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MATERNAL PLATELET SIZE AS A MARKER FOR FETAL TRISOMIES 18 AND 13

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SUMMARY

Maternal and fetal platelet size and glycoprotein expression were measured in 14 pregnancies complicated by fetal aneuploidy between 20 and 24 weeks' gestation. Flow cytometry was used to determine platelet size and surface glycoprotein Ib (GPIb) and GPIIIa expression both before and after stimulation with adenosine diphosphate (ADP). The data were compared with results obtained from 35 normal paired maternal and fetal controls. In fetuses affected with trisomies 18 and 13, but not trisomy 21, the maternal and fetal platelet sizes were significantly higher than those of the normal controls. Furthermore, the increase in fetal platelet size was significantly associated with the increase in maternal platelet size. Increase in maternal platelet size may be of potential value as a marker for fetal trisomies 18 and 13.

KEY WORDS: cordocentesis; fetal blood; fetal aneuploidy; maternal blood; platelet size.

INTRODUCTION

In normal pregnancy, there is a correlation between maternal and fetal platelet size suggesting the presence of a common factor influencing thrombocytopoiesis in both the maternal and the fetal haematopoietic tissues (Meher-Homji *et al.*, 1994a,b). Supportive evidence for this hypothesis was provided by the study of pathological pregnancies complicated by utero-placental insufficiency, where fetal platelet count is decreased and platelet size is increased; in this condition, the maternal platelet size is also increased and the correlation with fetal platelet size is unaltered (Thilaganathan *et al.*, 1995).

Chromosomally abnormal infants have abnormalities in platelet function and count (Lott *et al.*, 1972; McCoy and Enns 1978; Markenson *et al.*, 1975; Stuart and Kelton, 1987). If aneuploid fetuses also demonstrate platelet abnormalities and these are associated with alterations in maternal platelet turnover, measurement of the maternal platelet size may be of value as a screening tool for fetal aneuploidy. The aim of this study was to investigate fetal and maternal platelet count, function, and size in pregnancies complicated by fetal aneuploidy.

PATIENTS AND METHODS

This was a cross-sectional study of fetal and maternal blood samples obtained at 20–24 weeks' gestation from 14 pregnancies with fetal aneuploidy: trisomy 13 ($n=3$), trisomy 18 ($n=4$), trisomy 21 ($n=5$), Turner's syndrome ($n=1$), and triploidy ($n=1$). The indication for karyotyping was the presence of one or more markers of chromosomal abnormality (Nicolaides *et al.*, 1992). Ten of the 14 fetuses had cardiac defects and seven of the 14 showed ultrasonographic evidence of intrauterine growth retardation. Paired fetal and maternal blood samples were also obtained from 35 pregnancies at 20–24 weeks.

Cordocentesis was performed without maternal sedation or fetal paralysis. Kleihaur–Betke testing

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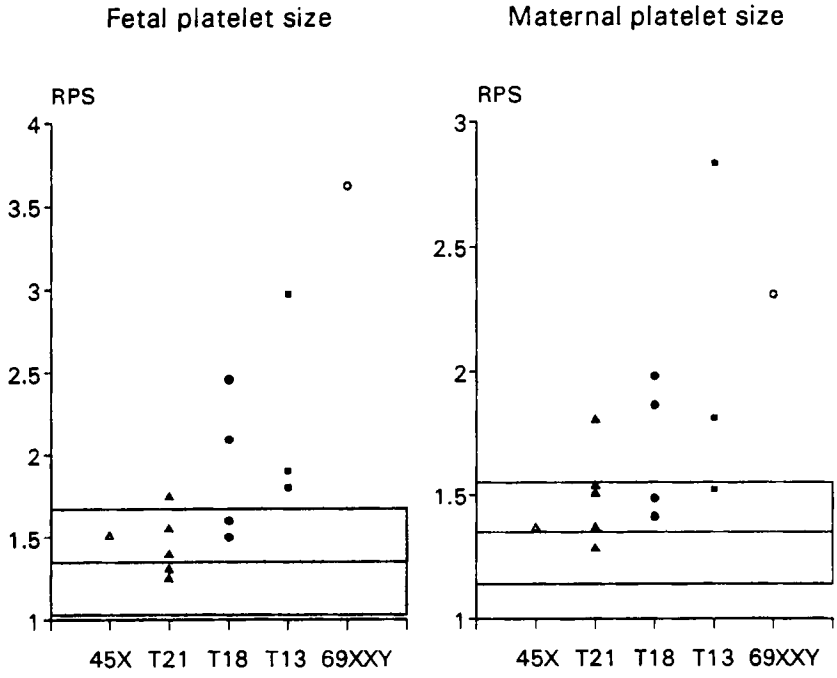

Fig. 1—Maternal and fetal platelet size (RPS) in 14 pregnancies with fetal aneuploidy plotted on the normal range (mean, 2·5th, and 97·5th percentiles) for 20–24 weeks' gestation

confirmed that all blood samples contained only fetal blood. Maternal blood samples were obtained from the ante-cubital vein. Blood samples (180 μl) were collected into 20 μl of isotonic edetic acid solution (0·5 mmol/l in 0·15 mmol/l sodium chloride) and the full blood count was determined using a Coulter S-Plus counter (Coulter Electronics, Luton, U.K.). Blood (500 μl) was also collected into 50 μl of sodium citrate (3·8 per cent w/v) for analysis by flow cytometry, which was performed on the same day of sampling as previously described (Meher-Homji *et al.*, 1994a).

Flow cytometry

In brief, platelet-rich plasma (PRP) was prepared by centrifugation (225 *g*, 10 min). PRP (20 μl) and fluorescent antibody (5 μl) were incubated in the absence or presence of 1 mM adenosine diphosphate (ADP; Sigma Chemical Company Ltd, Poole, U.K.). Fluorescein, isothiocyanate (FITC)-conjugated monoclonal mouse anti-human antibodies (Dako Ltd, High Wycombe, U.K.) were used for the determination of CD42b (GPIb) and CD61 (GPIIIa) positive cells. In order to prevent platelet aggregation, the reaction mixture was diluted with phosphate-buffered saline (PBS; Sigma Chemical Company Ltd, Poole, U.K.) and incubated for 30 min in the dark at room temperature. PBS (500 μl) was then added to each tube prior to analysis by flow cytometry, which was carried out using a fluorescence-activated cell sorter (FACscan) and Consort 32 software (Becton Dickinson, Oxford, U.K.). The flow cytometer was calibrated for size and fluorescence before each analysis using a flow cytometry fluorescence intensity standardization kit (Coulter Ltd, Luton, U.K.). Samples were gated using forward angle and 90° light-scattering properties to exclude leucocytes. Control staining of PRP with anti-mouse monoclonal IgG_{2a}-PE/IgG_1-FITC was performed on each sample and background readings of less than 1 per cent were obtained. A minimum of 5000 cells were acquired in the platelet fraction and analysed to calculate the percentages and the mean fluorescence intensity (MFI) of each sample. The density of surface glycoproteins was measured by calculating the relative fluorescence intensity (RFI), using the formula RFI=antilog(MFI/number of channels per decade). The platelet size was estimated by calculating the relative platelet size (RPS), using the formula RPS=antilog(median forward scatter/number of channels per decade).

Table I—Comparison of platelet size and count between the chromosomally normal and aneuploid pregnancies

	Maternal platelet size	Maternal platelet count	Fetal platelet size	Fetal platelet count
Trisomy 21	$z=0{\cdot}00$	$z=1{\cdot}03$	$z=0{\cdot}00$	$z=1{\cdot}03$
($n=5$)	NS	NS	NS	NS
Trisomy 18	$z=2{\cdot}79$	$z=0{\cdot}00$	$z=2{\cdot}79$	$z=2{\cdot}79$
($n=4$)	$P<0{\cdot}01$	NS	$P<0{\cdot}01$	$P<0{\cdot}01$
Trisomy 13	$z=2{\cdot}09$	$z=0{\cdot}00$	$z=2{\cdot}09$	$z=2{\cdot}09$
($n=3$)	$P<0{\cdot}05$	NS	$P<0{\cdot}05$	$P<0{\cdot}05$

NS=Not significant.

Statistics

The Mann–Whitney *U*-test was used to determine the significance of differences in measurements between the chromosomally normal and abnormal groups. Regression analysis was applied to examine whether the maternal and fetal platelet sizes were significantly related.

RESULTS

Platelet sizes in both fetal and maternal blood for trisomies 18 and 13, but not trisomy 21, were significantly higher and platelet counts were significantly lower than in the normal controls (Fig. 1 and Table I). There was a significant association between fetal and maternal platelet size in the chromosomally abnormal group (Fig. 2; all aneuploidies: $r=0{\cdot}856$, $P<0{\cdot}001$). The highest maternal and fetal platelet sizes were observed in those pregnancies where the fetuses were both growth-retarded and had congenital heart defects (Fig. 2).

In the chromosomally abnormal group, the percentage expression and surface density of platelet glycoproteins in maternal and fetal blood, both before and after stimulation with ADP, did not differ significantly from the values for the normal controls.

DISCUSSION

The data of this study demonstrate that in fetuses with trisomies 18 and 13, platelet size is increased and platelet count is decreased. These findings are compatible with data from postnatal studies in neonates and children with these trisomies (Markenson *et al.*, 1975; Stuart and Kelton, 1987) and demonstrate that increased platelet

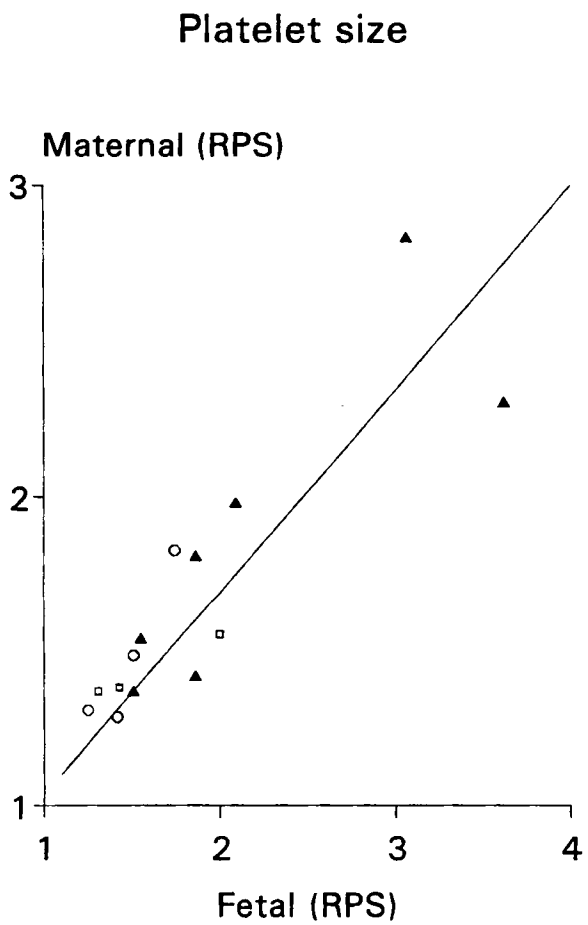

Fig. 2—Relationship of the maternal and fetal platelet size (RPS) in 14 pregnancies with fetal aneuploidy. In three cases, the fetuses were growth-retarded with normal hearts (□); in seven cases, they were growth-retarded and had heart defects (▲); and in four cases, they were appropriately grown and did not have heart defects (○)

turnover occurs prenatally. The finding of decreased platelet count and increased platelet size in these fetuses may be the consequence of the enzymatic and metabolic manifestations of the extra chromosome 18 or 13, as suggested by postnatal data (Markenson *et al.*, 1975; Stuart and Kelton, 1987).

Alternatively, the increase in fetal platelet size may be the consequence of either intrauterine growth retardation or congenital cardiac defects, since both of these conditions have also been shown to increase platelet consumption and turnover (Thilaganathan *et al.*, 1995; Pearson & McIntosh, 1978). The finding that fetal platelet glycoprotein expression is unaltered suggests that the increase in platelet size is the consequence of localized platelet consumption and not due to generalized platelet activation as occurs in diabetic pregnancy (Meher-Homji *et al.*, 1994a; Tschoepe *et al.*, 1990, 1991).

The finding that in pregnancies complicated by fetal trisomies 18 and 13 there is a significant association between the increase in fetal and maternal platelet size suggests that the factor responsible for altered thrombocytopoiesis in the fetus also affects maternal platelet production. The potential implication of this finding is that an increase in maternal platelet size may be of value as a marker for fetal trisomies 18 and 13.

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