Prenatal diagnosis of β -thalassaemia by fetal red cell concentration with anti-AB serum¹

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SUMMARY Prenatal diagnosis by globin chain synthesis analysis on fetal blood samples obtained by placental aspiration was performed in two pregnancies at risk for β -thalassaemia. Since both fathers had blood group AB, and both mothers had group O, fetal red cell concentration from maternal-fetal mixtures was obtained with the aid of anti-AB serum. With this approach it was possible to carry out globin chain synthesis analysis with very small amounts of fetal blood. Heterozygous and normal genotypes were ascertained and confirmed after birth.

In the last few years, prenatal diagnosis of inherited haemoglobin disorders by fetal blood sampling and globin chain synthesis analysis of fetal red cells has been found to be feasible (Kan *et al.*, 1975, 1977; Alter *et al.*, 1976; Alter and Nathan, 1978; Fairweather *et al.*, 1978; Kan, 1978).

The elimination of the maternal contribution to globin chain synthesis in mixed maternal-fetal blood samples has been carried out in three ways: (1) transfusion of the mother to suppress red cell production (Alter *et al.*, 1978; Fairweather *et al.*, 1978); (2) fetal red cell concentration with differential agglutination, using anti-i serum (Kan *et al.*, 1975, 1977); and (3) differential lysis of maternal cells with the Ørskov-Stewart-Jacobs reaction (Alter *et al.*, 1976). Since high titre anti-i is rare, given certain blood group combinations in the parents, fetal red cell enrichment could be obtained with other antibodies against blood group antigens (ABO, MNs, Rh systems).

This paper reports the results of prenatal diagnosis in two couples at risk for β -thalassaemia, carried out by differential agglutination of fetal red cells with anti-AB serum, as the fathers' blood groups were AB and the mothers' O.

Subjects and methods

Two couples, one of Sardinian origin (case 1) and

the other from Ferrara (case 2), at risk for β^0 - and β^+ -thalassaemia, respectively, requested prenatal diagnosis after genetic counselling. ABO blood group determination showed the mother to be group O and the father AB in both couples. In case 2, there was Rh (D) incompatibility, so anti-D gamma globulin was given 24 h after blood sampling.

Fetal blood sampling was carried out at 22 and 26 weeks' gestation, respectively, after placental localization by ultrasound (Picker model 80L). Fetal blood sampling was attempted twice in case 1. The second time, a blood sample of 20 μ l with 10% fetal red cells was obtained. In case 2, three blood samples of 16, 18, and 9 μ l containing 40, 7, and 7% fetal red cells, respectively, were drawn in the first attempt.

Previous experiments, carried out with a mixture of heterozygous β -thalassaemia group O blood samples and newborn group A or B blood samples at varying concentrations, showed that even with minimal newborn red cell concentration (less than 5%) it was possible to obtain satisfactory agglutination using anti-AB serum, when it was not possible with anti-i serum. It was found that this concentration procedure gave no modification of the β/γ ratio (unpublished results).

The placental blood was incubated with 3% leucine according to the method of Kan *et al.* (1969). Maternal venous blood, drawn before placental aspiration, was incubated in the same way. The placental samples were washed three times with normal saline (0.9 NaCl). A red cell suspension of 20% (v/v) in saline was prepared. A total of 2.5 vol of red cell preparation was mixed with 1 vol anti-AB

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serum (Ortho Diagnostic Inc., New Jersey, USA). The tubes were spun at 300 rpm for 2 min. The red cells were then suspended in 4 ml anti-AB serum

Fig. 1 Kleihauer stain after differential agglutination with anti-AB serum.

diluted 1:50 in saline. This suspension was left to sediment for 20 min, after which the supernatant was aspirated. This procedure was repeated until the supernatant was clear. After this, the samples were tested with the Kleihauer stain.

The globin extraction and globin chain synthesis analysis were carried out according to the method of Kan *et al.* (1969).

Results

The samples obtained with anti-AB differential agglutination were composed of a nearly pure fetal red cell preparation (Fig. 1).

In case 1, the β/γ ratio was 0.038 (Fig. 2) and in case 2 it was 0.10; in the latter case the β/γ ratio after differential agglutination with anti-i was 0.11. These values are in the range found in heterozygous β -thalassaemia and normal mid-trimester fetuses, respectively (Table). Both diagnoses were confirmed by globin chain synthesis analysis on cord blood. ABO blood group determination showed both newborn babies to be group A.

Table β/γ ratios in fetuses at risk for β -thalassaemia

	β/γ ratio
Case 1	0.038
Case 2	0.10
8 normal fetuses*	0.084–0.12
26 β -thalassaemia trait fetuses*	0.028–0.075
23 homozygous β -thalassaemia fetuses*	0.00†

*, unpublished results; †, in one case at risk for β^+ -thalassaemia a β/γ ratio of 0.05 was found.

Fig. 2 Chromatogram of a placental blood sample of case 1. — ^3H leucine incorporation; --- absorbance at 280 nm. Unlabelled newborn and sickle cell trait haemolysates were added to the sample as carriers.

Discussion

The study of these two pregnancies at risk for β -thalassaemia showed that the concentration of fetal red cells from maternal-fetal mixtures, for prenatal diagnosis of β -thalassaemia, can be carried out in selected cases with anti-AB serum instead of anti-i serum.

Differential agglutination with anti-AB serum was possible even with minimal fetal red cell concentration in very small blood samples; this would not have been possible with anti-i serum (Kan *et al.*, 1977). Anti-AB differential agglutination produces no modification in the β/γ ratio.

Complete blood group testing should be carried out in both members of a couple before attempting prenatal diagnosis of β -thalassaemia, in order to find genetic paternal-maternal blood group combinations which permit the use of antibodies other than anti-i for fetal red cell concentration.

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