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FETAL HAEMOGLOBIN MEASUREMENT IN THE ASSESSMENT OF RED CELL ISOIMMUNISATION

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Summary A reference range of fetal haemoglobin concentration (g/dl) was established from umbilical cord blood samples obtained by cordocentesis (n = 200) or at delivery (n = 10). In normal pregnancy the mean fetal haemoglobin increases linearly from 11 g/dl at 17 weeks' gestation to 15 g/dl at 40 weeks' gestation and one standard deviation is approximately 1 g/dl. The haemoglobin was also measured in fetal blood from 154 red cell isoimmunised pregnancies from 17 to 36 weeks' gestation. In 48 fetuses with ultrasound features of hydrops the haemoglobin was 7–10 g/dl below the normal mean for gestation. It is proposed that in pregnancies complicated by red cell isoimmunisation the severity of the disease should be assessed and treated on the basis of the deviation of the fetal haemoglobin from the normal mean for gestation into mild (haemoglobin deficit < 2 g/dl), moderate (deficit 2–7 g/dl), and severe (deficit > 7 g/dl).

Introduction

IN the management of pregnancies complicated by red cell isoimmunisation, the assessment of the degree of fetal anaemia is customarily indirect, by spectrophotometric measurement of the amniotic fluid bilirubin concentration.[1] Fetal blood sampling from an umbilical cord vessel now allows assessment of the severity of the disease directly by measurement of the haemoglobin concentration.[2-4] We provide here a reference range of normal fetal haemoglobin from 17 to 40 weeks' gestation and propose three zones, analogous to those of Liley for amniotic fluid, for determining the degree of fetal anaemia.

Patients and Methods

Pure fetal blood was obtained from an umbilical cord vessel at 17–36 weeks' gestation from 154 pregnant women with red cell isoimmunisation. Blood sampling was originally by fetoscopy (n = 90) and more recently by cordocentesis (n = 64).[3,4] These patients were referred to our unit from several centres in the UK and overseas for fetal blood sampling and intravascular fetal blood transfusions as necessary. Referrals were on the basis of the diagnosis of severe disease, as assessed by indirect methods including history of previous affected pregnancies, maternal haemolytic antibody levels, measurements of amniotic fluid optical density (ΔOD 450), or ultrasonographic evidence of fetal hydrops. The study was cross-sectional and the data were derived from fetuses that had not yet received transfusions. Gestational age was established from the history of the last menstrual period and from an ultrasonographic measurement of the fetal biparietal diameter at 16–18 weeks.

The presence or absence of fetal hydrops (skin oedema and ascites, pericardial or pleural effusions) was determined by ultrasonography at the time of fetal blood sampling. Fetal 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 haemoglobin concentration (g/dl) was determined by means of a Coulter S Plus counter. The direct Coombs test was positive in all

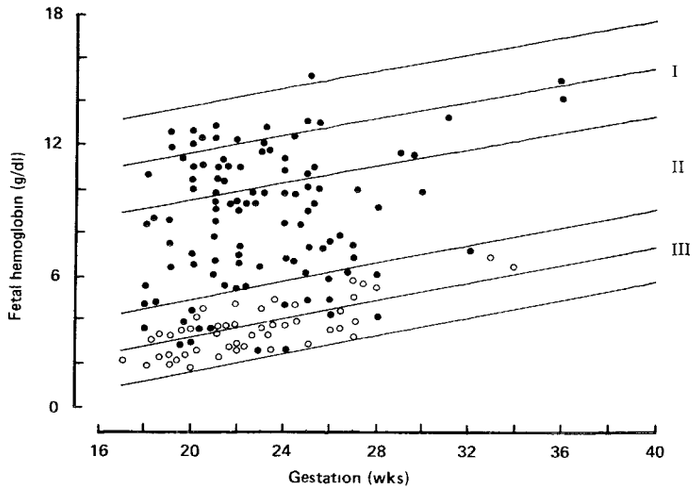

Fig 1—Fetal haemoglobin concentration of 48 hydropic (○) and 106 non-hydropic (●) fetuses from red cell isoimmunised pregnancies at time of first fetal blood sampling.

Values are plotted on the reference range of fetal haemoglobin for gestation. The individual 95% confidence intervals of the normal haemoglobin for gestation define zone I and the individual 95% confidence intervals of the haemoglobin for gestation of the hydropic fetuses define zone III. Zone II indicates moderate anaemia.

fetuses and on Kleihauer staining all samples contained only fetal red cells.

A reference range for normal fetal haemoglobin from 17 to 40 weeks' gestation was established from 200 fetal blood samples obtained by cordocentesis and 10 cord samples obtained after delivery at term. The fetuses sampled were undergoing prenatal diagnosis and were subsequently shown not to be affected by the condition under investigation. Regression analysis was used to calculate individual 95% confidence intervals for haemoglobin with gestational age.

Results

In normal fetuses the haemoglobin rose linearly with gestation ($n = 210$, correlation coefficient $r = 0{\cdot}74$, $p < 0{\cdot}0001$, constant = 7·9, slope 0·19). The 2·5th and 97·5th confidence intervals were nearly parallel and one standard deviation was approximately 1 g/dl (fig 1).

In the hydropic fetuses from the red cell isoimmunised pregnancies the haemoglobin rose linearly with gestation ($n = 48$, $r = 0{\cdot}76$, $p < 0{\cdot}0001$, constant = $-1{\cdot}9$, slope = 0·24). The 97·5th centile was approximately 7 g/dl and the 2·5th centile approximately 10 g/dl below the normal mean for gestation (fig 1).

The severity of red cell isoimmunisation was classified according to the fetal haemoglobin into three zones: zone I lies within the 95% confidence intervals for the normal haemoglobin for gestation; zone II lies between the 2·5th centile of the normal range and the 97·5th centile for the hydropic fetuses; and zone III is defined by the 95% confidence intervals for the hydropic fetuses. There were 38 fetuses whose haemoglobin concentration was in zone I, 50 in zone II, and 66 in zone III.

Volume of Donor Blood Necessary to Correct Fetal Anaemia

When a fetal blood transfusion is given by cordocentesis the volume of donor blood necessary to correct fetal anaemia can be calculated from the pretransfusion fetal haemoglobin concentration, the haemoglobin of the transfused blood, the desired post-transfusion haemoglobin (normal mean value for gestation), and the normal mean fetoplacental blood volume for that gestation,[15] as illustrated in fig 2. The neccessary calculations are given in the appendix.

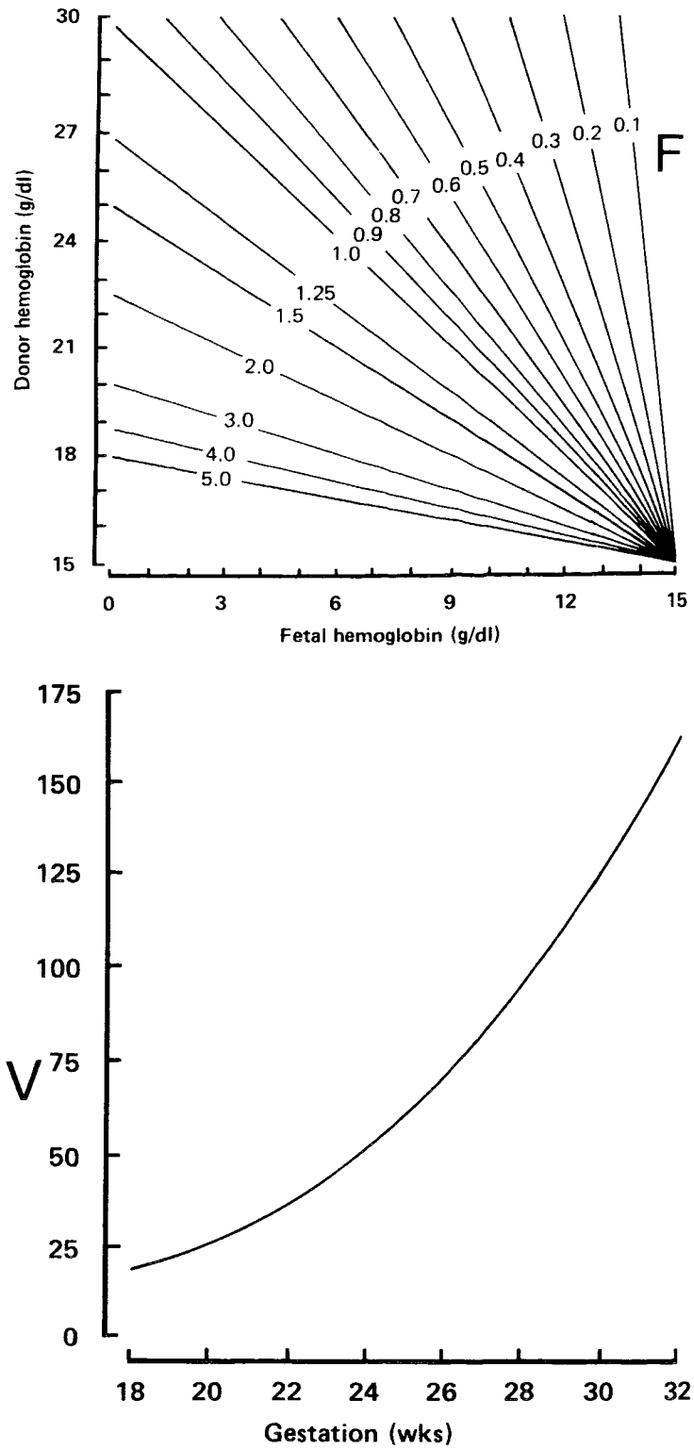

Fig 2—Nomogram for calculating volume of donor blood (ml) necessary to correct fetal anaemia.

The value F is multiplied by the value V. For example, for a fetus at 24 weeks' gestation the V value is 50. If the pretransfusion fetal haemoglobin is 5 g/dl and the donor blood haemoglobin is 26 g/dl (value F = 0·9), then 45 ml donor blood will need to be transfused to achieve a post-transfusion fetal haemoglobin of 12·5 g/dl (normal mean for gestation).

Discussion

In the normal fetus, haemoglobin increases with gestation, presumably to maintain a normal oxygen content despite the decreasing pO_2.[5] This finding is in accord with published data derived from newborn babies and second trimester fetuses that were sampled either at hysterotomy for elective abortion or fetoscopy for prenatal diagnosis.[2,6,7]

The data derived from the red cell isoimmunised pregnancies provide evidence that hydrops fetalis in this

condition is associated with severe anaemia. Although exceptions to this association have been reported, the cited cases had undergone previous intrauterine blood transfusions, whereas our data come from untreated fetuses.[8] Similarly, an explanation of the failure to produce hydrops by induction of anaemia in animals is that the anaemia achieved did not reach the critical level of haemoglobin deficit of 7 g/dl.[9] It appears that in red cell isoimmunised pregnancies the fetus compensates for moderate degrees of anaemia by haemodynamic adjustments.[10] However, when the haemoglobin deficit exceeds 7 g/dl the functional reserve of the cardiovascular system is exhausted and tissue hypoxia, evidenced by erythroblastosis, systemic lacticacidosis, and hydrops fetalis develops.[2,11] No fetuses were found with a haemoglobin deficit of more than 10 g/dl, presumably because this is the minimum haemoglobin necessary to ensure intrauterine survival.

The underlying pathophysiology of red cell isoimmunisation is fetal haemolysis and consequent anaemia and therefore the only accurate method for assessment of the severity of the disease is blood sampling by cordocentesis and measurement of the fetal haemoglobin.[12,13] When the fetal haemoglobin is within 2 g/dl of the normal mean for gestation and the Coombs test is positive, the fetus is only mildly affected and therefore not in need of intrauterine therapy or early delivery. However, such pregnancies should be monitored by serial determinations of maternal haemolytic antibody levels, an increase in which may indicate increased severity of the disease and the necessity for further cordocentesis (unpublished results). Fetuses with a haemoglobin deficit >2g/dl require blood transfusions, which can be given through the umbilical cord by cordocentesis.[4] The volume of donor blood necessary to correct the fetal anaemia is determined as shown in fig 2. After a fetal blood transfusion the mean rate of decrease in fetal haemoglobin is about 0·3 g/dl per day (haematocrit 0·01 per day).[4] Therefore subsequent transfusions can be given before the haemoglobin drops into zone III.

Despite the apparent advantages of cordocentesis in the management of red cell isoimmunised pregnancies, the indication for and the timing of fetal blood sampling in the context of this disease have not yet been defined adequately. The observation that the severity of the disease tends to be progressive with successive pregnancies has led to the tradition that an amniocentesis be perfomed 10 weeks before the time of the earliest previous fetal or neonatal death, fetal transfusion, or birth of a severely affected baby. In the absence of such a history, amniocentesis is undertaken when a "critical level" of maternal haemolytic antibody is reached.[16] However, at least in the second trimester of pregnancy, the data derived from measurement of the amniotic fluid ΔOD_{450} do not define accurately the severity of fetal anaemia.[12] Similarly, although ultrasonography allows the diagnosis of hydrops (thereby identifying a group of severely anaemic fetuses), in the absence of hydrops neither ultrasonographic measurements of placental thickness, umbilical vein diameter, fetal abdominal circumference, head to abdominal circumference ratio, or intraperitoneal volume, nor doppler assessment of the fetal circulation reliably distinguish mild from severe fetal disease.[10,13]

It could therefore be argued that for all patients with a history of severe disease and those with high haemolytic antibody levels cordocentesis rather than amniocentesis should be performed. In the hands of experienced operators the risk of fetal mortality from cordocentesis is only marginally greater than that from amniocentesis.[16] Decisions for or against the procedure—ie, whether the dangers of receiving inadequate information from ultrasonography and amniocentesis outweigh the risks of cordocentesis—will depend greatly on the skills available at the referral centre.

APPENDIX

H_F = Pre-transfusion fetal haemoglobin concentration; V_F = fetoplacental blood volume; H_D = donor blood haemoglobin concentration; V_D = donor blood volume; H_{Fn} = post-transfusion haemoglobin (mean normal gestational haemoglobin.

Fetal pre-transfusion haemoglobin mass = $H_F \times V_F$; donor blood haemoglobin mass = $H_D \times V_D$.

If post-transfusion fetal volume is $V_f + D_D$, then post-transfusional fetal haemoglobin mass will be H_{Fn} $(V_F + V_D)^* = (H_{Fn} \times V_F) + (H_D \times V_D)$. Therefore, $V_D = V_F (H_F - H_{Fn})/(H_{Fn} - H_D)$.

The oblique lines (F) in the nomogram depicted in fig 2a were plotted by expressing the above formula as: $V_F/V_D = (H_{Fn} - H_D)/(H_F - H_{Fn})$, and by making the following substitutions:

(1) H_{Fn} 15 g/dl (mean normal value at 40 weeks),

(2) H_F values from 1–15 g/dl (range of possible pretransfusion values), and

(3) H_D by values from 15 to 30 g/dl (range of possible packed donor blood values).

The values F represent the fractions of the fetoplacental blood volume that needs to be transfused to achieve a post-transfusional fetal haemoglobin of 15 g/dl, with donor packed red cells of known haemoglobin concentrations.

For an individual fetus requiring transfusion the F value is multiplied by the V value read off the ordinate in fig 2b. V is the adjusted fetoplacental blood volume, obtained by multiplying the mean fetoplacental blood volume at each gestational age[14] by the fraction: normal mean gestational haemoglobin divided by 15 ($V = V_F x H_{Fn}/15$).

*Assumes donor blood equilibrates fully with fetal blood.

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