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EVALUATION OF ERYTHROCYTE HYPERAGGREGATION IN FETAL BLOOD DRAWN BY CORDOCENTESIS AS A MARKER OF FETAL DISEASES

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ABSTRACT.

Fetal blood can now be routinely studied by intrauterine sampling. Low erythrocyte aggregability is one of its most prominent hemorheological characteristics before 32 wk gestation. We investigated whether increased RBC aggregability is a marker of fetal pathologies. After we established in 119 fetuses a control group for fetal blood rheology, we measured erythrocyte aggregation with the Myrenne apparatus (i.e. a micromethod using 1 droplet of blood) in 111 fetuses explored for various diseases. RBC aggregation was increased (>2 Sd for gestational age) in 24 fetal blood samples, with the following diseases: 12.5% toxoplasmosis; 12.5% rhesus immunization; 8.33% autoimmune thrombocytopenia; 8.33% kidney polycystosis; 8.33% old maternal age; 8.33% heart malformation; 8.33% fetal hypotrophia. In autoimmune diseases (rhesus; thrombocytopenia) RBC aggregation was increased in 5/10 cases (50%); in heart or kidney malformations 5/10 (50%); in toxoplasmosis seroconversion 3/23 i.e. 13% of the fetuses had increased aggregation. These data suggest that RBC aggregation is increased in several fetal diseases and may be a nonspecific marker of them.

key words: fetal blood, cordocentesis, hemorheology, erythrocyte deformability, blood viscosity, erythrocyte aggregation.

INTRODUCTION

The hemorheology of the normal fetus has been studied by several investigators (1-6), which observed that fetal blood was more viscous than the adult one (1-4, 5), because hematocrit is higher (5,6) and RBCs are less deformable (6). By contrast, fetuses have reduced erythrocyte aggregation and lowered plasma viscosity (7). These aspects of blood rheology may be important for blood circulation in the fetal organism, since vascular reactivity which counteracts in adults the effects of blood viscosity factors is immature (8). Consistent with this assumption, we recently reported a correlation between fetal blood viscosity and doppler resistance index in umbilical artery (9). The development of intrauterine sampling (cordocentesis) has allowed to obtain fetal blood for various measurements. With this technique, the previous studies on fetal blood rheology, which used indirect evidence given by cord blood drawn after delivery, have been confirmed (10-11), and the 'natural history' of fetal blood viscosity parameters during pregnancy has been described (9-11).

Whether some rheologic parameters may be biological markers of fetal diseases remains unknown. One of the most attractive of them was RBC aggregation index 'M', as measured with the Myrenne aggregometer. This parameter remains almost equal to zero before 32 wk gestation in our control series. It can be measured automatically on a simple droplet of blood. In this study, we investigated its relevance to several pathologic situations observed in fetal medicine.

MATERIAL AND METHODS

Patients

All fetuses included in this study underwent the cord puncture for the diagnosis of genetic abnormalities and/or recent infection by rubella or toxoplasmosis. Cordocentesis was performed in utero during pregnancy as previously reported (12). The method allowed an ambulatory sampling during a hospital visit performed in an operating room with surgical preparation of the abdomen. No premedication (e.g. maternal sedation) was administered. Bladder filling was unnecessary. The ultrasound device was a 3.5 MHz sectorial transducer (Combison Kretz 320) manipulated through a sterile bag. After local anesthesia (1 per cent xylocaïne), a 22.5 gauge needle fixed on a syringe was introduced in the plane of the ultrasound section through the abdominal wall, the uterine wall, the membranes, into the amniotic cavity, and finally into the umbilical cord. The 22.5 gauge needle was chosen in order to reduce cord bleeding when the needle was withdrawn. Fetal blood was aspirated after changing the syringe in order to avoid contamination of samples with maternal blood or amniotic fluid. The duration of funicular bleeding was noted at the withdrawal of the needle. Two hours after sampling, patients were

again examined ultrasonographically. Preventive antibiotic treatment consisting of 2g of cefotaxime daily was administered for 5 days, as well as an injection of anti-D gamma globulin if the mother was Rh-negative and fetus Rh-positive. Two methods were used for verifying purity of blood samples: the Kleihauer test and the measurement of the mean corpuscular volume on a Coulter Counter S Plus II. During the study period, samplings were performed by the same obstetrician and ultrasound guidance was performed by the same specialist.

The control group, partially described in our previous papers (9-11) includes 119 fetuses (between 25 and 30 week's gestation) in whom no pathology was found after the examination. In these fetuses, as far as the quantity of blood was sufficient, we measured blood viscosity, plasma viscosity, hematocrit and RBC aggregation.

The study group consisted of 111 fetuses who underwent an intrauterine blood sampling for antenatal diagnosis. One droplet of blood was employed for measuring RBC aggregation index with the Myrenne aggregometer.

Hemorheological measurements.

Blood was collected into potassium EDTA. Red cell aggregation was assessed photometrically in the Myrenne cone-plate aggregometer (Myrenne GmbH, Roetgen, Germany). Measurement is based on the light transmitting properties of aggregating suspensions (13). The amount of light transmitted is dependent on the shear rate applied. At high shear rates increased light transmission occurs due to the deformation of cells with flow streamlines and at low shear rates the increased light transmission with time is due to the cell-free gaps associated with rouleaux formation. The aggregometer consists of a transparent perspex cone and plastic plate, the latter being fixed in position and shearing of the sample achieved by rotation of the cone. Infrared light transmitted through the sample is measured with a photometer which produces photovoltages (mV) that are processed in a microprocessor unit incorporated within the machine. Twenty five micolitres of blood at 25°C was dropped onto the centre of the cone and the sample spun at 600 s^{-1} for 10 s to disperse aggregates before being stopped abruptly. The extent of aggregation in stasis, determined as the change in light transmission over 5 s, was integrated via the microprocessor unit and recorded digitally as the aggregation index 'M'. The aggregation index 'M1' represents the extent of aggregation measured also after dispersion at high shear rate, but RBCs were not submitted to stasis and were maintained at low shear rate (6 s^{-1}). The mean of two readings of each index was taken.

RESULTS

The control range for 'M' aggregation index is shown on fig.1. Before 32 wk gestation 'M' seems to be a 'constant' with a mean value of 0.033 $\pm$ 0.009 (n=73). Thus, the confidence interval (mean $\pm$ 2 SD) is between 0 and 0.18. Similarly 'M1' is

a constant in fetal blood throughout pregnancy with a mean value of 2.98 ± 0.26, i.e. the normal range is between 0 and 7.85. Data concerning M1 are not shown since this parameter did not give interesting results. In 111 fetuses explored for various diseases the values of M were investigated. This index of RBC aggregation was increased (> 0.18) in 24 fetal blood samples, with the following diseases: 12.5% toxoplasmosis; 12.5% rhesus immunization; 8.33% autoimmune thrombocytopenia; 8.33% kidney polycystosis; 8.33% old maternal age; 8.33% heart malformation; 8.33% fetal hypotrophia. In autoimmune diseases (rhesus; thrombocytopenia) RBC aggregation was increased in 5/10 cases (50%); in heart or kidney malformations 5/10 (50%); in toxoplasmosis seroconversion 3/23 i.e. 13% of the fetuses had increased aggregation.

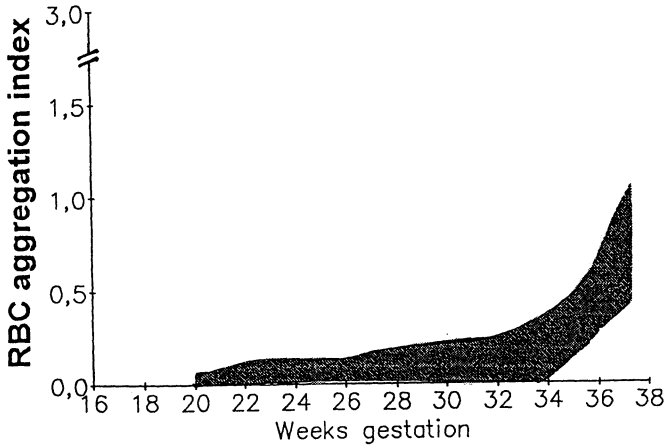

FIG.1
Control range of RBC aggregation 'M' index in fetal blood during pregnancy.

DISCUSSION

The very low erythrocyte aggregation of fetal blood, which results in values of 'M' index close from zero, is a well-documented property of fetal circulation (1, 2, 4). However, the physiological relevance of this hypoaggregation remains unclear. *In vitro* experiments on blood flow in tubes indicate that neonatal (and presumably fetal) erythrocytes have favorable flow properties, and exert a higher Fahraeus-Lindqvist effect than adult erythrocytes, even at high hematocrit values (14-15). Similarly, the cellular mechanisms explaining this hypoaggregation of fetal RBCs are not clearly elucidated.

Therefore, it is difficult to discuss the mechanism and the relevance of erythrocyte aggregation in fetal diseases. This preliminary work indicates that this finding is not uncommon, especially in autoimmune diseases (50%); in heart or kidney

malformations (50%). However, a study on a larger group is required to elucidate if hyperaggregation is a sensitive marker ('the fetologist's ESR') and if it is associated with some circulatory disturbances.

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