Clin. Lab. Haem.
2006, **28**, 14–16

Effect of sex difference on platelet aggregation using an optical method in healthy subjects

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Summary There are many studies reporting conflicting results of sex differences on various platelet functions. The purpose of this study was to investigate whether sex differences could affect platelet aggregation results using an optical method in healthy subjects. A total of 42 subjects, 21 males and 21 females, were included in the study. Platelet aggregation was induced by adenosine diphosphate (ADP; 5 μM), collagen (2 μg/ml), and epinephrine (10 μM). Optical aggregation was performed using a turbidometric method. In all platelet aggregation tests ADP, collagen and epinephrine were studied; there was no significant difference between females and males in platelet aggregation amplitudes and slopes. As a result, sex difference does not affect platelet aggregation performed with this optical method in healthy subjects. This result supports that there is no need for sex differentiation while composing control groups in platelet aggregation studies using the optical method.

Keywords Platelets, platelet aggregation, platelet function, sex, sex characteristics

Introduction

There are animal studies which report that sex differences could affect various functions of platelets (Morikawa *et al.*, 1985; Torres Duarte, Ramwell & Myers, 1986; Jayachandran *et al.*, 2004; Leng *et al.*, 2004). But there are a few critics about whether these results obtained from animal studies match the mechanisms of human beings (Kasjanovova *et al.*, 1993). The purpose of this study was to investigate whether sex differences could affect platelet aggregation results using an optical method in healthy subjects.

Materials and methods

This study was carried out on 21 healthy females, ages ranging from 20 to 40 (mean ± SD: 27.52 ± 5.96 years) and 21 healthy males, ages ranging from 21 to 42 (28.76 ± 5.79 years).

Received 21 June 2005; accepted for publication 10 October 2005
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There was no history of coagulopathy in any subject and no case had taken either prophylaxis for thromboembolic disease or any other medication that affects platelet aggregation for at least 2 weeks. No female subject had received combined oral contraceptives. All studies were done in the morning with the patients fasting overnight. The venous blood samples were drawn without a tourniquet with a 20-gauge needle from the antecubital vein. These samples were anticoagulated with 3.8% 0.130 M sodium citrate solution (blood to anticoagulant ratio: 9/1). There was no haemolysis in samples. They were kept at room temperature and tested within 60 min of collection. Platelets were counted using an automated cell counter device (Abbott Cell-Dyne 4000; Abbott Park, Chicago, IL, USA). Platelet aggregation was induced by adenosine diphosphate (ADP; 5 μM), collagen (2 μg/ml; native collagen fibrils-type I- from equine tendons suspended in isotonic glucose solution of pH 2.7) and epinephrine (10 μM). Optical aggregation was performed using a turbidometric method (Chrono-log Corporation, Model 560-Ca; Havertown, PA, USA) according to the protocol of Sigma Diagnostics, procedure no.885. Platelet aggregation was performed using Sigma platelet aggregating reagents (Sigma Diagnostic Corporation, St Louis, MO, USA, Code No 885/A. For ADP: 885/3, for collagen: 885/1, and for epinephrine: 885/5). Blood

Table 1. Platelet aggregation results and statistical comparison of two groups

		Female		Male		
Reagent	Parameter	Median	Range	Median	Range	*P*-value
ADP	Amplitude (%)	89	53–118	78	47–118	>0.05
	Slope	108	62–156	97	49–176	>0.05
Collagen	Amplitude (%)	69	57–84	70	54–86	>0.05
	Slope	90	55–134	97	60–123	>0.05
Epinephrine	Amplitude (%)	68	25–91	69	28–85	>0.05
	Slope	53	31–141	58	29–97	>0.05

samples were centrifuged (250 ***g***, 10 min) to isolate platelet-rich plasma (PRP) in the supernatant. The remainder of the blood was centrifuged again (1500 ***g***, 10 min) to prepare platelet-poor plasma (PPP) as previously described (Sirridge & Shannon, 1983). The PRP was diluted with the PPP to yield test PRP with a final count of $250 \pm 50 \times 10^9$/l. Dose-response curves were calculated automatically by the device and evaluated using amplitude and slope. Statistical analysis was done with the Mann–Whitney *U*-test. $P < 0.05$ were considered significant.

Results

No statistically significant difference was found between groups when compared for age. In all platelet aggregation tests, which were performed with ADP, collagen or epinephrine, there was no significant difference between females and males in platelet aggregation amplitudes and slopes (Table 1).

Discussion

There are several studies investigating the effects of sex on platelet functions. Torres Duarte, Ramwell & Myers (1986) investigated the effect of sex on platelet aggregation in mice. In this study, male platelets exhibited a greater response than female platelets to both ADP (15 μM) and arachidonate (0.3 mM) in PRP. Contrary to this finding, Leng *et al.* (2004) reported that platelets of female mice were intrinsically more sensitive to agonists than platelets of males. In this study, female platelets also demonstrated greater aggregation in response to ADP and collagen-related peptide. In our study, we did not observe any difference between ADP and collagen. The differences between our study and the others might be the differences of the final concentrations of aggregation inducers used and the performance of our study on human beings.

Jayachandran *et al.* (2004) reported that sex-related differences had been observed in platelet aggregation and secretion with sexual maturation in pigs. In this study, platelet aggregation and ATP secretion decreased in females but increased in males with maturity. Results indicate that changes in platelet aggregation and secretion change with sexual maturity differently in females and males. The investigators suggest human studies based on their results. According to our study results, sex does not affect platelet aggregation in healthy adults. For this reason, it is possible to suggest that results in pigs do not reflect the condition present in humans.

Morikawa *et al.* (1985) reported that the effect of aspirin on platelet aggregation in rats was influenced by sex differences. The results of this study suggest that there is a sex difference in rat platelet cyclooxygenase activity, and it is closely related to the sex difference in the antiplatelet effect of aspirin. Effects of sex differences in healthy adults have been investigated in our study. Human studies which focus on whether the effect of aspirin is influenced by sex differences in healthy adults are needed to criticize the results of Morikawa *et al.* (1985).

In another human study performed by Kasjanovova *et al.* (1993), the effects of sex and age on platelet aggregation and the relationship with hematocrit were investigated. According to results of this study, there were no differences between men and women in the platelet aggregation and thromboxane B_2 production of young probands with similar hematocrit values. The results of this study support our findings. Finally, sex difference does not affect platelet aggregation with the optical method in healthy adults. This result supports that there is no need for sex differentiation while composing control groups in platelet aggregation studies using the optical method.

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