

DETECTION OF FETAL-MATERNAL TRANSFUSION BY ACID ELUTION OF ADULT RED CELL HEMOGLOBIN*

Ömer Bedir, M.D.**

The possibility that the passage of erythrocytes from the fetus to the mother caused certain hemolytic disorders in the newborn was suggested as early as 1941. These disorders are characterized by the presence of fetal hemoglobin in the maternal circulation. Further investigations were made to determine whether fetal red cells cross the placenta only in hemolytic disorders or do so under normal conditions also. Levine and associates¹ suggested that the invasion of the mother's circulation by fetal red cells could occur during normal pregnancies. Other investigators, however, held that fetal-maternal passage of red blood cells occurs only through a damaged placental barrier.

The investigations of the last decade have proved that the infant's red blood cells can invade the maternal circulation in a variety of ways either before or during delivery.

Estimates of the amount of fetal-maternal transfusions have hitherto been based on different investigating procedures. Chown,² Colbatch, Pitt and Maddison,³ and a few other investigators have reported their observations on fetal-maternal transfusions. Their conclusions are based on measurements of the percentage of fetal hemoglobin in the mother's blood. It is doubtful, however, that the reported increases of fetal hemoglobin in maternal blood can be used for confirmation, since in cases of small transfusions, the level of fetal hemoglobin may be close to normal.

Methods of minor red cell population detection which depend upon the removal by differential agglutination of the major population⁴ cannot be employed when the minor population represents less than three or four per cent of the total number of cells present in the reaction.⁴ In these small proportions the antisera fail to agglu-

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** Assistant Professor of Pediatrics, University of Istanbul Pediatrics Clinic.

minate the major red cell population. Hence the differential agglutination procedures have serious limitations even when there are antigenic differences antagonistic to the fetal red cells, and are satisfactory only in instances where there is a major blood group difference between the mother and infant.

The recently described mixed agglutination method of Jones and Silver⁵ is based on the phenomenon whereby separated minor red cell populations take part in the formation of a lattice with other sensitized detector erythrocytes through the medium of specific bivalent antibodies. Again, this method suffers from the limitations on any procedure based on blood group antigenic differences.

The opportunity to observe two cases of severe fetal-maternal transfusion (in one of which both the mother and the child were isogroup, namely, having the same O cDE cde antigenic properties) led us to search for a more precise and efficient investigating procedure. In this quite infrequent situation, the previously used conventional methods were inadequate to detect the presence of fetal red cells in the mother's blood.

An attempt was made to adapt the observation of Kleihauer and his associates⁶ that adult red cell hemoglobin on a fixed smear could be eluted by an acid-phosphate buffer without disturbing fetal cells in the same mixture. This report indicates the clinical practicability of this method applied in a modified form to the detection and semi-quantitation of fetal erythrocytes independently of any blood group antigens and its successful use in a mixture containing a variety of cells from both normal and abnormal adult type hemoglobin with fetal hemoglobin levels as high as 20 per cent.

Materials and Methods

1. *Collection of Blood*: Capillary blood obtained by finger prick was drawn into plastic tubes treated with EDTA. The blood specimens were thoroughly mixed and then diluted to 1 in 4 with normal saline. Smears were prepared of this saline suspension.

For semi-quantitation of fetal cells, 0.01 ml of the specimen was added to 0.04 ml of saline, again thoroughly mixed and similar smears prepared. These two types of smears were dried at room temperature and fixed in absolute alcohol for two minutes.

2. *Elution of Adult Hemoglobin*: After fixation and drying, the slides were placed in equal parts of citric acid and phosphate buffer

(0.20 M citric acid and 0.16 M K_2HPO_4 + 0.02 M K H_2PO_4 containing molar phosphate buffer) with a pH of 3.4 to 3.8, for 30 minutes at 37 C. They were washed three times before staining with Wright's stain or May-Grünwald, and then washed until practically no stain could be seen with the naked eye. After drying at room temperature the slides were scanned under low and high power microscope. Adult cells appeared faint and ghost-like, whereas fetal erythrocytes were correspondingly bright pink or dark intact cells.

Blood smears of 20 normal individuals, selected at random, were also tested. In all of these specimens the red cell hemoglobin could be eluted and no fetal cells were found. On the other hand, in experiments using the blood of two people with sickle-cell traits, the abnormal hemoglobin-S could be completely eluted from the fixed smears at the same speed.

Among ten newborns, the elution of hemoglobin failed in two cases of Thalassemia major and in two cases of sickle-cell anemia, in both of which the fetal hemoglobin concentrations were about 20 per cent.

Case Reports

As stated above, we had the opportunity to observe two cases in which massive fetal-maternal transfusion took place.

Case 1. H. A., a two-day-old baby girl was admitted to the Pediatric Clinic of the Istanbul University because of palor and anemia following delivery. On admission, the baby's condition was fairly good, but she was severely pale and disinclined to movement. She had not cried since birth. There was marked muscular hypotonia. The abdomen was small, the heart rate and the respiration were rapid, and respiration was shallow. A complete blood count yielded the following results: hemoglobin, 7.8 gm per 100 ml; bilirubin, 0.76 mg per 100 ml; reticulocyte count, 1.8. Many normoblasts, mild polychromasia and anisopoikilocytosis could be seen on peripheral smear. The direct Coombs' test was negative. A presumptive diagnosis of anemia due to blood loss was made. The baby's blood group was O Rh + cDe cde.

This was the mother's first child and the pregnancy had been normal. There had been no obvious bleeding before or at the time of delivery. The mother's blood group was B Rh + CDe cde. When the mother's blood was tested with anti C serum, clumps were seen among the mother's unagglutinated red cells. The Ashby technique reduced her red cell count by about 12,400 per ml. The definitive diagnosis was established as fetal-maternal transfusion. The amount of fetal cells present in the mother's blood indicated that approximately 90 ml

Note:

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of the infant's blood had been lost from her circulation. The isoagglutinin level of the mother's blood was found to be 1:1024. Immune antibodies could not be detected ten days later because of lack of laboratory facilities.

Case 2. C.H., a three-day-old baby boy, was brought to the Pediatric Clinic at Istanbul University because of pallor and anemia. On admission the baby was extremely pale and profoundly anemic. He had not cried since birth. His heart rate was rapid and respiration was shallow. The abdomen was small, the liver and spleen were not enlarged. There was an obvious muscular hypotonia. A complete blood count showed hemoglobin to be 6.8 mg per cent; bilirubin, 0.85 mg per cent; reticulocyte count, 1.2 per cent. The blood smear showed many normoblasts, marked anisopoikilocytosis and mild polychromasia. The direct Coombs' test was negative. A tentative diagnosis of anemia due to blood loss was made in this case, too. The baby's blood group was O Rh + cDE cde.

The patient was the mother's second baby. Pregnancy had been uneventful, and membranes had ruptured about 15 hours before delivery. No open bleeding was noted before or during labor, and the mother had not had any subjective complaints concerning labor. The mother's blood type was O Rh + cDE cde. The mother's anti-A and anti-B titers were 1:512.... The Ashby technique was not performed.

In this instance we applied the elution technique to detection of fetal cells and, indeed, the examination confirmed their presence in the maternal blood. The results of low and high power microscopic examinations are shown in Figures 1 and 2.

Semi-quantitation of fetal cells was calculated to be 18,600 per milliliter of maternal blood. This amount corresponds to the loss of approximately 125 ml of fetal blood. Since there was no blood incompatibility between the mother and child, fetal hemoglobin could be detected in the maternal blood even on the 85th day after delivery.

In an additional study, blood specimens of 100 pregnant women, randomly selected without reference to blood groups of the mother and child, were examined on the first day after the beginning of labor and on the first postnatal day. A summary of the data is given in Table 1. These findings, obtained by a relatively simple technique, are in agreement with those suggested by the more complex methods previously used.

TABLE 1
FETAL CELLS FOUND IN BLOOD SMEARS OF 102 RANDOMLY
SELECTED PREGNANT WOMEN BEFORE AND AFTER DELIVERY

No. of women	Delivery	No. of women in whose smears fetal cells were found			
		Before Delivery No.	%	After Delivery No.	%
81	Normal	17	22.4	18	22.2
8	Forceps	2	25.0	2	25.0
4	Difficult Labor	1	25.0	2	50.0
2	Breech	0	0	1	50.0
4	Cesarian	1	25.0	4	100.0
1	Twins	0	0	0	0
2	Abortion	0	0	1	50.0
Total 102		21	(24 %)	28	(27.8 %)

Discussion

The cases presented here are rather characteristic of massive transplacental passage of the fetal red cells to the maternal circulation. These cases seem to offer definite proof that any delivery is itself an event which may cause the passage of fetal blood across the placental barrier. Lesser degrees of hemorrhage, indeed, occur frequently.

Obstetric intervention, and long and complicated labor are frequently the cause of fetal-maternal transfusion. However, it is quite possible under normal conditions that red cells in small amounts may pass from the fetus to the mother.

Although the exact mechanism by which adult type hemoglobins are eluted in a citric acid-phosphate buffer has not been fully explained, it is well established that the method can be used for the detection of transplacental passage of human fetal cells under all conditions irrespective of the number of cells and blood group antigens.

In normal individuals, the initial hemolysis of adult red cells occurs between pH of 5.10 and 4.10. The pH values are somewhat lower for fetal red cells (pH 4.8 to 3.6 at 37 C). However, fetal cells treated with alcohol have a slightly increased pH resistance (4.5 to 3.4) while fetal hemoglobin is completely denatured in these ranges by citric acid-phosphate buffer.

The hydrogen ion concentration of the medium alone, or of the phosphate, may play an important role in the elution of hemoglobins

from the red cells. Since lowering of the pH decreases the negative charge of the phosphate ions and results in an increase in non-ionized H_3PO_4 molecules (the latter being capable of penetrating the red cell membrane and causing changes in membrane permeability) the relation between the hydrogen ions and water and other cations, such as Na^+ and K^+ might be primary in the mechanism of hemoglobin elution.

Whatever the mechanism is, it is obvious that the method can be used as a reliable routine method for clinical purposes without any reference to blood group antigens in detecting fetal cells in the maternal blood stream.

Summary

The author presents observations on the occurrence of severe fetal-maternal transfusion in two cases, in one of which both the child and the mother were of the same blood group. In the second case, conventional investigating procedures based upon differential agglutination failed to demonstrate fetal erythrocytes in the maternal circulation. In this case, the elution of adult red cell hemoglobin on fixed smears prepared from the mother's peripheral blood, confirmed the presence of her infant's intact red cell in her circulation.

In an additional study, labor-induced transplacental passage of fetal red cells was investigated and a brief discussion of the diagnostic procedure is offered.

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