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Reduced spontaneous platelet aggregation: a novel risk factor for adverse pregnancy outcome

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ABSTRACT

Objective: Spontaneous platelet aggregation has not been adequately assessed as a potential risk factor for adverse outcomes in pregnancy. Therefore the objective of this study was to assess spontaneous platelet aggregation (SPA), measured via a novel functional assay, as a risk factor for hypertensive disease and intra-uterine growth restriction (IUGR).

Study design: This was a prospective longitudinal study. Spontaneous platelet aggregation was assessed as a marker of platelet reactivity using a modification of light transmission aggregometry. Platelet reactivity was assessed in four groups: non-pregnant healthy female volunteers ($n = 30$), longitudinally in normal uncomplicated pregnancy ($n = 50$), hypertensive disorder ($n = 40$) and IUGR ($n = 30$). The mean percentage SPA was plotted and compared across all groups.

Results: Spontaneous platelet aggregation was significantly reduced in the first trimester compared to the non-pregnant group (p-value = 0.003). The mean aggregation for the hypertensive group was 1.9%, (95% CI −0.08 to 4.02) and for the IUGR group was 1.6%, (95% CI −0.6 to 3.72). Platelet aggregation in the hypertensive group was significantly reduced compared to the normal pregnant group ($p < 0.05$). Spontaneous platelet aggregation was also reduced in the IUGR group compared to normal pregnancy ($p < 0.05$).

Conclusion: This study demonstrates that a reduction of spontaneous platelet aggregation may be a novel risk factor for adverse pregnancy outcomes such as pre-eclampsia and IUGR. The most clinically significant finding is that SPA is significantly lower in pregnancies complicated by hypertension and IUGR compared to those who had a normal pregnancy outcome. Further studies should be carried out to asses if spontaneous platelet aggregation may be a clinically useful tool for the prediction of pre-eclampsia and IUGR.

Introduction

Platelet function testing has become more widespread. With this, the concept of 'platelet hyper-reactivity' and the term "increased platelet reactivity" have emerged. Platelet reactivity can be defined as an aggregatory response to a platelet agonist but can also be assessed by measuring the degree of aggregation that occurs in platelets in the absence of an agonist, known as spontaneous platelet aggregation (SPA). In patients with cardiovascular disease increased platelet reactivity and increased SPA are both associated with an increased incidence of major adverse cardiovascular events [1]. To our knowledge, SPA has not previously been assessed as a potential risk factor for adverse pregnancy outcomes. Abnormal pregnancy states, such as pre-eclampsia and IUGR are often described as exaggerated responses of normal physiological pathways [2] and platelets have been implicated as mediators of these disease processes, but have not been adequately examined as predictors of disease. Studies have reported a number of conflicting results in respect to platelet

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function in pregnancy, with many finding increased platelet activation and aggregation [3–10], some researchers showed no difference in platelet function in pregnancy [11–14] while others found reduced aggregation or reactivity [15–22].

We have shown previously that there is an increased response to arachidonic acid in platelets from patients with recurrent miscarriage [23]. This finding suggested that platelet hyperreactivity may be a risk factor for abnormal pregnancies and we hypothesized that there may be changes in platelet reactivity or SPA between normal pregnancies and those affected by gestational hypertension, pre-eclampsia and IUGR.

Therefore, the objective of this study was to assess platelet reactivity in a normal non-pregnant female population and compare these results to each trimester of healthy pregnancies and to pregnancies complicated by PET, gestational hypertension and IUGR. The 3rd trimester results of those who had complicated pregnancies were compared to those with a normal healthy pregnancy.

Materials and methods

Participants

The study cohort consisted of 150 participants who were either staff volunteers or patients attending the Rotunda Hospital for antenatal care. This study was a prospective study with normal non-pregnant healthy female volunteers assessed at one time point and normal pregnancy participants assessed longitudinally at three separate time points (1st, 2nd and 3rd trimesters). Other participants included those with a diagnosis of a hypertensive disorder of pregnancy or IUGR assessed at one time point in the third trimester. All participants were controlled for a number of factors known to affect platelet function tests. To minimize several factors that can affect platelet function such as circadian rhythm, smoking, coffee and food intake blood was drawn from all subjects in the morning between 07.00 and 11.00. All subjects were fasting and were asked to avoid coffee and strenuous exercise for 24 h prior to phlebotomy. Subjects were excluded if there was any history of non-steroidal anti-inflammatory or aspirin use within the preceding seven days or had a diagnosis of thrombocytopenia, spherocytosis or coagulopathy. Specific inclusion and exclusion criteria for each group are outlined as follows:

Thirty non-pregnant healthy female volunteers were recruited to assess spontaneous platelet aggregation. All participants had a history of a prior normal pregnancy outcome with no obstetric complications and no more than two prior miscarriages. Since cyclic variation in hormones may affect platelet function blood samples were taken from all these subjects under the standard conditions described above, in addition samples were drawn while subjects were in the follicular phase of the menstrual cycle to control for progesterone levels. Participants were excluded if using hormonal contraception.

Fifty pregnant participants were recruited from the initial antenatal clinic visit in the Rotunda Hospital. Selection criteria included non-smokers (as smoking is known to cause increased platelet reactivity), with a body mass index (BMI) $\leq$30 with a singleton pregnancy $\leq 14^{+6}$ weeks' gestation at time of first blood draw. Nulliparous and multiparous women were both included. If a participant developed a pregnancy complication (PET, IUGR, preterm labor and gestational diabetes) they were then excluded from this arm of the study. Repeat phlebotomy was scheduled at the second trimester ultrasound visit (between 20 and 22 weeks' gestation) and again at their 28 or 34 week antenatal visits.

Forty participants who developed a hypertensive disorder of pregnancy as defined by new onset hypertension over 20 weeks gestation, with a blood pressure reading of 140/90 on two occasions 6 h apart, with or without significant proteinuria were recruited from the antenatal ward in the Rotunda Hospital. A blood sample was obtained at the time of diagnosis. Magnesium Sulphate therapy was the only extra additional exclusion criteria for this group.

Thirty pregnant participants with a suspected diagnosis of IUGR were also recruited to the study. Only participants with a confirmed delivery of an infant with a birth weight less than the 10th centile for gestational age were included in the final analysis (n = 24). Patients were excluded if there was also a concomitant diagnosis of gestational hypertension or PET. Smoking was not an exclusion criterion from this group as it was determined an important aetiological factor in IUGR pregnancies.

Blood was collected via a 19 gauge butterfly needle from an uncuffed arm into a 30 ml syringe containing 3 mls of 3.2% Sodium citrate. The first 5 mls was used to perform a complete blood count and confirm normal platelet count. Blood was the centrifuged for 10 min at 150$\times$ g. Platelet rich plasma (PRP) was aspirated from the supernatant and using a multichannel pipette dispensed into 4 wells in a plate reader (PerkinElmer, Wellesley, MA, USA).

To obtain platelet poor plasma (PPP), the PRP was centrifuged for 1 min at maximum speed in a microfuge; supernatant was again aspirated and dispensed into the plate reader adjacent to the PRP.

Assay

A modification of light transmission aggregometry was used to assess spontaneous platelet aggregation. Platelet rich and platelet poor plasma were added to 8 wells of a plate reader, four containing PRP and four with PPP. Aggregation was calculated on the basis that that the initial light absorbance reading for PRP at time 0 was classed as 100 percent aggregation while that for PPP was 0 percent. Light absorbance data at time 0 and 18 of each well was measured at 572 nm using a Victor3V Multilabel Counter plate reader (PerkinElmer, Wellesley, MA, USA). Between these times 0 and 18 min, the plate was rotated at 1000 rpm through a 0.1 mm orbit. The percentage change in platelet aggregation from time 0 to 18 min was calculated from the light absorbance readings. The spontaneous platelet aggregation for each individual in each group was calculated and plotted on scatter charts. The mean spontaneous platelet aggregation was calculated and compared for each group.

Statistical methods and analysis

The study was powered (80%) to detect a 5% change in platelet aggregation across the three time-points in normal pregnancy and an 8% change for the complicated pregnancies. Comparisons between spontaneous platelet aggregation assessments in the three time points of normal pregnancy were made using the Friedman test mixed effects model (ANOVA-like method) with study participant as a random-effect, allowing for possible correlations between the repeated assessments. The Kruskal Wallis test was then used to compare the non-pregnant group to each of the three trimesters of pregnancy. The two complicated pregnancy groups were compared individually with the third trimester normal pregnancy results using Mann Whitney two tailed t tests.

Results

Main findings

The demographic details for each of the three pregnant groups are shown in Table 1. There were no participants excluded from the non-pregnant control group. Sixteen patients were excluded from