CLINICAL RESEARCH

Fetal haematology in rhesus isoimmunisation

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Abstract

Haematological studies were carried out in pure fetal blood samples obtained fetoscopically in 29 rhesus isoimmunised pregnancies at 18-24 weeks' gestation, and the values were compared with those obtained in 62 normal control pregnancies. Fetal reticulocytosis or erythroblastemia was seen only in association with a haemoglobin concentration of 4 g/dl or less. Ten of the 14 fetuses with a haemoglobin concentration below 4 g/dl showed ultrasonographic evidence of hydrops.

Introduction

Although several innovative approaches in the management of rhesus isoimmunised pregnancies have been undertaken during the past 40 years,¹ the underlying pathophysiology of the disease and of hydrops or intrauterine death in severely affected fetuses remains obscure. The development of a fairly safe technique for obtaining pure fetal blood samples by fetoscopy²⁻⁴ has provided the opportunity to study the haematological profile of rhesus isoimmunised fetuses and hence to gain a better understanding of the pathophysiology of this condition.

This report describes the haematological findings in fetal blood specimens obtained in 29 rhesus isoimmunised pregnancies and compares the values with those in 62 normal controls. The blood values were further correlated with the presence or absence of fetal hydrops as determined by ultrasonography.

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Patients and methods

Twenty nine Rh negative pregnant women at 18-24 weeks' gestation who were considered to be severely rhesus isoimmunised on the basis of traditional indirect methods of assessment were referred from several centres in the United Kingdom and abroad for direct intravascular fetal blood transfusion by fetoscopy.^{5,6} These women had had a total of 122 previous pregnancies, which had resulted in 36 abortions, 12 intrauterine deaths at 20-28 weeks' gestation, 18 stillbirths, 14 neonatal deaths, and only 42 surviving children (perinatal mortality 432/1000). In their present pregnancy all patients, including 15 undergoing intensive plasmapheresis, had high anti-D concentrations (mean 76 IU/ml; range 9-690) and in 16 the measurements of amniotic fluid deviation in optical density at a wavelength of 450 nm were above the extrapolated 80% Liley's zone II.⁷

Linear array real time ultrasound scanning was performed to estimate gestational age, exclude external and internal anatomical defects,⁸ measure fetal heart rate, and detect fetal scalp or general skin oedema, ascites, and pericardial or pleural effusions. Fetoscopy was performed and a pure fetal blood sample taken^{3,4} with a 1.7 mm diameter Olympus Selfoscope or Dyonics Needlescope; after assessment of the degree of fetal anaemia an intravascular fetal blood transfusion was given. Further fetal blood transfusions were given (range 1-6; mean 3) and 25 (86%) of the fetuses survived, including eight with hydrops. In this study only the fetal samples taken before the first intravascular blood transfusion were investigated.

Fetal blood samples (180 μ l) were collected in 20 μ l isotonic edetic acid solution (0.5 mmol/l (15 mg/ml) in 0.15M sodium chloride). Full blood counts were determined with a Coulter S Plus counter. Films were stained with Jenner's Giemsa on an automatic processing machine and differential white cell and nucleated red cell counts determined for every 100 white blood cells. Reticulocyte preparations were made using brilliant cresyl blue; 500 red cells were counted, and reticulocytes were reported as percentages. The fetal blood group was also determined from the same sample using standard serological techniques. All fetuses were Rh D positive, and a fetal direct Coombs test gave positive results.

A normal range of haematological values was established from blood samples obtained fetoscopically in 62 normal fetuses at 18-24 weeks' gestation.

Results

The mean (SD) normal ranges in 62 fetuses at 18-24 weeks' gestation were: haemoglobin concentration 12.1 (1.2) g/dl; red cell count $2.97 (0.41) \times 10^{12}/l$; reticulocytes 11.0 (5.0)% of red cells; erythroblasts 56 (50)/100 white cells; white cell count $2.5 (0.7) \times 10^9/l$; and platelet count 208 (51) $\times 10^9/l$.

Figure 1 shows the fetal haemoglobin concentration in the rhesus isoimmunised pregnancies plotted against gestational age. Ultrasonographic evidence of hydrops (fetal skin oedema and serous effusions) was found in 10 of the 14 fetuses with a haemoglobin concentration of 4 g/dl or less; none of the fetuses with a higher haemoglobin concentration had hydrops.

Figure 2 shows the fetal red cell count and the percentages of

FIG 1—Fetal haemoglobin concentration in 62 normal controls (mean (2 SD), indicated by hatched area) and in 29 rhesus isoimmunised pregnancies with (○) and without (●) fetal hydrops.

FIG 2—Fetal red cell count and percentages of reticulocytes and erythroblasts plotted against fetal haemoglobin concentration in 29 rhesus isoimmunised fetuses (●). Ten fetuses showed ultrasonographic evidence of hydrops (○).

reticulocytes and erythroblasts plotted against the fetal haemoglobin concentration in the rhesus isoimmunised pregnancies. Of the 14 fetuses with a haemoglobin concentration of 4 g/dl or less, 10 had reticulocytosis, one leucocytosis, three leucopenia, and three thrombocytopenia. In the fetuses with a haemoglobin concentration above 4 g/dl all haematological values and cell counts, other than the red cell count, were normal.

There was no difference in the fetal heart rate between the rhesus isoimmunised fetuses, irrespective of the degree of anaemia, and the 62 normal controls matched for gestational age.

Discussion

In this study a normal range of fetal haematological values was established in samples obtained fetoscopically from live fetuses in the late second trimester of pregnancy. Compared with blood from normal newborn infants at term,⁹ the haemoglobin concentration and red cell, white cell, and platelet counts were lower and the percentages of reticulocytes and erythroblasts were higher. These findings are similar to those reported in previous studies on samples from abortuses^{10,11} and agree with the accepted concepts of fetal haemopoiesis.⁹

We found that in midtrimester fetuses in rhesus isoimmunised pregnancies both the development of hydrops and stimulation of active erythropoiesis, as reflected by reticulocytosis and erythroblastemia, are associated with a haemoglobin concentration of 4 g/dl or less. Conversely, as the primary stimulus for increased erythropoiesis is tissue hypoxia¹² these data provide indirect evidence that the fetus can maintain tissue oxygenation despite a reduction of its haemoglobin concentration to one third of the normal value. This may be achieved by adjustments of fetal haemodynamic variables other than the heart rate, which does not change with fetal anaemia. In recent studies in rhesus isoimmunised pregnancies blood flow measurements in the umbilical cord vein¹³ and fetal descending aorta¹⁴ were found to be directly correlated with the degree of fetal anaemia. Other mechanisms that may operate to maintain tissue oxygenation despite severe anaemia, including the possible preferential synthesis of haemoglobin A, with low oxygen affinity, or an increase in the red cell content of 2,3-diphosphoglycerate, are currently under investigation.

Fetal hydrops may occur in rhesus diseases when the functional reserve of the fetal cardiovascular system cannot compensate for the fall in haemoglobin concentration—usually at concentrations below 4 g/dl. The result may be high output cardiac failure with a secondary increase in venous and capillary hydrostatic pressure. Furthermore, at this degree of anaemia tissue oxygenation could be compromised, leading to hypoxic arteriolar dilatation and increased capillary permeability. The skin oedema and serous effusions of hydrops may well result from either or both of these mechanisms. The alternative hypothesis of hydrops resulting from fetal hypoproteinaemia due to hepatic dysfunction,¹⁵ a consequence of extensive hepatic erythropoiesis and distortion of hepatic parenchyma,¹⁶ also finds support in our data, which show an association of hydrops with a critical degree of fetal anaemia at which increased erythropoiesis is evident.

Pronounced leucocytosis, showing the increased activity of the reticuloendothelial system, is found in many cases of acute haemolytic anaemia¹⁷ and has been reported in rhesus isoimmunised infants.^{18,19} This was noted in one of the most severely affected hydropic fetuses in our study. Neutrophils predominated, and many immature cells, particularly metamyelocytes and myelocytes, were present. Leucopenia or thrombocytopenia was noted in five of the 14 fetuses with severe anaemia. These findings suggest that the enormous erythropoietic activity of the fetal bone marrow, liver, and spleen may ultimately diminish formation of granulocytes and megakaryocytes. In one of the hydropic fetuses, however, leucopenia was found in the absence of reticulocytosis and erythroblastemia.

The accepted cause of fetal anaemia in rhesus isoimmunisation

is the reduced life span of erythrocytes coated with antibody, presumably from phagocytosis by reticuloendothelial cells. A further possibility, however, is the relative failure of erythropoiesis or haemopoiesis in general. This might explain the leucopenia and thrombocytopenia in some more severely affected fetuses, with an absence in some of them of reticulocytosis or erythroblastemia. A megaloblastic crisis, where an arrest of haemopoiesis due to folic acid or vitamin B occurs, is a recognised complication of chronic haemolytic anaemia.^{20 21} Although in theory this could apply to some cases of erythroblastosis, one study showed that administering high doses of vitamin B, folic acid, and other known haematinics to the mother did not noticeably benefit the fetal blood.²²

Our findings suggest that in the late mid-trimester the rhesus isoimmunised fetus has a large adaptive functional reserve that maintains tissue oxygenation despite severe anaemia. When the haemoglobin concentration is reduced to one third of the normal value, however, tissue hypoxia, indicated by increased erythropoiesis, becomes apparent. Evidence suggests that more severe tissue hypoxia ultimately leads to widespread haemopoietic failure.

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Hepatitis B virus DNA and e antigen in serum from blood donors in the United Kingdom positive for hepatitis B surface antigen

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Abstract

Serum samples from 214 blood donors in the United Kingdom who were carriers of hepatitis B surface antigen (HBsAg) were examined for hepatitis B virus deoxyribonucleic acid (DNA) by DNA:DNA hybridisation and for hepatitis B e antigen (HBeAg) and its antibody. One fifth of the donors carried infectious virus in their circulation. The presence of hepatitis B virus DNA correlated well with that of HBeAg, although hepatitis B virus DNA was found in five serum samples that were negative for HBeAg.

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It is concluded that analysis of serum samples for hepatitis B virus DNA by hybridisation should be the method of choice for determining whether carriers of HBsAg are infectious.

Introduction

In carriers of hepatitis B surface antigen (HBsAg) viral replication may be continuing in the hepatocytes, with release of infectious virus into the blood stream, or may have stopped, with subsequent clearance of virus from the circulation. Hepatitis B virions may be detected in samples of serum or plasma by assaying for endogenous deoxyribonucleic acid (DNA) polymerase,^{1 2} although a more direct assay, based on the hybridisation of a cloned hepatitis B virus DNA probe to extracts of such samples, has become the method of choice because of its greater sensitivity and ease of application.³⁻⁵

The serological presence of hepatitis B e antigen (HBeAg) is correlated with that of infectious virus,^{6 7} and clearance of virus from the circulation often results in seroconversion with the appearance of antibodies to HBeAg (anti-HBe).⁸ Recent reports, however, have established that an appreciable proportion of patients with anti-HBe have detectable amounts of hepatitis B virus DNA in the serum and so are potentially infectious.⁹⁻¹¹ We examined serum samples from 214 blood donors, who were positive for HBsAg, for the presence of hepatitis B virus DNA