently been described [13, 14]. Published data show that the incidence of clopidogrel hypo-responders is different from one study to another. Müller et al. [13] found that 26% of patients were hypo-responders to clopedogril. By using 5 μmol/l of adenosine diphosphate Serebruany et al. [15] show that the prevalence of hypo-responders to clopidogrel was 4.2%. Matetzky et al. found that up to 25% of STEMI patients undergoing primary PCI with stent placement are hypo-responders to clopidogrel; these patients may be at an increased risk for recurrent cardiovascular events [16]. Hyunjung et al. revealed that the prevalence of clopidogrel non-responsiveness in patients with CAD or ischemic cerebrovascular disease account for about 38.8% [17]. Another study revealed 44% clopidogrel hypo-responders [14]. Thus, our definition of hypo-responsiveness to clopidogrel corresponds well with the published rliterature. This wide range may be related to the laboratory methods used (different methods or different concentrations of agonists) or it may occur as a result of different definitions of resistance or reduced response to clopidogrel [18, 19].

In brief, the results from this study have clearly demonstrated that clopidogrel effectiveness may be highly variable among Iraqi patients. The effect of CYP 2C19 polymorphism on clopidogrel effectiveness must be studied. Further studies are necessary to verify this fact.

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