# PRE-LABOUR DETERMINATION OF FOETAL BLOOD GROUPS

by

SHASHI RAMESH,* M.S.

E. PETERS,** F.R.C.O.G.

and

S. M. DUGAR,*** D.M.J.

As early as 1956 it was reported by Fuchs *et al* that the ABO as well as rhesus grouping of the foetus can be done by examination of the 'amniotic-fluid', but as there was no practical application, transabdominal amniocentesis was regarded as a procedure not free from risk (Ducos, 1958; Ducos and Marty 1964). However, recent work has demonstrated that transabdominal amniocentesis is as safe a procedure as any other surgical procedure, and the same has been widely used in the management of rhesus sensitization (Mackey, 1961; Liley, 1963; Whitfield *et al* 1968).

The present study was undertaken in collaboration with the department of Forensic Medicine, S.M.S. Medical College Jaipur, who were interested in solving the cases of disputed paternity at the intrauterine stage also. However, the opportunity was also utilised to confirm the studies of sex determination from amniotic fluid specimens. Moreover, it was suggested that if this can be a reliable technique for the determination of the rhesus group of the foetus, it can further help the decision to undertake intrauterine transfusion in case of the critically affected foetus requiring immediate transfusion.

*Material and Methods*

Assessment was done in 400 cases in which the pregnancy had progressed to about 34 weeks, at Zanana Hospital, Jaipur, between February 1969 and April 1969. Amniotic fluid was collected during labour in 280 cases, in 110 cases it was collected while vaginal amniotomy was being performed. In 10 cases it was collected by abdominal amniocentesis. Heavily blood-stained specimens were discarded. Samples giving positive occult blood test were also discarded.

The collected samples were centrifuged immediately and the amniotic cells thus obtained were used for:

A. ABO grouping by the indirect antibodies absorption technique;

B. Determination of the sex chromatin by examination of stained smear.

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*Reader in Obst. & Gynec.

**Prof. and Head, Dept. of Obst. & Gynec.

***Chemical Examiner to the Govt. of Rajasthan.

M.S. Medical College, Jaipur.

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C. Rhesus (D) grouping by the indirect antibodies absorption technique.

The clear supernatant fluid thus obtained was also used for ABO grouping by 'Principle of Mixed Agglutination Inhibition'. Standard antisera for A B & O blood groups were diluted with normal saline so as to obtain a dilution of 1:5, and were placed in three sets of test tubes. A small amount of supernatant fluid was added to one set of test tubes, using various dilutions. Saline as a control was added to another set of test tubes having antisera. After 30 minutes of incubation at room temperature, a small quantity of known 2 to 5% suspension in saline of freshly washed red cells of A, B, and O groups were added separately with a Pasteur pipette. All the sets of tubes were allowed to stand in an incubator at 37°C, for fifteen minutes, and then centrifuged for 30 seconds at 3,000. rpm. Results were determined after a few minutes by observing agglutination. If such clumpings were present in both the tubes having saline and one having supernatant amniotic fluid, the blood group substance was not present. If in the control (saline) tube agglutination occurred, with none in the tubes having supernatant amniotic fluid, the blood group of the latter had inhibited the antisera activity. It, therefore was the type present on the foetal cells (Guy and Taylor, 1966). The contents of each tube not showing obvious agglutination were removed, spread evenly on a microscope slide and examined under the low power of a microscope for confirmation. Control tubes were always read first, and only when these were satisfactory others were read.

ABO Grouping from amniotic cells was done by the indirect antibody absorption technique described by Duros, (1958). Briefly, the amniotic cells to be tested (obtained after centrifugation of the amniotic fluid) were washed and incubated with the antiserum overnight, together with a control specimen of antiserum. After centrifugation the antiserum incubated with the cells was titrated against the control; if the amniotic cells carry the appropriate antigen the antiserum shows a lower titre than the control.

After birth of the baby the blood was collected from the umbilical cord and confirmatory ABO and rhesus grouping on the red cells were carried out. The phenotype sex of the child was also confirmed.

*Results*

A. Determination of A, B and O group.

Correct results were obtained in correlation to amniotic cells in liqour specimen and from the cord blood specimen in all the 400 cases. However, cells obtained from the liqu : collected during labour or near te l reacted poorly.

Complete correlation existed between A, B and O group as determined from the supernatant fluid separated out from the liquor specimen and the cord blood. No difficulty was encountered even when the amniotic fluid was collected durin labour.

B. Nuclear Chromatin Deter tion:

Showed complete correlation with the phenotypic sex. Sacs containing male babies had all chromatin negative specimens. Chromatin positive specimen cells came from sacs having female babies.

C. Rhesus (D) group determination:

We were able to determine the rhesus group in 200 cases only. Correlation obtained between the determinations of cord blood and amniotic squames were far from satisfactory with the rhesus grouping (the figures are shown in Table I). It is evident that only 92 cases were correct while 108 were incorrect. Sometimes liquor predictions were negative and blood was positive and vice versa.

TABLE I

*Determination of rhesus group from examination of amniotic squames*

| Total cases | Correct | | Incorrect | |
|---|---|---|---|---|
| | Positive | Negative | Positive (or negative) | Nagative (or positive). |
| 200 | 88 | 4 | 74 | 34 |

*Discussion*

We were able to confirm the determination of the foetal ABO blood groups from amniotic cells, as already reported by Fuchs *et al* (1956) and Ducos (1958). However, to us it appeared that the determination of the foetal blood group from the clear amniotic fluid is 30% more accurate and easy than the previous tests (Guy and Taylor 1966), as the procedure operates on the simple principle of haemagglutination inhibition (Wiener, 1963; Roy and Chaterjea, 1965 and Guy and Taylor, 1966). Virtually all cells, including those shed off by the foetus, contain blood group antigens. However, such cells are not present in large quantities in the amniotic fluid and cells collected at the time of delivery or near term have been found to react poorly. Blood antigen being soluble expresses itself as a whole in the amniotic fluid (Guy and Taylor 1966). The ability to determine the foetal ABO blood groups can be used with advantage for excluding parentage. The mode of inheritance of the ABO system was formulated by Bernstein (1924, 1925). The characters A, B and O are inherited by means of three allelomorphic genes, also called A, B and O. Every individual inherits 2 of these genes, 1 from each parent, so that the the possible ABO genotypes are AA, AO, BB, BO and OO.

The population can be divided into the 4 groups by using the two antisera, anti-A and anti-B., that is Group A (phenotype A) comprising 2 genotypes AA (homozygous) and AO (heterozygous); group B (phenotype B) comprising 2 genotypes BB (homozygous) and BO (heterozygous); group O (phenotype O), genotype OO (homozygous) and group AB, where the phenotype and the genotype are both AB (heterozygous). A and B behave as dominant genes, so it follows that these agglutinogens cannot appear in the blood of a child unless present in the blood

of one or both parents. Although the position of the O gene is controversial but, from the practical point of view in problems of paternity it is regarded as recessive.

Table II shows the various ABO matings and the children which can arise from them. While Table III shows phenotype matings and the possible and impossible phenotypes of children arising therefrom.

The reliability of the ABO system for the cases of doubtful paternity has been convincingly demonstrated before. The inheritance of this system has been well worked out and entirely reliable. It has been used as decisive by many courts of law (Race and Sanger 1962; Sussman 1965). However, Haselhorst and Lauer, in 1930, reported a case of apparent mutation, in which a mother of group AB had a child of group O. But, in view of the more modern work this can be explained by the presence of a suppressor gene operating in the child.

The technique may be used for making provision of like-grouped blood for immediate exchange transfusion in case the child is affected by haemolytic disease. The only consideration to be made is for mother's agglutinins from the point of view of the haemolysis of the donor's blood, as agglutinins in the donor's blood may increase the haemolysis of the recipient's blood of a different ABO group. The procedure can also help in having like-grouped blood for intrauterine transfusions.

The ABO groups have also been suspected of contributing to miscarriages. McNeil (1954) reported that compared with other groups a relatively larger proportion of abortions occurred among B incompatible marriages. This was further proved by Allen (1964). However, the claim was not accepted without some reservations by Race and Sanger (1962).

We were able to confirm the earlier reports of Riis and Fuchs (1960), Serr and Margolis (1964) and others that a correct prediction of foetal sex can be made in utero, by the examination of the amniotic fluid. The technique can be used for selecting the blood of the opposite sex for intrauterine transfusion in order that any persistence of transfused lymphocytes may be detectable by chromosome analysis as suggested by Jones, (1968). This can also help in taking a decision for selective therapeutic abortion in case of sex-linked diseases.

Witebsky and Mohn (1945), Roy and chatterjea (1961) and Ohlert *et al* (1962) have made studies for the detection of traces of rhesus antigen in amniotic fluid, believing it to have a soluble form and thus present in the amniotic fluid, while others tried to prove the presence of the existence of rhesus antigen on other cells than those of the erythropoietic system, (Boorman and Dodd, 1943, Mohn and Witebsky, 1948, Jankovic and Lincoln, 1959, Skrzypulec and Skorezynski, 1968). However, it is now generally accepted that rhesus antigens are only detectable in red blood cells and not in other secretions or cells (Walker and Bailey, 1956, Levine and Celano, 1961, Lawler and Shatwell, 1962).

Ducos (1958), Freiesleben *et al* (1958), Ducos and Marty (1964), Skrzypolec and Skorczynski (1968) and Ducos *et al* (1966) have tried to

TABLE II

*The Various ABO matings and the children which can arise from them*

| Mating | | Children | |
|---|---|---|---|
| Phenotypes | Genotypes | Genotypes | Phenotypes |
| A X A | (1) AA X AA<br>(2) AA X AO<br>(3) AO X AO | (1) AA<br>(2) AA and AO<br>(3) AA, AO, and OO | A and O. |
| A X B | (1) AA X BB<br>(2) AA X BO<br>(3) AO X BB<br>(4) AO X BO | (1) AB<br>(2) AB and AO<br>(3) AB and BO<br>(4) AB, AO, BO and OO | A, B, AB and O. |
| A X AB | (1) AA X AB<br>(2) AO X AB | (1) AA and AB<br>(2) AA, AB, AO, and BO | A, B and AB |
| A X O | (1) AA X OO<br>(2) AO X OO | (1) AO<br>(2) AO and OO | A and O |
| B X B | (1) BB X BB<br>(2) BB X BO<br>(3) BO X BO | (1) BB<br>(2) BB and BO<br>(3) BB, BO and OO | B and O |
| B X AB | (1) BB X AB<br>(2) BO X AB | (1) AB and BB<br>(2) AB, BB, AO, and BO | A, B, and AB |
| B X O | (1) BB X OO<br>(2) BO X OO | (1) BO<br>(2) BO and OO | B,& O. |
| AB X AB | (1) AB X AB | (1) AA, AB, and BB | A, B and AB |
| AB X O | (1) AB X OO | (1) AO and BO | A and B. |
| O X O | (1) OO X OO | (1) OO | O. |

TABLE III

*ABO matings showing possible and impossible phenotypes of children*

| Mating | Possible phenotypes of children | Impossible phenotypes of children. |
|---|---|---|
| A X A | A and O | B and AB. |
| A X B | A, B, O, and AB | None. |
| A X AB | A, B, and AB | O. |
| A X O | A and O. | B and AB. |
| B X B | B and O. | A and AB. |
| B X AB | A, B, and AB | O. |
| B X O | B and O. | A and AB. |
| AB X AB | A, B and AB | O. |
| AB X O | A and B | AB and O |
| O X O | O | A, B, and AB. |

demonstrate the presence of rhesus antigen on the amniotic cells. However, in our present study we are not able to confirm the claim that the rhesus antigen is present on amniotic cells. Although out of 200 cases 92 were correct suggesting that it cannot be by chance, the low accuracy of this technique has very much limited its clinical importance.

*Summary*

Clear amniotic fluid and the desquamated cells from the centrifuged specimens of liquor amnii were studied and examined for ABO group while desquamated cells alone were examined for nuclear chromatin and rhesus (D) group. We were able to confirm the previous claims that ABO blood group and phenotypic sex of the foetus in utero can be predicted accurately. However, we were not able to confirm the claims regarding the reliable prediction of rhesus group. The advantages and the application of the techniques are discussed.

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