

Clopidogrel Resistance in Stroke Cases

Inme Olgularında Klopidoğrel Rezistansı

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ABSTRACT

Introduction: Clopidogrel treatment is one of the standard treatments in terms of reducing mortality and morbidity in patients with cerebrovascular disease diagnosed with large artery atherosclerosis. However, resistance to clopidogrel treatment is a significant problem today. In this study, we aimed to retrospectively investigate clopidogrel resistance (CR) and related factors in patients with detected large artery atherosclerosis who were evaluated for cerebrovascular disease.

Methods: A total of 96 patients, including 31 females and 65 males, were evaluated in the neurology and neuroradiology clinics with the diagnosis of cerebrovascular disease. Age, gender, presence of CR, and complete blood count values [platelet count (PLT), plateletcrit (PCT), mean platelet volume, white blood cells, platelet distribution width] were evaluated. Impedance Aggregometry was used to evaluate CR in the study. The results were given as the area under the curve. An adenosine diphosphate value higher than 46 U was taken as a resistance indicator. The relationship between blood tests and CR was investigated.

Results: CR was detected in 33.3% (n=32) of 96 patients. PLT (295.7±12.4) and PCT values (0.3±0.01) were significantly higher in patients with resistance than those without resistance (p<0.005).

Conclusion: This study shows that high PLT and PCT values can be used to predict CR.

Keywords: Cerebrovascular diseases, clopidogrel, resistance, platelet count, plateletcrit

ÖZ

Amaç: Beyin damar hastalığı tanısı ile değerlendirilen ve büyük arter aterosklerozu saptanan hastalarda mortalite ve morbititenin azaltılması yönünden klopidoğrel tedavisi standart tedaviler içinde yer almaktadır. Bununla beraber klopidoğrel tedavisine karşı gelişen rezistans günümüzde önemli bir sorundur. Bu çalışmada, beyin damar hastalığı nedeni ile değerlendirilen ve büyük arter aterosklerozu saptanan hastalarda klopidoğrel rezistansının (KR) incelenmesi ve ilişkili faktörlerin retrospektif olarak gözden geçirilmesi amaçlandı.

Yöntemler: Çalışmaya nöroloji ve nöroradyoloji kliniklerinde beyin damar hastalığı tanısı ile değerlendirilen KR'nin incelendiği, 31 kadın ve 65 erkek olmak üzere toplam 96 hasta dahil edildi. Tüm hastaların yaş, cinsiyet, KR varlığı, hemogram değerleri [platelet sayısı (PLT), plateletkrit (PCT), ortalama trombosit hacmi, beyaz kan hücreleri, trombosit dağılım genişliği] retrospektif olarak değerlendirildi. Çalışmada KR'nin değerlendirilmesinde impedans agregometri kullanıldı. Sonuçlar eğri altında kalan alan cinsinden verildi. Adenozin difosfat değerinin 46 U değerinden yüksek olması rezistans göstergesi olarak alındı. KR varlığı ile incelenen tüm parametreler arasındaki ilişki değerlendirildi.

Bulgular: Toplamda 96 hastanın %33,3'ünde (n=32) KR saptandı. Rezistans gösteren hastaların PLT (295,7±12,4) ve PCT değerleri (0,3±0,01) rezistans göstermeyen hastalara göre anlamlı oranda daha yüksek bulundu (p<0,005).

Sonuç: Bu çalışma yüksek PLT ve PCT değerlerinin KR'yi öngörmeye kullanılabilecek parametreler olduğunu göstermektedir.

Anahtar Kelimeler: Serebrovasküler hastalıklar, klopidoğrel, rezistans, platelet sayısı, plateletkrit

Introduction

Platelets (PLTs) play an essential role in the development of ischemic stroke in terms of pathophysiology of thrombosis formation (1). Clopidogrel, an antithrombotic agent that inhibits PLT activation

via adenosine diphosphate (ADP), has proven efficacy and safety in preventing recurrent ischemic strokes (1). Clopidogrel is a second-generation thienopyridine derivative prodrug and is converted to its active metabolite by cytochrome P450 enzyme system (mainly CYP2C19)



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in the liver. It shows its antithrombotic effect by irreversibly inhibiting the ADP-P2Y₁₂ receptor located in the PLT membrane (2-4). Clopidogrel given at the standard dose does not show a complete P2Y₁₂ antagonism; in other words, it inhibits ADP-mediated PLT aggregation by 50% (5). Therefore, some patients receiving clopidogrel therapy do not have an adequate therapeutic response to the drug, and this phenomenon is also described as clopidogrel resistance (CR). This phenomenon, also known as the absence of ADP-mediated PLT inhibition with the use of therapeutic doses of clopidogrel, can be attributed to individual variability in PLT response to clopidogrel treatment and may be associated with recurrent thrombotic events or poor prognosis incidence due to treatment insufficiency (6).

The frequency of patients who do not respond to clopidogrel treatment varies between 4-30%, depending on the clinical use indications, the dose of the drug, the time of treatment initiation, and the test method to evaluate PLT functions (5,7). Currently, CR is characterized by PLT function (a measurement of the degree of PLT aggregation induced by *in vitro* ADP) and genetic polymorphism analysis (5,6). Evaluation of PLT function in cardiovascular and cerebrovascular diseases is important for predicting clinical outcomes and prognosis and determining the efficacy of antithrombotic therapy (2). In these studies, basal PLT parameters such as high PLT and plateletcrit (PCT), mean PLT volume (MPV), PLT distribution width (PDW), have been tried to correlate with CR (8,9), but the results are still controversial (10,11).

This study aimed to evaluate CR in patients diagnosed with large artery atherosclerosis during evaluation for stroke and to determine the routine biochemical parameters before clopidogrel treatment, thus evaluating whether biochemical markers could predict early PLT response and future clinical outcomes that might be associated with resistance.

Methods

Patients who were diagnosed clinically and radiologically with magnetic resonance imaging with cerebrovascular disease at İstanbul Training and Research Hospital, Clinic of Neurology and Neuroradiology between 29.09.2015 and 30.11.2017, and who were also diagnosed with large artery atherosclerosis or stenosis by brain computed tomography (CT) or MR angiography examinations during further evaluation were included in the study. A total of 96 patients (31 women and 65 men) with CR were evaluated retrospectively. The study was approved by the İstanbul Training and Research Hospital Ethics Committee of clinical trials (decision no: 1687). As the study included retrospective file scanning, informed consent was not obtained from the patients.

The patients were divided into two groups as patients with CR (CR+) and without CR (CR-). Age, gender, complete blood count parameters [PLT count, PCT, MPV, white blood cell count, PDW], and prothrombin time (PT-INR) were recorded retrospectively from patient files.

Patients with severe anemia, active hemorrhage, bone marrow disease, heparin-induced thrombocytopenia, blood transfusion, anticoagulant medication other than clopidogrel, or drug use that would affect PLT count were not included in the study.

Evaluation of Clopidogrel Resistance

Impedance aggregometry (Multiplate Analyzer, Dynabyte, Munich, Germany) was used to evaluate CR. For the test, blood was collected in 4 cc tubes containing hirudin and incubated for 30 minutes at room temperature. Blood was diluted 1:2 with 0.9% NaCl and stirred at 37 °C for 3 minutes. Then, 20 µl of ADP (6.4 Mmol) was added. Resistance changes caused by PLTs, which were aggregated and adhered to the electrodes in the test cell, were recorded with two pairs of electrodes. Increased resistance by PLTs adhering to the electrodes was converted to the aggregation unit (AU) by the device, and the time-aggregation graph was plotted. The area under the aggregation line Area Under the Curve, the parameter that best reflects platelet activity, was calculated. An ADP value of >46 U indicates no suppression, a value between 19-46 U indicates adequate suppression, and <19 indicates over suppression.

In our study, there was no control group to determine the reference range of the PLT function test. Test results may vary between races, with each center determining its own reference range will give more accurate results.

Statistical Analysis

The data of our study were expressed as mean ± standard error of the mean. The difference between CR+ and CR- patients was evaluated by the Mann-Whitney U test. A chi-square test was used to compare qualitative data. P<0.05 was considered statistically significant.

Results

Impedance Aggregometry Results: Clopidogrel Resistance

CR was detected in 32 (33.3%) of 96 patients (31 women and 65 men) diagnosed with large artery atherosclerosis according to the impedance aggregometry results. The mean age of CR+ patients was 70.4±1.5 years, and the mean age of CR- patients was 68.9±1.4 years. There was no difference between the two groups in terms of mean age (p>0.05). Fifty-nine percent of CR+ patients were male, and 41% were female, and 72% of CR- patients were male, and 28% were female. There was no significant relationship between CR and gender (p=0.25) (Table 1). When CR+ patients (n=32) were evaluated for possible co-morbidities, ten patients (31.25%) were found to have both hypertension and diabetes, eight patients (25%) had only hypertension, four patients (12.5%) had only diabetes, and ten patients had no chronic disease.

Biochemical Parameters

Routine complete blood count parameters and PT-INR values requested from the patients were compared between CR+ and CR- patients and the values of both groups are summarized in Table 1. The mean ADP value of CR+ patients was 67.5±4.4 U and thrombin receptor activating peptide (TRAP) value was 116.6±4, whereas the mean ADP value of CR- patients was 23.5±3.5 U and TRAP value was 88.7±3.5 (Table 1). As these two parameters are indicators supporting the accuracy of the Impedance Aggregometry test, a significant difference was found between the two groups as expected (p<0.05).

The mean PLT value of CR+ patients was 295.7±12.4, and the mean PLT value of CR- patients was 259.0±8.8 (Figure 1A). The mean PCT value

of CR+ patients was 0.3 ± 0.01 , and the mean PCT value of CR- patients was 0.27 ± 0.01 (Figure 1B). Platelet and PCT values were higher in CR+ patients, and a statistically significant difference was found between the two groups ($p=0.02$ and $p=0.0015$) (Figure 1, Table 1).

Discussion

At present, antiplatelet drugs are proven treatment of atherothrombotic stroke in the prevention of secondary stroke. In the last 30 years, studies have shown that aspirin, ticlopidine, clopidogrel, and dipyridamole are effective in preventing recurrent stroke in patients with atherothrombotic stroke (12,13). Studies have shown that approximately 10-20% of patients undergoing antiplatelet therapy have a recurrent stroke (14). Repeated thrombotic event despite antiplatelet therapy raises antiplatelet drug resistance. Other commonly used definitions of this resistance are insufficiency of antiplatelet therapy, non-responsiveness, or inadequate efficacy.

In our study, 33.3% of 96 cases had CR. Studies on CR have reported different rates. In the study of Notarangelo et al. (15), this rate was stated as 30% and in the study by Wang et al. (7) as 11%. In general, CR varies between 4-30% depending on the clinical indications, starting dose, maintenance dose, time to start treatment, and PLT function test methods (16).

Studies have reported higher CR in women (17,18). However, although the rate of female patients in the CR+ group was higher than the rate of female patients in the CR- group, no significant relationship was found between CR and gender (Table 1). When the mean age was examined,

no significant difference was found between the CR+ and CR- groups in support of previous studies (17,18).

The main finding of our study was that the PLT and PCT values of the patients with CR were significantly higher than those without resistance. In studies conducted to date, baseline PLT values, which can easily be obtained from routine laboratory tests, are shown as simple and useful markers that can be used to predict CR (6). Our findings suggest that PLT count, and PCT, which is a measure of total platelet mass, can be used as parameters to predict CR.

Thrombus formation and PLT activation play an important role in the pathogenesis of ischemic cerebrovascular diseases (19,20). Estimates of PLT volume and count give information about PLT function and activation (20). PLT is a parameter reflecting PLT production, function, and aging (8), and high PLT values are noteworthy in patients showing CR in support of our study (6). Therefore, patients with elevated PLT levels before treatment may be prone to thrombosis.

It is assumed that PCT shows PLT count circulating in a unit of blood, similar to the hematocrit value for erythrocytes (21). In our study, PCT values were significantly higher in patients with CR. It has been proposed that high PCT values can be used as a useful parameter in predicting coronary slow-flow phenomenon associated with cardiovascular pathologies such as recurrent angina pectoris, acute myocardial infarction, and hypertension, and may be considered as a marker for more aggressive antiplatelet therapy (22). In support of this hypothesis, the findings of our study suggest that PCT value can be used as another parameter that can be evaluated together with the PLT count in predicting CR. However, larger scale and comprehensive studies are needed to establish the relationship between CR and high PCT values.

MPV is another commonly used parameter to evaluate PLT size and function (8). In the studies performed, MPV values were found to be

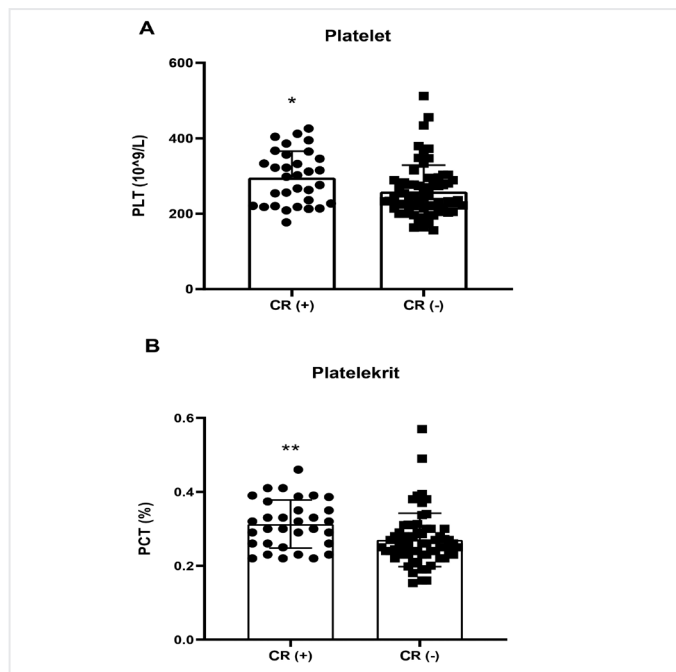


Figure 1. Platelet A) and plateletcrit B) values in patients with (CR+) and without (CR-) clopidogrel resistance. Data were expressed as mean \pm standard error of the mean, and the comparison was made with the Mann-Whitney U test. * $P < 0.05$. Individual data of CR+ and CR- patients were placed in round and square bar graphs, respectively

PLT: platelet, PCT: plateletcrit, CR: clopidogrel resistance

Table 1. Clinical and laboratory features of CR+ and CR- patients

Parametre	CR+ (n=32)	CR- (n=64)	p
Age	70.4 \pm 1.5	68.9 \pm 1.4	NS
Female, n (%)	12 (41)	18 (28)	NS
MPV (fL)	10.7 \pm 0.2	10.4 \pm 0.1	NS
PLT (10 ⁹ /L)	295.7 \pm 12.4*	259.0 \pm 8.8	0.01
PCT (%)	0.3 \pm 0.01**	0.27 \pm 0.01	0.0015
PDW (%)	13.2 \pm 0.5	14.4 \pm 1.8	NS
HB (g/dL)	12.9 \pm 0.3	13.4 \pm 0.2	NS
WBC (10 ⁹ /L)	8.8 \pm 0.4	8.2 \pm 0.3	NS
RBC (10 ¹² /L)	4.5 \pm 0.1	4.7 \pm 0.07	NS
HCT (%)	39.4 \pm 0.8	40.7 \pm 0.6	NS
INR	0.95 \pm 0.02	1.02 \pm 0.04	NS
ADP (U)	67.5 \pm 4.4***	23.5 \pm 1.3	<0.001
TRAP (U)	116.6 \pm 4***	88.7 \pm 3.5	<0.001

Quantitative data were expressed as mean \pm SEM, and the comparison was made with the Mann-Whitney U test. * $p < 0.05$. Qualitative data (age) were given as n (%) and evaluated by the chi-square test

CR: clopidogrel resistance, MPV: mean platelet volume, PLT: platelet count, PCT: plateletcrit, PDW: platelet distribution width, HB: hemoglobin, WBC: white blood cells, RBC: red blood cells, HCT: hematocrit, INR: international normalized ratio, ADP: adenosine diphosphate, TRAP: thrombin receptor activating peptide

significantly higher in patients with CR compared to patients responding to treatment (23,24). In a prospective study by Li et al. (25), PLT and MPV values were found to be significantly higher in the clopidogrel-resistant group in 152 coronary artery patients (CAD) treated with clopidogrel. It was observed that CR+ patients had more cardiovascular events in the 53-month follow-up period, and it was concluded that the increase in PLT and MPV was a risk factor for the development of CR in CAD (25). In our study, no statistically significant difference was found between CR+ and CR- patients in terms of MPV, whereas the PLT value was significantly higher in the CR+ group.

Study Limitations

The limitations of our study include the small number of patients and lack of evaluation of several related factors (especially CYP2C19 enzyme activity and genetic polymorphism, inflammation-related biochemical parameters such as C-reactive protein) due to the retrospective nature of our study.

Conclusion

We found a significant relationship between CR and PLT and PCT values in our study. This finding suggests that PLT and PCT values can be considered as parameters that can be used to predict CR. Considering that CR examination is costly and not performed in many centers, it may be appropriate to evaluate these findings with more large-scale prospective studies.

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Ethics Committee Approval: The study was approved by the Istanbul Training and Research Hospital Ethics Committee of clinical trials (decision no: 1687).

Informed Consent: As the study included retrospective file scanning, informed consent was not obtained from the patients.

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