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SHORT REPORT

The Incidence and Methods for Detecting Aspirin Resistance in Pediatric Patients

Hansamon Poparn[1,2], Yaowaree Kittikalayawong[1,2], Piti Techavichit[1,2], Supanun Lauhasurayotin[1,2], Kanhatai Chiengthong[1,2], Phumin Chaweephisal[2], Darintr Sosothikul[1,2]

[1]Division of Pediatric Hematology and Oncology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, 10330, Thailand; [2]Integrative and Innovative Hematology/Oncology Research Unit, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, 10330, Thailand

Correspondence: Darintr Sosothikul, Division of Pediatric Hematology and Oncology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, 10330, Thailand, Tel/Fax +66 2-256-4949, Email dsosothikul@hotmail.com; Darintr.S@chula.ac.th

Abstract: Since aspirin resistance is rarely assessed in pediatric patients and thrombosis might cause damage in vital organs, such as the myocardium or brain, we aimed to determine its incidence and the pivotal role of routine screening. The point-of-care test by platelet function analyzer (PFA-200) and bedside bleeding time (BT) was compared to standard whole blood impedance aggregometry (IA), the time-consuming and sophisticated assays. This single-center cross-sectional study was investigated in Thai children (≤15 years). All participants received at least five-day administrations of aspirin (3 to 5 mg/kg/day or equivalent to a single tablet of 81 mg) for any prior thrombotic risks. Platelet aggregation >5 ohms on IA with 0.5 mM arachidonic acid, closure time <180 seconds on collagen/epinephrine PFA-200, and modified Ivy BT ≤7 minutes, defined resistance. Of 37 patients, 2.7% had confirmed aspirin resistance to IA. Despite the 100% sensitivity, PFA-200 showed higher specificity than BT (83.3% vs 36.1%). However, both were not comparable (exact McNemar $P < 0.05$), with a slight/fair reliability (κ=0.215 vs κ=0.030 respectively). Aspirin resistance is uncommon in Thai children. Routine screening is discouraged but recommended only in cases with recurrent thrombosis despite good aspirin compliance or the presence of resistant risk factors. Although the gold standard IA could not be replaced, the rapid assay of PFA-200, not bedside BT, can potentially be considered a point-of-care alternative screening test to detect aspirin resistance in children.

Keywords: aspirin, pediatrics, platelet function tests

Introduction

Acetylsalicylic acid, or aspirin, is one of the antiplatelet prototypes, generally used for the primary prevention of arterial thrombus formation in pediatric patients with a high risk for thrombosis, including congenital heart disease with shunt, post-splenectomy thalassemia, and Kawasaki disease, and for the secondary prevention of arterial thrombosis including non-cardioembolic ischemic stroke.[1] Based upon its mechanism of action in irreversible inhibition of cyclooxygenase (COX)-1 in platelets and megakaryocytes, aspirin can reduce prostaglandin H_2 and subsequently downstream thromboxane A_2 (TXA_2) production. The inhibition of TXA_2 amplification ultimately impairs G protein-mediated signaling effects on platelet shape change, degranulation, and fibrinogen/von Willebrand factor (VWF)-mediated aggregation.[2]

Aspirin resistance, or aspirin unresponsiveness, is defined by both clinically recurrent thrombosis and evidence of suboptimal platelet inhibition on platelet aggregation test, despite good compliance with aspirin.[3,4] A previous study of pediatric patients undergoing cardiac surgery showed that 60% of patients with aspirin resistance identified by VerifyNow® platelet aggregation system (Accumetrics Inc., San Diego, CA) developed clinical thrombosis, compared to the lower incidence of only 1.2% in patients with adequate platelet inhibition.[5] Especially in infants aged 2 to 4 weeks or with low body weight (<5 kg) who used aspirin <81 mg/day, aspirin resistance was more common (incidence 57.1% vs 5.3%). Generally, the etiology of this condition can be categorized into two groups, COX-1 related or COX-1 unrelated. COX-1-related causes include coadministration of nonsteroidal anti-inflammatory drugs competing for binding with

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COX-1, reduced absorption by concomitant use of a proton pump inhibitor, increased platelet turnover associated with stress conditions, COX-1 polymorphisms, and non-compliance. While COX-1 unrelated causes include upregulation of the COX-2 pathway, increased platelet activation by other coexisting conditions, and genetic polymorphisms of other proteins/enzymes in the platelet activation cascade.[4] Apart from studies among pediatric patients undergoing cardiac surgery,[5,6] another study in patients with splenectomized thalassemia reported the incidence of approximately 30% resistance while taking 2 mg/kg/day aspirin.[7] The adult data who take aspirin for coronary artery disease show a similar result of 2.4% aspirin resistance by light transmission aggregometry (LTA)[8] but higher in recurrent ischemic cerebrovascular disease as high as 20%.[9] Although the studies in adult patients were variable, ranging between 5% and 75.3%.[10,11]

The platelet function test determined by optical light transmission aggregometry (LTA) or whole blood (WB) impedance aggregometry (IA) is considered a gold standard.[12,13] Aspirin alters the effect of arachidonic acid (0.5 to 1.6 mM) but also other platelet agonists, such as epinephrine (10 to 20 μg/mL) and collagen (1 to 25 μg/mL).[14] The concept of LTA methodology is to detect light transmission through stirred platelet-rich plasma, with 100% optical density at the resting stage but reduced by degrees of platelet aggregation. On the contrary, the IA method detects platelet aggregation based upon changes in electrical impedance between two metal electrodes immersed in WB after adding the agonists.[12,13]

Since LTA and IA are time-consuming and sophisticated assays, a platelet function analyzer (PFA-200) has been developed to simply and rapidly assess platelet function.[15] Briefly, the WB sample was added into cartridges containing collagen/epinephrine (CEPI) and collagen/ADP (CADP)-coated membranes for activating platelets, leading to an occlusion of membrane aperture. The closure time of >300 seconds specified for CEPI but not for CADP indicates aspirin-like defects.[15] Compared to conventional bedside bleeding time (BT) using the modified Ivy method to assess primary hemostasis defect,[16] PA-200 provides better diagnostic performance (81.3% sensitivity and 95.9% specificity) for von Willebrand disease and platelet dysfunctions, including Glanzmann's thrombasthenia, and aspirin-like defects, in children.[17] The detection challenge and future implication emphasize that, is routine screening essential for all aspirin-taking patients? Because thrombosis causes high morbidity and mortality, the standard IA is complex and rarely available in the general setting. Therefore, based upon the aforementioned reasons, this study is conducted to determine the incidence of aspirin resistance in Thai pediatric patients with a high risk of thrombosis using the gold standard IA and to compare PFA-200 and conventional bedside BT with the gold standard assay in evaluating this condition.

Materials and Methods

Subjects and Study Design

In this single-center cross-sectional study, patients aged ≤15 years receiving aspirin were recruited between October 2018 and February 2019. Aspirin was administered orally in antiplatelet doses (3 to 5 mg/kg/day or equivalent to a single tablet of 81 mg) in order to prevent arterial thrombosis in the presence of any conditions with increased thrombotic risk. Exclusion criteria included: (1) coadministration with any other antiplatelets, (2) inability to collect blood samples, (3) thrombocytopenia (platelet count <150 $x10^9$/L) or thrombocytosis (platelet count >500 $x10^9$/L), and (4) having other known causes of platelet dysfunction.

The study protocol was ethically approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University (IRB no. 781/61). The study complied with the Declaration of Helsinki. Informed consent was obtained from each patient and their parents/guardians before participation in the study.

Assessment of Aspirin Resistance

The platelet function was assessed using three different WB-based methods: (1) IA (Chrono-log® 700 lumi-aggregometer; Chrono-log Corporation, Havertown, PA) considered as the gold standard tool, (2) PFA-200 (Siemens Healthcare Diagnostics, Marburg, Germany), and (3) bedside BT by the modified Ivy method.

Peripheral blood samples were collected from participants once after receiving aspirin for at least 5 days. A 4-mL WB sample was drawn by direct venipuncture into 3.2% sodium citrate tubes. Citrated WB was loaded into a prewarmed

cuvette with a stir bar for IA (450 µL of WB diluted with physiological saline) and PFA-200 cartridges (800 µL of WB for both CEPI and CADP cartridges) upon arrival at the laboratory. For IA, arachidonic acid (0.5 mM) was added during the assay.

Bedside BT was performed by the professional laboratory scientist (Y.K.) using the modified Ivy procedure after WB sampling. As previously described,[16] forearm skin was incised with a lancet at 1-mm depth and 10-mm length during inflating of a blood pressure cuff to 40 mmHg in the ipsilateral arm. Bedside BT was measured by blotting blood oozing twice a minute until the bleeding completely stopped.

Based upon all three methods of assessment, aspirin resistance was defined by (1) normal response to arachidonic acid on IA (aggregation >5 ohms), (2) normal closure time in CEPI cartridge (closure time < 180 seconds), or (3) normal BT ≤ 7 minutes.

Statistical Analysis

All analyses with descriptive statistics were performed using GraphPad Prism v10.2.1 (GraphPad Software Inc., Boston, MA) and SPSS v29.0 (SPSS Inc., Chicago, IL). The exact McNemar significance probability test was used to assess diagnostic performance between paired data of two different tests. Cohen's kappa analysis was used to assess the reliability of PFA-200 and bedside BT compared with IA results. Fisher's exact test was performed by comparing alternative methods to IA.

Results

Within the study period, 37 pediatric patients were enrolled. Two were excluded due to age > 15 years old. The median age was 8.5 years (range, 1 to 15), and 73.0% (n = 27) were over 6 years old (Supplementary Tables 1 and 2). Most of the patients were male (54%; n = 20), predominantly with congenital cyanotic heart disease with prosthetic materials (62.2%; n = 23). The other conditions for the use of aspirin were Moyamoya disease (13.5%; n = 5), ischemic stroke due to other causes (16.2%; n = 6), post-splenectomy thalassemia (5.4%; n = 2), and renal artery stenosis implanted with arterial stent (2.7%; n = 1). The median aspirin dose was 3.9 mg/kg/day (interquartile range; 3.4 to 4.3) demonstrated separately (Supplementary Table 2).

The incidence of aspirin resistance measured by the gold standard IA was only 2.7% (n = 1) (Table 1). No clinical thrombosis was observed during the study period. In detail, the patient with aspirin resistance was a middle childhood girl with β-thalassemia/hemoglobin E disease post-splenectomy. Despite good compliance with aspirin (3.2 mg/kg/day), she also received other concomitant medications, including phenytoin, hydrocortisone, multivitamin, folic acid, omeprazole, and required red cell transfusion every 4 weeks. The aspirin-like effects were not demonstrated on IA (17 ohms aggregation induced by arachidonic acid), PFA-200 (CEPI closure time of 165 seconds), and bedside BT (7 minutes) in

Table 1 Detection of Aspirin Resistance Using three Different Methods of Assessment

Methods	Assay/Procedure Interpretation (n, %)		Aspirin Responsive on IA (n, %)	Aspirin Resistance* on IA (n, %)	Alternative Method Compared to IA
IA	Aspirin responsive	36 (97.3)	36 (100)	0 (0)	
	Aspirin resistance*	1 (2.7)	0 (0)	1 (100)	
PFA-200	Aspirin responsive	30 (81.1)	30 (83.3)	0 (0)	P = 0.189
	Aspirin resistance**	7 (18.9)	6 (16.7)	1 (100)	
Bedside BT	Aspirin responsive	13 (35.1)	13 (36.1)	0 (0)	P = 1.000
	Aspirin resistance†	24 (64.9)	23 (63.9)	1 (100)	

Notes: *Normal platelet function on IA is defined by positive platelet aggregation >5 ohms when activated by arachidonic acid. **Normal platelet function on PFA-200 is defined by closure time in collagen/epinephrine cartridge <180 seconds.† Normal platelet function on bedside BT is defined by BT ≤7 seconds.
Abbreviations: BT, bleeding time; IA, impedance aggregometry; PFA, platelet function analyzer.

this patient. This may be due to drug interactions, especially proton pump inhibitors, and high platelet turnover rate in thalassemia.

Based upon PFA-200, 18.9% of the patients (n = 7) elicited no aspirin-like defects by normal closure time in the CEPI cartridge. While bedside BT showed no significantly impaired primary hemostasis in 64.9% of the patients (n = 24; Table 1). These results indicated 100% sensitivity for both methods but 83.3% and 36.1% specificity for PFA-200 and bedside BT to detect aspirin resistance, respectively (Table 2). The diagnostic accuracy of PFA-200 was 83.8%, while that of bedside BT was only 37.8%. The results from each case were demonstrated (Supplementary Table 2). Considering the exact McNemar significance probability test, the null hypothesis that probabilities for IA and for either PFA-200 or bedside BT were the same could be rejected, advocating that the alternative tools provided a significantly inferior ability. Parallelly, Cohen's kappa analysis exhibited a fair reliability for PFA-200 (κ=0.215) and a slight reliability for bedside BT (κ=0.030), relative to IA (Table 2).

Discussion

This study assesses the incidence of aspirin resistance in pediatric patients by using IA, which is one of the gold standard assays for measuring platelet functions. Although its incidence (2.7%) in our study population is lower than previously reported,[5–7] this study reveals that PFA-200 and bedside BT as alternative methods produce poorer diagnostic performance. However, due to its 100% sensitivity and 83.3% specificity, PFA-200 may be considered as a screening test for aspirin resistance in children before undergoing IA as a confirmatory test.

Since aspirin resistance tends to occur during infancy rather than childhood,[5] our study with patients older than early childhood in >70% of the study population could reduce the probability of enrolling patients with positive results. Additionally, several confounders can affect platelet inhibition by aspirin in real-world settings. In this study, one patient with confirmed aspirin resistance concomitantly received omeprazole, a proton pump inhibitor, which could interfere with aspirin absorption,[4] although no clinical thrombosis developed. Therefore, routine screening for aspirin resistance by any method is not recommended for patients with good compliance with aspirin and no drug–drug interactions. Nonetheless, among cases with recurrent thrombosis, pre- and post-treatment evaluations could be considered to further assist clinical decision-making on antithrombotic strategies after clinical response failure to aspirin monotherapy.

Although aspirin can impair responses to epinephrine and collagen of platelets, leading to the utilization of a CEPI cartridge in PFA-200 to indirectly evaluate aspirin-like defects, PFA-200 does not directly determine platelet response to

Table 2 Diagnostic Performance of Platelet Function Analyzer (PFA-200) and Bedside Bleeding Time (BT) to Detect Aspirin Resistance Compared to the Gold Standard Whole Blood Impedance Aggregometry (IA)

Statistics	PFA-200	Bedside BT
Sensitivity (95% CI)	100% (100% to 100%)	100% (100% to 100%)
Specificity (95% CI)	83.3% (71.3% to 95.3%)	36.1% (20.6% to 51.6%)
Positive predictive value (95% CI)	14.3% (3.0% to 25.6%)	4.2% (−2.3% to 10.6%)
Negative predictive value (95 CI)	100% (100% to 100%)	100% (100% to 100%)
Accuracy	83.8%	37.8%
Number needed to treat	7.0	24.0
Exact McNemar significance probability (P-value)*	0.031	<0.001
Cohen's kappa coefficient (κ)**	0.215	0.030

Notes: *In exact McNemar significance probability, P-value <0.05 rejects the null hypothesis that there are no differences between the standard IA and the alternative methods (alternative test is not comparable). **In Cohen's kappa analysis, kappa coefficient (κ) ≤0 indicates no agreement, 0.01 to 0.20 as none to slight, 0.21 to 0.40 as fair, 0.41 to 0.60 as moderate, 0.61 to 0.80 as substantial, and 0.81 to 1.00 as almost perfect agreements.
Abbreviations: BT, bleeding time; CI, confidence interval; PFA, platelet function analyzer.

arachidonic acid which is specific for COX-1 inhibition. Shortened closure times in cases with confirmed aspirin responsiveness probably reflect increased platelet sensitivity to epinephrine and/or polymorphisms in glycoproteins of platelets. Furthermore, bedside BT using the modified Ivy method is less accurate in detecting specific types of platelet dysfunction.[18] It is because BT represents gross properties of primary hemostasis; therefore, VWF, vascular endothelium, and their interactions with platelets and each other could confound the results. As shown in this study, bedside BT was normal in 63.9% of cases with positive aspirin responsiveness to the gold standard IA. Consequently, the normal closure time on the CEPI cartridge of PFA-200 and normal BT could not exclude aspirin responsiveness. However, with a simpler assay technique and its fair reliability to the results of the gold standard IA, PFA-200 could be reasonably used for screening aspirin resistance in pediatric patients with high clinical probability. The normal closure time on the CEPI cartridge of PFA-200 should indicate retesting with IA in the presence of arachidonic acid to confirm the diagnosis of aspirin resistance.

Genetic thrombophilia (eg upregulation COX-2 pathway, upregulation platelet function by collagen, ADP, von Willebrand factor[4] eg platelet type vWD, etc.) might be considered as confounding factors, especially in pediatrics. Since the test is seldom performed before aspirin initiation. The poor compliance was not a confounding factor because the five days of aspirin administration was ensured to the patient before blood collection. This study has some limitations. A single-center study design with a small sample size lessens the chance of recruiting patients with a high clinical probability of aspirin resistance. The low incidence of true positive cases thus impacts the positive and the negative predictive values of the test. Since no infantile patients (<1 year) were included in this study and most of the patients were older than early childhood, the generalizability of data to all specific age groups of the pediatric population would subsequently be limited. Point-of-care multiple electrode aggregometry (MEA), a new generation WB platelet aggregometry with reportedly good performance in diagnosing aspirin resistance, was also not available at the study site, while the study was being conducted.[19] Multicenter studies comparing the diagnostic performance of conventional IA, MEA, and PFA-200 would provide more robust information on the updated technologies in clinical practice.

Conclusion

In conclusion, the incidence of aspirin resistance among pediatric patients with increased thrombotic risk is as low as 2.7% in Thailand. Routine screening is discouraged but recommended only in cases with recurrent thrombosis despite good aspirin compliance or the presence of resistant risk factors. Genetic thrombophilia might be considered a confounding factor, especially in pediatrics. Compared to WB IA, which is the gold standard diagnostic modality, the rapid assay of PFA-200 can potentially be considered a point-of-care screening test to detect aspirin resistance in childhood.

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Disclosure

All the authors declare that they have no conflicts of interest.

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