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ORIGINAL ARTICLES

Aspirin resistance among patients with recurrent non-cardioembolic stroke detected by rapid platelet function analyzer

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Abstract

Background and Objective: The prevalence of aspirin resistance amongst patients with cardiovascular disease and in the healthy population has been reported to range from 5% to 45%. Lately, rapid platelet function analyzer (RPFA) a point-of-care determination of platelet aggregability has been introduced for rapid determination of aspirin resistant patients. The purpose of this paper is to report the prevalence of aspirin resistance among patients with recurrent non cardioembolic ischemic stroke as detected by RPFA (Ultegra®). *Methods:* Seventy-seven patients with mean age of 61.2 ± 10.4 (range 33-87 years) who developed recurrent non-cardioembolic ischemic stroke were consecutively included in the study. Fifty-seven (74%) were males. Aspirin resistance was determined using the RPFA (Ultegra®) machine. Patients with an aspirin reaction unit (ARU) value above 550 were identified as aspirin resistant. *Results:* Following this method, the prevalence of aspirin resistance was determined to be 10.4% (95% CI: 1% to17%). Comparison of baseline characteristics between aspirin resistant and aspirin responsive patients did not show any significant difference.
Conclusion: The prevalence of aspirin resistance in this study was 10.4% amongst patients with recurrent non-cardioembolic ischemic stroke. The study has shown the feasibility of utilizing RPFA (Ultegra®) machine in detecting aspirin resistance.

INTRODUCTION

The concept of aspirin resistance has been introduced during the past few years in the medical literature. The first few papers on this subject were published in the early 1990's, mostly on patients with cardiovascular disease.[1-4] Several authors defined aspirin resistance in biochemical terms, i.e, a failure of this medication to affect aggregation of platelets as detected by various laboratory methods.[5-12] Others defined aspirin resistance in clinical terms, i.e., a failure of aspirin to protect individuals from atherothrombotic complications leading to stroke, myocardial infarction, or other vascular occlusion.[11-17]

Studies have reported an extremely wide range of prevalence of this condition, ranging from 5% to 45%.[12] This has been attributed to several reasons, such as the variable definition of aspirin resistance among different authors; the non-uniformity of tests utilized to detect aspirin resistance; and the different populations studied. Some authors consider the detection of urinary 11-dehydroxy TXB2 to be a more reliable technique that may correlate with outcome, however the procedure is not yet available for routine clinical application.[18-20]

Lately, rapid platelet function analyzer (RPFA), a point-of-care determination of platelet aggregability has been introduced for rapid determination of aspirin resistance.[21] RPFA has already been utilized to determine aspirin resistance among patients with stable coronary artery disease and in those undergoing elective percutaneous coronary intervention.[10,21] However, this technique has not yet been applied to patients with recurrent ischemic stroke. This study aims to determine the frequency of aspirin resistance among patients who developed recurrent non-cardioembolic ischemic stroke or transient ischaemic attack as detected by a commercially available RPFA (Ultegra®) machine.

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METHODS

Consecutive patients who developed a recurrent non-cardioembolic ischemic stroke while taking aspirin for at least 6 months were included in this study. Patients who presented with ischemic stroke following the definition set by the World Health Organization (WHO) were recruited.[23] The ischemic event must be confirmed by cranial tomography (CT) scan or magnetic resonance imaging (MRI). These patients should have been compliant with the intake of aspirin at a dose of 80-325 mg per day.

A written informed consent was obtained from the patients. Demographic information, co-morbid medical conditions such as hypertension, diabetes mellitus, smoking, alcohol intake, as well as medications were recorded. Likewise, laboratory data such as fasting blood sugar, HbA_1c and lipid profile were determined. These baseline characteristics were compared between aspirin resistant patients and aspirin responders.

A 2-ml blood sample was taken from the patients using a 3.2% sodium citrate vacuum tube within two hours after the intake of aspirin. These patients should have be consistently taking aspirin for at least 5 days prior to blood extraction. The sample was then placed in the aspirin (RPFA-ASA®) test cartridge. The citrated test tube with blood was then inserted into the Ultegra RPFA-ASA® machine and after 10 minutes, the machine provided a read-out. The analysis was performed within 4 hours of sample collection. The results were interpreted based on the extent of platelet aggregation and were reported as Aspirin Reaction Unit (ARU). ARU values of 550 and above were consistent with aspirin resistance.[24] Once identified as a non-responder, the treatment options were left to the discretion of the primary physician.

RESULTS

Seventy-seven patients with non-cardioembolic ischemic stroke were included in this study over a period of 22 months. The mean age was 61.2 ± 10.4 years with a range of 33 to 87 years (Table 1). Fifty seven (74%) of these patients were males. Sixty-seven patients (87%) were hypertensive, and 43 (55%) had diabetes mellitus. Twenty-nine (37%)

Table 1: Baseline demographic characteristics of the study subjects

	Aspirin Responders	Aspirin Non-responders	P value
No. of patients	69 (89.6%)	8 (10.4%)	
Age in years (mean ± SD)	60.5 ± 10.4	67 ± 10.4	0.088
Male	53 (76.8%)	4 (50%)	0.432
Risk factors			
Hypertension	60 (87%)	7 (86%)	1.000
Diabetes Mellitus	36 (52%)	7 (86%)	0.091
Current smoker	27 (39%)	2 (25%)	0.703
Alcohol	18 (26%)	1 (13%)	0.671
Hypercholesterolemia	35 (90%)	4 (10%)	1.000
Hypertriglyceridemia	42 (86%)	6 (14%)	0.703
No. of recurrence/s			
First (44)	39 (87%)	5 (11%)	1.000
Second (24)	22 (92%)	2 (8%)	1.000
Third (4)	3 (75%)	1 (25%)	0.361
Fourth (5)	5 (100%)	0	1.000
ARU's (mean ± SD)	431.3 ± 67.9	592.8 ± 32.4	<0.001

ARU: Aspirin Reaction Unit

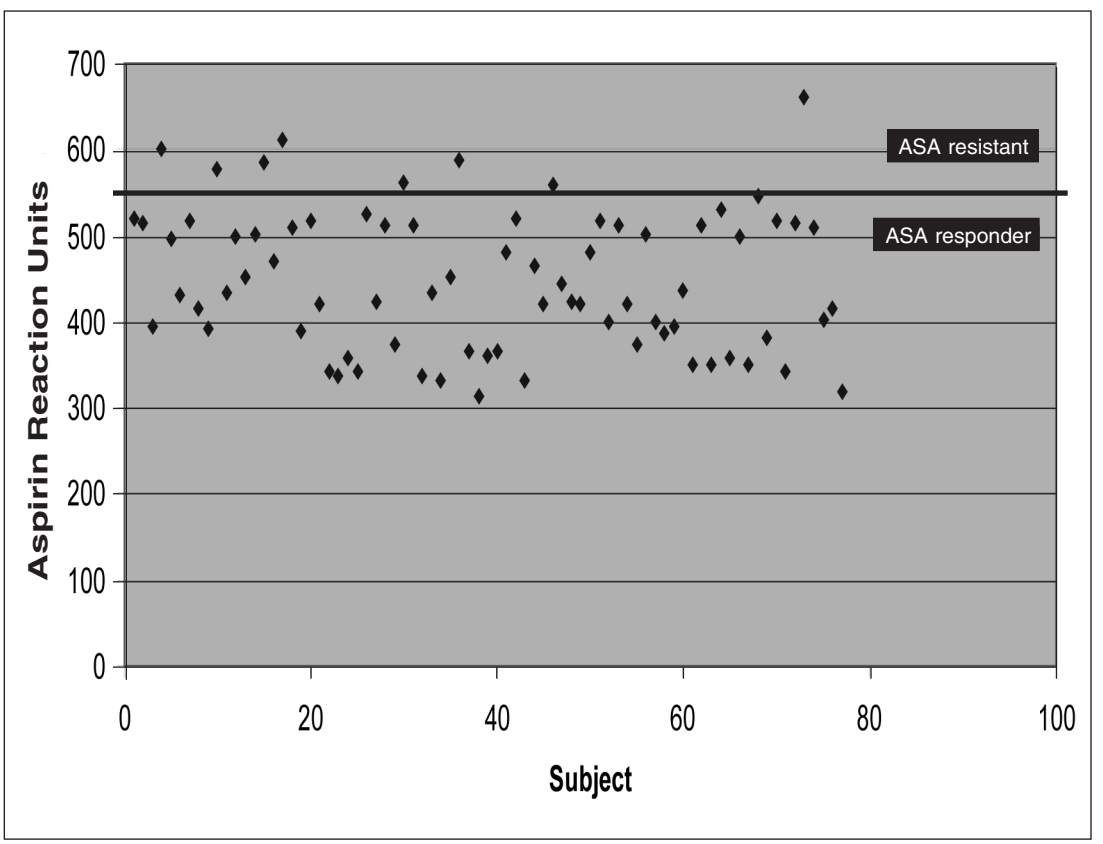
and 19 (25%) of the subjects were smokers and alcoholic beverage drinkers, respectively. More than half of the patients had hypercholesterolemia (51%) and hypertriglyceridemia (62%). Sixty nine patients (89.6%) obtained an ARU below 550. The mean ARU among all the subjects was 448. 2 ± 81.7. Among aspirin responders, the mean ARU was 431.3 ± 81.7. On the other hand, 8 (10.4%) patients had an ARU level of 550 and above, consistent with aspirin resistance. Mean ARU values for aspirin resistant patients was 592.9 ± 32.4. Figure 1 shows the distribution of ARU obtained among the subjects.

Hypertension was observed in 60 (87%) patients who were aspirin responders, compared with 7 (86%) aspirin resistant patients. Diabetes mellitus was seen in 36 (52%) aspirin responders compared with 7 (87%) aspirin resistant patients. In the aspirin responder group, 27 (39%) were current smokers compared with 2 (25%) in the aspirin non-responders. A total of 44 patients experienced a first stroke recurrence, 39 (87%) of whom were aspirin responders when measured on the Ultegra RPFA-ASA® machine and 5 (11%) of whom were aspirin resistant. Except for the ARU values, there was no significant difference in clinical characteristics between aspirin resistant patients and aspirin responders.

DISCUSSION

Our study determined the prevalence of aspirin resistance among patients with recurrent non-cardioembolic ischemic infarction. Until recently, few studies have studied the prevalence of aspirin failure among stroke patients.[1,3] These older studies made use of optical aggregometry and platelet reactivity to detect aspirin resistance; a prevalence rate of 25% and 30% was obtained among post-stroke patients using these methods, respectively (Table 2). A more recent study using platelet aggregometry reported a prevalence of 7%.[25] Using PFA-100, the prevalence of aspirin resistance among post-stroke patients has been reported to range from 0-37%.[26-31] The most

Figure 1: Distribution of aspirin reaction units (ARU) obtained from the subjects



ARU values obtained from each subject using the Ultegra® Rapid Platelet Function Analyzer are shown below. Values above 550 denote the presence of aspirin (ASA) resistance

Table 2. Studies on the prevalence of aspirin resistance among stroke patient

References	Type of Study	Population	Method	Aspirin dose/day	Prevalence of aspirin non-responders
Grotemeyer[1]	Prospective	180 post-stroke patients	Platelet reactivity	1500mg	30%
Helgason[3]	Prospective	306 post-stroke patients	Optical platelet aggregometry	325-1300mg	25%
Grau [26]	Case-crossover	31 post-stroke patients	PFA-100	300mg	16%
Grundmann[29]	Prospective	18 post-stroke vs. 25 recurrent stroke patients	PFA-100	100mg	0% of asymptomatic post-stroke patients; 35% of recurrent stroke patients
Alberts[27]	Prospective, observational	129 patients who developed ischemic stroke or TIA	PFA-100	81mg vs. 325mg	37%
Macchi [28]	Observational	37 post- stroke	PFA 100	160mg	24%
Harrison [31]	Observational	100 TIA or post-stroke	PFA-100 vs. RPFA vs. LTA	75-150mg	5-22%
McCabe[30]	Observational	100 post-stroke	PFA-100	75-300mg	78%
Berrouschot[25]	Prospective	291 post-stroke	Optical platelet aggregometry	300mg	7%
Navarro	Prospective, observational	77 recurrent stroke	RPFA (Ultegra)	80mg/day	10.4%

TIA: transient ischaemic attack

recent study, which made use of whole blood aggregometry, reported a rate of 29% among 62 post stroke patients.[32]

It is notable that all the above studies depended on a biochemical definition of aspirin resistance among patients with a prior stroke.[11] The study by Grundmann compared the prevalence of aspirin resistance among patients with a prior stroke to patients who developed a recurrent ischemic stroke while taking aspirin. Using PFA-100, a rate of 34% AR was obtained among recurrent stroke patients compared with none for the asymptomatic post-stroke patients.[29] In the present study, we attempted to incorporate the clinical definition of aspirin resistance by focusing solely on patients with a recurrent stroke in spite of aspirin therapy.[11] We then used RPFA to determine the presence of biochemical aspirin resistance. Patrono has proposed that rather than being termed "clinical aspirin resistance", patients with recurrent vascular events should be identified as "treatment failure".[33]

In this particular study, only 10% of "clinical" aspirin resistant patients had concomitant biochemical evidence of aspirin resistance. This prevalence rate is relatively low compared with previous studies. Various factors may account for this discrepancy. The method used in this study, Ultegra® RPFA-ASA, differs from that employed in previous studies. The wide range of prevalence rates across various studies on a similar population highlights the fact that these values are highly dependent on the type of method employed, although a previous study had shown comparable

prevalence rates obtained between PFA-100 and RPFA among patients with TIA or stroke.[31] RPFA had been used previously among patients with coronary artery disease and those undergoing percutaneous coronary intervention.[10,22] To our knowledge, this method has not yet been reported among patients with recurrent ischemic stroke. The test consists of a turbidimetric-based optical detection system that measures platelet-induced aggregation in citrated whole blood. It induces platelet aggregation utilizing cationic propyl gallate and platelet agglutination through fibrinogen-coated microparticles. Subsequently, the platelet aggregation is measured by light transmission depending on the number of available platelet receptors that would occur by adhesion.[34] Numerous other methods have been used to determine aspirin resistance. The determination of bleeding time is perhaps the most commonly used method for clinical purposes and has the advantage of simplicity and availability. Unfortunately, it is neither sensitive nor specific, is operator dependent, and is not easily reproducible.[13,34] Optical platelet aggregation methods, on the other hand, have the advantage of being widely available and have been shown to correlate with clinical events. However, it is labor intensive, and its sensitivity and specificity are uncertain.[13,34] The determination of urinary 11-dehydroxy TXB2 has been shown to be a more reliable technique in detecting aspirin resistance, but also has uncertain sensitivity and specificity.[13] In addition, it has not been widely evaluated. In a busy office practice where immediate information of patients' response to aspirin is necessary, the use of RPFA in the point-of-care setting could be of significant value.

Other factors that may account for the low prevalence rate in this study are the male predominance of the population, genetic variables and the timing of the test. Some studies have reported that aspirin resistance is more common among women and the elderly[27,35] while other studies have found no such correlation.[25] In addition, genetic factors, such as mutations and/or polymorphisms of the COX-1 gene and glycoprotein IIb/IIIa receptor have been postulated to play a role.[12] Whether race is a factor in the frequency of these mutations remains to be studied. Previous studies have not found any predisposition to aspirin resistance based on race or ethnicity.[35] In addition, the response to aspirin has been shown to vary with time.[3,25] In one study, 8-33% of patients receiving aspirin developed some degree of resistance to aspirin over 6-33 months, a phenomenon termed "secondary aspirin resistance".[3] Many of the subjects underwent laboratory determination for aspirin resistance during the early phase of stroke.

There was no significant difference in the clinical characteristics of aspirin resistant patients and aspirin responders, which is consistent with the findings of Berrouschot.[25] Previous studies, however, have noted that older age, female gender, and hypertension tended to be more common among aspirin resistant patients.[27,28] In the absence of clinically significant differences between the two groups in this study, a possible role for aspirin resistance as a mechanism in stroke recurrence is hypothesized. Although a causal relationship cannot be established, previous studies have highlighted the role of aspirin resistance in the incidence of recurrent vascular events.[1,19,29,36,37] While it is still too early to recommend routine determination of AR among post-stroke patients, clinicians should be aware of this entity among patients with recurrent stroke.

There are several limitations in this study. First, intake of aspirin was not under the direct supervision of the clinician, so that the investigators had to rely on information provided by the patient. Second, some of the patients included in this study had been on a combination of antiplatelet medications, such as clopidogrel and dipyridamole. These medications have been reported to influence the results obtained by RPFA.[10,34] Third, the treatment of patients was left to the discretion of the attending physician, so that data regarding changes in therapeutic practice following detection of aspirin resistance were not included in the scope of the study. Fourth, long-term outcome of patients with aspirin resistance was not determined. These limitations may be addressed in future studies.

In summary, this investigation has shown the prevalence of aspirin resistance among patients with recurrent non-cardioembolic ischemic stroke. The condition was detected by RPFA, a simple, rapid, point-of-care method that can provide relevant information regarding the possibility of aspirin resistance. The feasibility of utilizing RPFA (Ultegra®) has likewise been shown in this study.

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