SPECTROPHOTOMETRIC ANALYSIS OF LIQUOR AMNII IN Rh IMMUNISED WOMEN

by

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Introduction

In the management of pregnancy of the Rh immunized mother, the obstetrician often has to resort to timely induction to save the foetus. Too early an induction increases the risk of prematurity, while delayed induction increases the risk of death due to haemolysis. It is important to balance these two risks by correctly assessing the severity of haemolytic disease.

The assessment of the severity of haemolytic disease has been based mainly on past obstetric history and maternal antibody titre. Within the present decade, spectrophotometric analysis of amniotic fluid has been found to be an important tool to assess the foetal condition more reliably. Bevis (1953) showed a close relationship between the pigment content of the amniotic fluid and the severity of haemolytic disease in the newborn. Liley (1961) and Freda (1966) evolved systems of spectrophotometric analysis and grading whereby the condition of the foetus in utero could be assessed.

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That the antibody titre of the mother does not give a fully reliable picture of the haemolytic disease, is shown in Table 1 covering 94 immunized cases.

Even when the titre was low, 1:16 or less, there were 2 cases out of 26 which resulted in perinatal loss. A more reliable assessment of foetal condition and timely induction would have helped for the 20 cases which died soon after birth, and for the 14 cases of full term stillbirths. The 8 cases of premature stillbirth could perhaps have been helped by intrauterine transfusion.

Spectrophotometric Analysis

The breakdown products of haemoglobin and bilirubin in the amniotic fluid, i.e. each one of the related bile pigments, absorbs monochromatic light somewhere in the wavelength range between 525 mu and 375 mu and the summation of their light absorptions plotted against wavelength forms the spectrophotometric curve. In a 'normal' spectrophotometric graph, the curve is smooth and sweeping upwards in the lower wavelength zone due to turbidity, vernix, uric acid etc. With increasing concentration of bile pigments in the amniotic fluid, a hump occurs on the curve at 450 mu. The height of the hump above the expected slope of the curve at 450

TABLE 1

Outcome of Pregnancy in Relation to Maternal Antibody Titre

Titre	Total No. of cases	F.T. del. with mild jaundice	Severe jaundice; lived with exch. transfusion	Died	F.T., S.B.	Pre. S.B.
1: 16 and less	26	20	4	0	0	2
1: 32 to 1: 64	20	5	7	5	1	2
1: 128 to 1: 256	22	0	10	5	6	1
1: 512 and greater	26	0	6	10	7	3
Total	94	25	27	20	14	8

mμ is used as an indication of the severity of haemolytic disease (Fig. 1). By plotting the curve on semi-logarithmic paper, the height of the hump can be more conveniently measured.

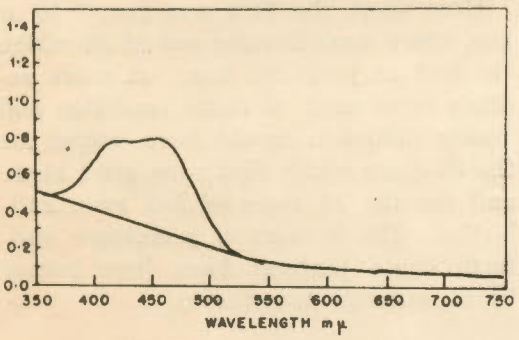

Fig. 1

The spectrophometric curve of liquor amnii of an immunised woman with affected baby.

Liley's Chart

By examining a number of specimens of liquor obtained at various periods of gestation from immunized patients and correlating them with the foetal condition at birth and cord blood findings, Liley has graded the height of the hump into 3 zones (Fig. 2). As the height of the hump decreases with increasing maturity, he has taken the gestational period also into consideration while fixing the 3 zones.

If the height of the hump (optical density) falls in the lower zone, the foetus may be considered little or mildly affected. If the height of the hump falls in the upper zone, the foetus may be severely affected with intrauterine death imminent. The middle zone indicates foetus which requires close observation and follow up study of amniotic fluid in subsequent weeks.

Material and Methods

Our study comprises of 148 spectrophotometric curves of liquor amnii, 119 from normal non-immunized patients and 29 from Rh immunized women. In the non-immunized cases amniotic fluid was collected when the patients were in early labour as we did not wish to take any risk of premature onset of labour, infection, placental separation, etc. which might jeopardize the outcome of the pregnancy. In the immunized Rh-ve patients, the amniotic fluid was studied of those with antibody titre of 1:16 or more. Amniocentesis was performed between 33 weeks and full term, taking into consideration the past foetal outcomes and antibody titre.

The patient was made to empty her bladder and then examined for the foetal presentation, position and foetal heart sounds. Perfect aseptic precautions were taken. The site for the puncture was selected in the space occupied by the foetal limbs or the space just posterior to the neck of the foetus. A small quantity of local anaesthetic was injected and a 21 or 22 number spinal needle (3½") was inserted in the uterine cavity. About 10 ml. of fluid was gently withdrawn and collected in a clean test tube covered with black paper or in a brown coloured bottle, so as not to lose any pigment colouration by exposure to light.

Premedication with Inj. Pethidine 100 mg. intramuscularly was given to only 9 apprehensive patients.

Seven of our taps were frank blood and four of these were successful when another site was selected to avoid the placenta. Seven other taps were blood stained liquor.

Five ml. of amniotic fluid spun at 4,000 r.p.m. for 15 minutes, was filtered with Whatman filter paper. The clear fluid was placed in the cuvette and analysed spectrophotometrically, as explained earlier.

Results

Table II demonstrates the relationship of antibody titre to the 29 spectrophotometric findings of immunized patients graded according to Liley's chart.

Table III demonstrates the relationship of the spectrophotometric findings to the foetal outcome.

One hundred and nineteen spectrophotometric curves of normal non-immunized patients were studied. Most of these patients were tapped in early labour and all of them were 38 weeks' or more. All had normal graphs except 7 cases which showed abnormal humps on the graph. Of these 3 had meconium-stained liquor and all three were in early labour. Four had contamination of amniotic fluid by blood.

An example of the usefulness of spectrophotometric analysis is the case of Mrs. B. A. Her antibody titre was 1:64 and she had history of previous full-term stillbirth. Amniocentesis and spectrophotometric analysis of liquor done twice showed the optical density peak at 450 u. (See fig. 2). going closer to the critical zone. Labour was induced at 36 weeks and immediate exchange transfusion was done. Because of the severity of haemolytic disease the baby's bilirubin continued to rise (see Fig. 3) and two further exchange transfusions were given and the baby survived.

TABLE II

Relationship of Antibody Titre to the Spectrophotometric Findings

Titre	Total analysis	Number of liquors with 450 mu peak		
		In safe zone	In observation zone	In critical zone
1:32	8	7	1	—
1:64 and 1:128	10	3	7	—
1:256 or greater	11	2	4	5

TABLE III

Spectrophotometric Finding and Outcome of Pregnancy

Spectrophotometric result from Liley's Chart Figure 2	No. of cases	Cord blood haemoglobin Range gms. %	Cord bilirubin Range mg. %	Outcome
Safe zone	12	11-17	2.5-4.5	7 lived without treatment 5 required exchange transfusion, of them 1 died, 4 lived.
Middle zone	12	6-12	4.0-8.5	11 required exchange transfusion, all lived 1 intra-uterine death
Critical zone	5	Cord blood not available	Cord blood not available	1 required exchange transfusion, but died 1 died immediately after birth. 3 intra-uterine deaths.

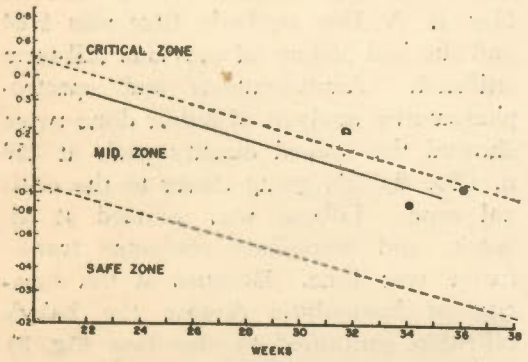

Fig. 2

Liley's chart showing the value of peaks plotted on it. The baby was going closer to the critical zone.

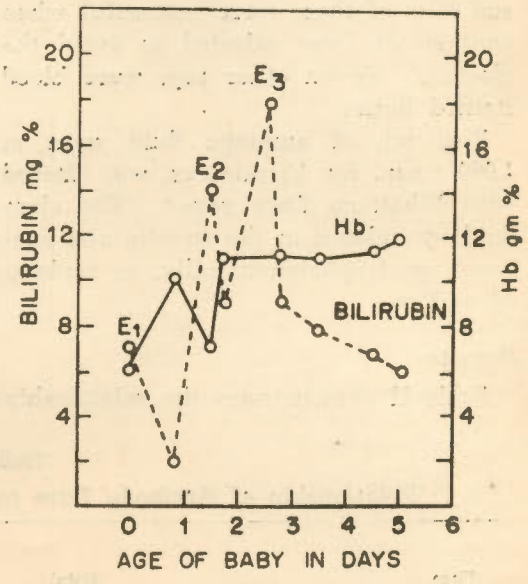

Fig. 3

This shows the level of bilirubin, Hb% of the affected baby who received exchange transfusions.

Discussion

Thls preliminary study convinces us that spectrophotometric analysis of liquor amnii is very helpful in the management of pregnancies of Rh immunized women. In this small series of 29 graphs of liquor amnii of immunized women we find 27 correlate with the severity of haemolytic disease of the newborn.

No doubt these studies would be more useful if started from early third trimester in selected cases and if tappings

were repeated more often (about once in 7 to 14 days). Until we gained more experience, we were conscious of the hazards of amniocentesis and avoided early and repeated taps.

The hazards (complications) of amniocentesis are:—

1—Introduction of infection
2—Premature onset of labour
3—Foeto-maternal haemorrhage and rise of antibody titre
4—Trauma to the foetus
5—Bloody taps from uterine wall or placenta. If placental localisation were possible, the incidence of bloody taps, injury to placenta and foeto-maternal haemorrhage would be minimised.

Errors in the assessment of haemolytic disease from amniotic fluid analysis could be due to:

Wrong fluid, which would be maternal urine or foetal ascitic fluid. In case of multiple pregnancy the condition of the two foetuses may be different and therefore analysis of fluid from one sac will not indicate the condition of both foetuses. There is also the rare possibility of obtaining the wrong fluid from an amniotic cyst.

Wrong pigment or source of pigment. This may be serum and haemoglobin from the foetus or mother. The graph is much more misleading if there is contamination with heavily pigmented serum of an affected foetus. Meconium staining of amniotic fluid can cause an error of assessment but this is easily identified, as the curve has a peak at 450 mu, but has a taller peak at 410 mu. Meconium staining is not likely to occur except when patient is in labour or close to term.

The perinatal mortality of babies of Rh immunized women has been brought down from about 20% to 9% by amniotic fluid analysis and pre-term induction in necessary cases. Selective premature delivery to prevent stillbirths or delivery of severely anaemic or moribund neonates has been practised for many years. It is very essential to reliably assess the intrauterine condition of the foetus so that the best compromise between prematurity and anaemia is achieved. A further development in intrauterine treatment of an anaemic foetus is intraperitoneal packed cell transfusion given to prevent intra-uterine death and postpone induction of labour of a very premature foetus. Spectrophotometric assessment of liquor amnii is an important tool for judging the correct timing for such intraperitoneal transfusion. as well.

Summary

The method of spectrophotometric analysis and method of assessment of the severity of haemolytic disease of the newborn is presented. One hundred and nineteen graphs of the liquor from normal non-immunized women and 29 graphs of liquor from Rh immunized women have been studied. The correlation of these graphs with the maternal antibody titre and the foetal outcome is discussed. Hazards of amniocentesis and errors of spectrophotometric assessment are briefly mentioned. The usefulness of spectrophotometric analysis for timely induction of labour for affected foetuses, is stressed.

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