AMNIOCENTESIS AND SPECTROPHOTOMETRIC ANALYSIS OF AMNIOTIC FLUID IN RH NEGATIVE CASES

by

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Safety of amniocentesis now has been proved by various authors. Amniocentesis is no longer carried out for the academic purpose only and its implication in obstetric practice in the management of Rh immunized patients is remarkable. Although serological evidence of sensitization in Rh negative woman indicates the possibility of hemolytic disease, the probability of event is difficult to assess (Krishna, 1967). The assessment of foetal condition requires a test on foetus and the requirement is met by analysing amniotic fluid which is most valuable in forecasting the condition of newborn due to the close proximity. Since the introduction of it's relation with hemolytic disease (Bevis 1950) and by speculation of concept of △ 0.D. 450 mu by Liley (1961), the perinatal mortality due to Rh incompatibility has been considerably lowered.

Material and Methods

This included 30 Rh negative patients on whom spectrophotometric analysis of amniotic fluid was performed. Out of 30 cases, 6 were immunized. Amniotic fluid was obtained by amniocentesis. The patients were hospitalized a day prior to the procedure. Abdominal palpation was done to find the position of foetus and foetal heart sounds were checked. After taking aseptic measures spinal needle 21 was inserted through abdominal wall in suprapubic region in midline while presenting part was pushed up. About 10-15 cc of amniotic fluid was aspirated and stored in brown coloured bottle in order to avoid adverse effect of light. Amniotic fluid was filtered through Watman filter paper No. 1 and centrifuged at 4000 rpm for 15 minutes. Spectrophotometric analysis was either done on the same day or the amniotic fluid was stored a 4°C. Helger's spectrophotometer was used for this study. The principle of this involves the amount of visible, ultraviolet and infra-red rays at definite wave length which when absorbed by amniotic fluid gave the information about optical density of amniotic fluid under test which can be read on the scale. The optical density of various wave lengths from 350-750 mu was determined by using distilled water as blank and the results were plotted on semilog paper. The optical density at 450 mu was calculated by drawing a straight line between 350 mu—550 mu (Fig. 1) the difference in optical density between this and actual reading at 450 mu was termed the peak △ O.D. 450 mu. In isoimmuniz-

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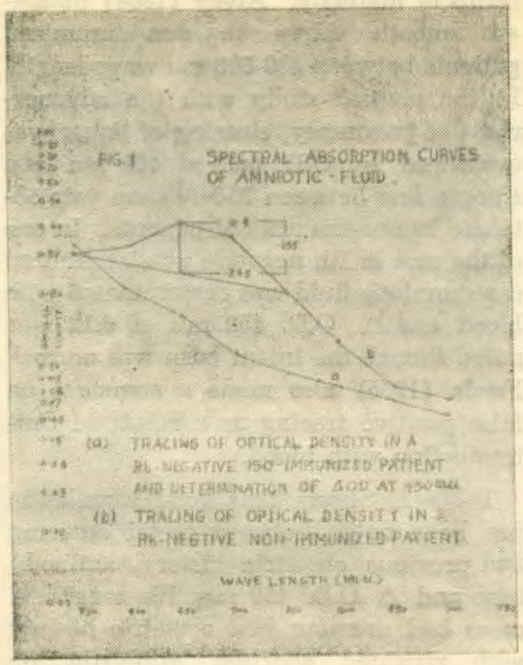


ed patients a definite peak at 450 mu wave length was obtained. This peak was plotted against the period of gestation in weeks on semilog paper as has been devised by Liley (1961). The peaks grouped themselves into three zones depending upon severity of disease. The results were divided into two groups.

Group I. Rh negative cases.

Group II. Rh immunized cases.

Observations and Discussion

Success and Failure Rate

Thirty patients were subjected to amniocentesis during varying period of gestation from 32 to 40 weeks. Out of 30 cases only in 1 case it was not possible to get amniotic fluid thus giving a failure rate of 3.3%. In 2 cases second attempt was required to aspirate fluid. Mackay (1961) reported 77 failures among 300 cases, whereas Walker (1957) had failure in 11.6% of his cases. Cary (1960) reported 6 failures in 89 cases, whereas Alvey (1964) could not aspirate amniotic fluid in 9 out of 200 cases. Fairweather and Walker in 1964 reported a failure rate of 8.9%.

Complications

Though amniocentesis has been proved to be a very safe procedure by Bevis (1953), Liley (1961) Freda (1963) Robertson (1964) and Waston (1965), various foetal complications had been encountered. Aspiration of blood was reported by various workers. Liley (1961) aspirated blood in 9 out of 101 cases, and Walker and Jennison (1963) reported 5% bloody taps. In our study out of 30 cases in 3 cases (10%) blood was aspirated. Fairweather and Walker (1964) and Queenan and Adam (1965) reported a very high percentage of cases 52% and 66% respectively where blood was aspirated. This has an important bearing in rise of antibody titre in case the placenta is injured (Zipursky 1963 and Hibbard 1963). Hence location of placental site has been advised before amniocentesis. In the present study blood was aspirated in 3 non-immunized Rh negative patients but these developed no demonstrable antibodies. Mackay (1961) noted rise of maternal antibodies occasionally following amniocentesis. Zipursky (1963) noted that out of the 4, 3 immunized patients in whom blood was aspirated during amniocentesis showed rise of antibody titre. Queenan and Adam (1965) observed rise of titre in 4 out of 5 cases where blood was aspirated, whereas Freda (1965) noted rise in 5% of total 379 taps. Hibbard (1963) claimed, "The maternal antibody titre may be boosted and the woman is condemned to repeat still birth and neo-natal death".

No foetal complication was encounter-

ed during the course of this study. Fairweather and Walker (1964) reported direct injury to the foetus and 2 deaths have been reported 1 each by Robertson (1964) and Fairweather and Walker (1964).

No maternal complication occured except that pain in lower abdomen was complained of by most patients and one patient had tachycardia and giddiness. There was no instance of intrauterine infection, abruptio placentae and premature labour.

Spectrophotometric Analysis
Spectral Absorption Curves

Optical density at the varying wave lengths from 350-750 mu each at the interval of 50 mu wave length was plotted on semi log paper for each case. Fig. 3 shows the tracing of liquor amnii as a smooth curved line between 350-750 mu which sweeped upwards in lower wave length range. This is due to turbidity, vernix and uric acid which are normally present in the amniotic fluid. Bevis (1953) has propagated that oxyhaemoglobin present in amniotic fluid gave bands at 415 mu, 540 mu and 575 mu wave length. Bile pigmants present in amniotic fluid are able to absorb nonchromatic light in the wave length ranging between 525-375 mu and the summation of their respective light. Absorption produces a characteristic abnormality in spectrophotometric tracing. This abnormality is readily recognised as a 'broad hump' which increases in the amplitude with the increasing concentration of pigment (Freda 1965). The deviation at 450 mu due to increased optical density was attributed to the bilirubin concentration which reflects antibodies.

Liley (1961) measured the peak at 450 mu and established limits of normality in terms of maturity. Alvey (1864) obtained smooth curve in non-immunized patients between 400-600 mu wave length. In the present study with the advancement of pregnancy, clearing of liquor was evidenced by O.D. 0.00 at 450 mu and smooth line between 350-700 mu was obtained in non-immunized patients. In one of the case of Rh negative non-immunized case amniotic fluid was contaminated with blood and △ O.D. 450 mu of 0.12 was noted though the infant born was normal. Freda (1965) also made a comment on false positive tracing as a result of contamination with blood.

Table 1. Shows that good carrelation has been noted between foetal outcome and previous obstetric history, antibody titre and △ O.D. 450 mu. Rh sensitized cases had previous bad obstetric history with repeated abortion, stillbirths or neonatal deaths due to jaundice. These cases also had high peak of △ O.D. 450 mu and all these new born were afflicted with hemolytic jaundice and required exchange transfusion.

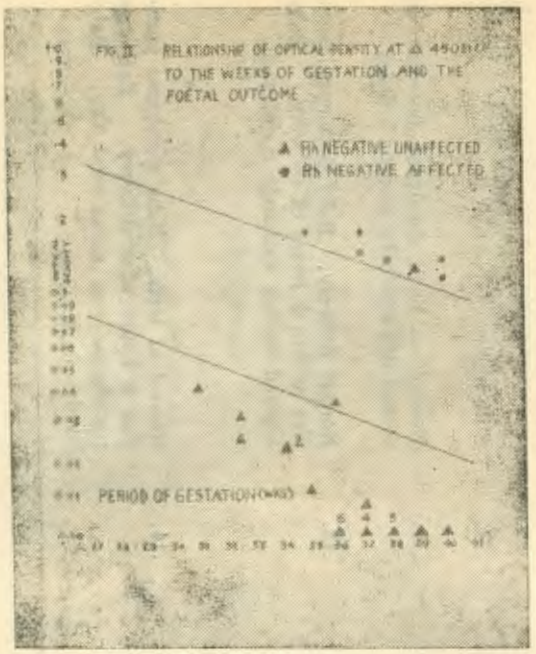
Relationship of Optical Density at 450 *mu at Weeks of Gestation to the Foetal Outcome*

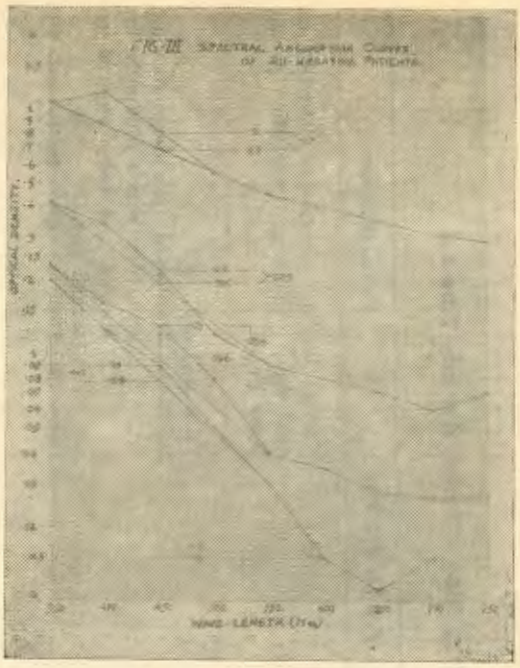
It is evident from Fig. 2 that the patients above and at 36 weeks of gestation had 0.00 optical density. In this study 6 patients were immunized with varying titre of 1:16 to 1:128 Good correlation was observed between higher level of antibody titre and △ O.D. 450 mu. All immunized cases were grouped in upper zone. Out of the 6, 1 patient had still birth and rest of the new born babies developed jaundice soon after delivery and required exchange transfusion. Out of these—1 new born died during exchange transfusion. There was 1 non-immunized case grouped in upper

TABLE I

Relationship of Foetal Outcome With Previous Obstetrics History, Parity, Antibody Titre and O.D. 450 mu.

S. No.	Previous Obstetric History	Titre	Parity	△ 0.D. 450 mu:	Foetal Outcome
1.	(i) F.T.N.D. (ii) F.T.N.D. (iii) F.T.N.D.	1:64	III	.13.	Normal birth. Developed jaundice on next day. Exchange transfusion given. Baby lived.
2.	(i) F.T.N.D. died at age of 4 years. (ii) Still Birth. (iii) Still Birth. (iv) Still Birth. (v) F.T.N.D. (vi) Still Birth. (vii) Died of jaundice. (viii) Died of jaundice. (ix) Died of jaundice.	1:64	IX	.17.	Twin delivery. Baby—A: Developed jaundice on the same day which gradually increased and baby expired on second day. Baby—B: Died of prematurity on 3rd day.
3.	Still Birth.	1:128	Ist	.17.	Still birth.
4.	F.T.N.D. died of jaundice on 2nd day of delivery.	1:64	Ist	.11.	Normal at birth developed jaundice on second day and baby died on 3rd day.
5.	(i) F.T.N.D. died at age of 9 months. (ii) F.T.N.D. died of jaundice. (iii) F.T.N.D. died of jaundice. (iv) F.T.N.D. died of jaundice. (v) F.T.N.D. died of jaundice.	1:16	V	.13.	Normal delivery. Baby developed jaundice within 2 hours of delivery. Baby expired during exchange transfusion.
6.	F.T.N.D. baby developed jaundice next day and had exchange transfusion.	1:8	Ist	.14.	Normal at birth. Baby developed jaundice 4 hours after delivery. Exchange transfusion was given next day. Baby lived.





zone and baby born was unaffected, in this case amniotic fluid was blood stained. Only 1 patient was in lower middle zone. This patient had full term normal delivery and baby had no jaundice. In this case also amniotic fluid was contaminated with blood. All the other patients were grouped in lower zone. In this group 2 new born babies developed jaundice where no demonstrable antibodies could be detected in mother's serum. Liley (1961) compiled excellent charts so as to guide the management of Rh iso-immunized patients. Peak at 450 mu O.D. in the bottom zone included unaffected infants or infants in whom affliction was very mild. Peaks in top zone represented very severely affected babies and cases where intrauterine death was iminent whereas peak in low middle zone was associated with mild hemolytic disease.

Interpretation of spectrophotometric analysis tracing has been used in prediction of severity of hemolytic disease (Walker 1957 and 1963) but this correlation is not a good one and had not been of much clinical value. Amniocentesis should be utilized in assessing the present state of foetus in terms of danger or death or probable survival. At the most prediction is made before 2 weeks for ensuring survival. Hence repeat procedure is of great value. Freda (1965) remarked that marked rise in amplitude of peak and shift of peak from 450 mu to 420 mu indicated foetal death within 7-10 days. Macbeth (1961), Bowes (1965) and Westberg (1965) recommended more than one test on amnoitic fluid. Clinical importance of observation is that essential value of test lies in prediction of infant survival rather than diagnosing hemolytic disease. Pomerance (1968) noted downward trend of value as pregnancy advanced in 95% of unaffected infants.

Liley (1965) commented on the use of amniocentesis in guiding foetal transfu-

sion and reported that 70% of otherwise fatally affected erythroblastotic infants could be saved. Warren (1965) remarked that patient in whom peak fell in upper zone prior to 34 weeks should be subjected to intrauterine transfusion, whereas Freda (1965) recommended it only if there was history of previous stillbirth. MacCutcheen (1967) noted that intrauterine death was not imminent as long as O.D. 450 mu was 0.18, when value higher than this should be considered for intrauterine transfusion.

Summary and Conclusion

Amniocentesis was performed on 30 Rh negative cases out of which 6 were Rh sensitized. In 3.3% of cases amniotic fluid could not be aspirated. Blood was aspirated in 3 out of 30 (10%) in Rh negative non-immunized cases, but these cases developed no demonstrable antibodies. No maternal or foetal complication was encountered in this study. Spectrophotometric analysis of amniotic fluid revealed that with the advancement of pregnancy optical density of 0.00 at 450 mu was obtained. All the 6 immunized cases were grouped in upper zone of Liley's chart. New born babies in this group developed jaundice and required exchange transfusion. Good correlation was observed between high antibody titre and Δ O.D. 450 mu and foetal outcome at present pregnancy.