COMPARATIVE STUDY OF LECITHIN AND SPHINGOMYELIN AS A GUIDE TO FETAL LUNG MATURITY

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Obstetrical practice is engaged in furthering the well being of the mother and the fetus during pregnancy so as to bring the fetus to a state of maturity. This is also to deliver the fetus with a wide margin of safety so that it adopts satisfactorily to the extra-uterine environment.

Birth before term is associated with a perinatal mortality which is 30-33 times higher than for normal gestation, whereas births following prolonged pregnancy have roughly twice the mortality rate. The uncertainty about the gestational age only helps in making things worse. Premature infants have also higher mortality and morbidity from infection than full term infants. This is specially true in the first few weeks of life. This may in great part be related to the immunoglobulin levels of the infants.

Since many neonatal deaths are due to respiratory disorders, the association of respiratory distress syndrome with prematurity is inevitable. R.D.S. due to progressive atelectasis of hyaline membrane disease is a leading cause of death in neonates. Typically, it occurs in immature babies born after spontaneous premature labour or when early induction of labour is carried out owing to some obstetrical problems. Therefore, fetal lung maturity test would be desirable in management of this type of obstetrical problem.

Clements *et al* (1957) identified the presence of surfactant in the lung tissue. This substance is a complex of lipid, protein and carbohydrate. A major portion of lipid is dipalmitoyl phosphatidyl choline or lecithin, which is hydrophilic due to its choline portion. It is thus capable of lowering surface tension, which prevents the collapse of lung tissue. It is released into the lung from inclusion bodies of alveolar type II cells (Morgan, 1971).

Adams *et al* (1965) showed that the alveolar phospholipid levels equated with lung tissue levels. Several investigators have noted that pulmonary tract of the fetus communicates with amniotic cavity and contributes to its content. Others believed that there is a passage of fetal respiratory tract secretion into the amniotic cavity helped by respiratory movements.

The present work was undertaken to estimate the maturity of the fetal lung by study of lecithin-sphingomyelin spots by thin layer chromatography. A rapid method (foam test) described by Clements *et al* (1972) has also been made

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use of here. A comparative study is also done between these two procedures to find out the accuracy and the error between the two, the fine and the crude test, as to use the rapid test if chromatography is not available.

Material and Methods

The present study is based on 55 patients from the department of Obstetrics & Gynaecology, S.S. Hospital, Banaras Hindu University, Varanasi. In 39 patients amniotic fluid samples were collected through transcervical route in the first stage of labour and in the rest by performing abdominal amniocentesis.

Liquor Amnii Study: Each liquor amnii sample was subjected to two types of test:

(i) Foam test: This was performed as described by Clements *et al* (1972) and modified by Edward and Baillie (1973).

(ii) Estimation of lecithin/sphingomyelin ratio by thin layer chromatography: The method described by Lipshaw *et al* (1973) was followed here, which is a modified method of Gluck *et al* (1971). Data of all the tests were recorded and statistical analysis was made. Value of lecithin and sphingomyelin ratio obtained by chromatography was compared with that of obtained by the Foam test, physical criteria and last date of menstrual period to find out the lung maturity, gestational age and the outcome of the fetus.

Observation and Analysis

Fifty-five samples of amniotic fluid were studied. Sequential study was not done to avoid infection. The cases included in this series are shown in Table I.

TABLE I
Indications for Amniocentesis

	Indications	Number of patients
1.	Elective	27
2.	Elective caesarean section	4
3.	Rh diseases	1
4.	Toxaemia	5
5.	Postmaturity	2
6.	Maturity	5
7.	Polyhydramnios and twin	3
8.	Hepatic coma	1
9.	Severe anaemia	2
10.	Prematurity	5

Table II shows the amniotic fluid lecithin/sphingomyelin ratio grouped by gestational age. There was considerable overlap among the different gestational groups with regard to the range of lecithin/sphingomyelin ratio. Sample from each of the pregnancies less than 31 weeks gestation had L/S ratio of 0.7 or less.

TABLE II
Amniotic Fluid Lecithin-Sphingomyelin Radio Grouped by Gestational Age

Gestational age in weeks	Number of samples	Mean	Range
28 to 30	2	0.57	0.45 to 0.7
31 to 32	6	0.85	0.50 to 1.8
33 to 34	3	1.16	0.50 to 2.0
35 to 36	7	2.30	1.50 to 3.0
37 to 38	10	2.86	2.10 to 3.8
39 to 40	25	3.02	1.50 to 4.1
Above 40	2	3.45	2.80 to 4.1

Individual ratio of less than 1.0 were obtained from subjects at 31 to 34 weeks gestation, L/S ratio tended to be higher and the range to be broader for each gestational group with increasing duration.

The clinical status of the new born and the increase in mortality can be seen in Table III of the pregnancy whose liquor amnii showed L/S ratio of less than 2. From our analysis, we can also say that findings were in agreement with Gluck *et al* (1971) the severity of R.D.S. was inversely related to the L/S ratio.

A correlation of the rapid test with the respiratory status of the new born is shown in Table IV. Thirty samples gave

TABLE III

Clinical States of Neonates With Amniotic Fluid Lecithin to Sphingomyelin Ratio Less Than 2

Neonate		Weeks of pregnancy	Weigght of infants in lbs.	L/S ratio	Clinical State
T	1	28	1.0	0.70	Died after 16 hrs. with R.D.S.
w i n	2	28	1.8	0.70	Died after 42 hrs. with R.D.S.
T	3	30	1.5	0.45	Died after 41 hrs. with R.D.S.
w i n	4	30	1.8	0.45	Died after 36 hrs. with R.D.S.
	5	32	3.0	1.80	Died of neonatal septicemia
	6	32	2.5	0.75	Still birth
T	7	32	3.5	0.50	Mild R.D.S.
w	8	34	5.5	0.75	No R.D.S.
i n	9	34	4.5	0.75	No R.D.S.
	10	32	4.0	0.50	Died of R.D.S. after 52 hours
	11	34	5.0	1.00	No R.D.S.
	12	36	4.5	1,50	No R.D.S.

TABLE IV

Correlation of the Rapid Test for Surfactant With the Respiratory Status of the Newborn

Test results	Respiratory distress present	Transitional respiratory distress	No respiratory distress
1. Immature (positive in undiluted or negative in all tubes)	2	1	8
2. Intermediate (positive in undiluted or 0.75 dilution only)	2	—	9
3. Mature (positive at all dilutions or undiluted 0.75 and 0.5 dilutions)	1	—	29

a mature test (positive in all dilutions or positive in undiluted 0.75 and 0.5 dilution only). Twenty-nine out of 30 newborns were perfectly normal and did not show any sign of respiratory distress. Only one newborn with mature shake test born to severely anaemic mother showed sign of respiratory distress and died. In all cases giving intermediate test (Positive in undiluted and 0.75 dilution only), nine newborns were absolutely normal and showed no sign of respiratory distress. Eleven samples showed immature test (positive in undiluted only or negative in all dilutions). Eight babies had no sign of respiratory distress. One baby had transitional respiratory distress and improved with oxygen inhalation. Two died due to respiratory distress and 2 showing immature test were stillborn. One baby delivered at 32nd week gestation with immature test died of neonatal septicaemia.

Table V shows a correlation between the rapid test for the surfactant with the (mature L/S ratio). This shows a wide variation of L/S ratio in different types of rapid surfactant test, so that no definite opinion can be resorted by one type of rapid surfactant test.

Discussion

Review of the literature shows many tests suggested for finding out the general maturity of the fetus and also certain for specific organs. No test is yet of much help in the evaluation of the maturity of the fetal lung, whose importance cannot be denied from point of view of fetal safety.

The only concept that has stood the test of time is "R.D.S. is a phenomena of development and not a disease at all". There are biochemical evidences to show that there is marked dissinuition into concentration of major surface active compound lecithin in fetal lung. This association between the respiratory distress syndrome and viability of the pre-

TABLE V

Comparison of Rapid Test for Surfactant (R.ST.) and Lecithin Sphingomyelin Ratio (L/S ratio)

R.S.T.	L/S ratio	No. of cases	% percentage
Immature	Immature L S	4	28.57
Immature	Intermediate L = S	1	7.13
Immature	Mature L S	9	64.28
Intermediate	Intermediate	—	—
Intermediate	Immature	3	27.27
Intermediate	Mature	8	72.72
Mature	Mature	30	100.00
Mature	Intermediate	—	—
Mature	Immature	—	—

ranges of L/S ratio. There were 14 samples giving immature R.S.T. This L/S ratio ranged from 0.5 to 2.5. Eleven samples showed intermediate R.S.T., L/S ratio ranging from 0.45 to 3.0. Thirty samples showed clearly mature R.S.T. with L/S ratio ranging from 2.0 to 4.1

mature infants, once born to synthesize enough surface active lecithin is important. This association was first suggested by Avery and Mead (1959) who showed the ultimate lack of surface activity in saline extract of minced lung from infants dying of H.M.D. as compared to those in-

fants who died from causes other than pulmonary disease. They also showed that atelectases occurring in H.M.D. is due to an absence or critical depletion of pulmonary surfactant, or impairment in synthesis or excretion, inhibition of previously formed surfactant.

The L/S ratio determination has come to stay as a valuable tool in the hands of obstetrician for estimating fetal maturity in general and pulmonary maturity in particular. Hobbin *et al* (1972) studied L/S ratio in cases of high-risk pregnancy and found it clinically useful in 71.7 per cent of cases. In present study 50.9 per cent clinical usefulness of the L/S ratio was observed.

Gluck *et al* (1971) saw a surge in lecithin concentration at 36th week of gestation, heralding maturity of fetal lung. Clinical interpretation was made in thin-layer chromatography a lecithin spot clearly larger than that of sphingomyelin marked pulmonary maturity in the fetus. Hobbins *et al* (1972) could predict the respiratory outcome of the newborn by studying lecithin sphingomyelin spots on T.L.C. Their results were very similar to Gluck *et al* (1971), Clements *et al* (1972). They observed an abrupt rise in the titre of the surfactant at around 35th week of gestation. This study has revealed the same pattern.

In agreement with Gluck *et al* (1972) the severity of R.D.S. was inversely related to L/S Ratio. No constant relation could be established between neonatal weight or gestational age and lung maturity. In cases, studied by Spellacy and Buhi (1972) L/S ratio and infant birth weight correlated significantly. A ratio of more than 2 always indicated a baby born at that point will be free from R.D.S. According to Whitfield *et al* (1972) L/S ratio above 2.0 can be regarded as safe from the view point of pulmonary function. They held a ratio in the range of 1.5 to 2.0 as an index of transitional level of pulmonary maturity, with chance of R.D.S. after delivery.

It is clear that a L/S ratio of 2.0 always indicates a mature fetal lung. If delivery is allowed to occur at this stage, neonate might fall a victim to R.D.S. A value below this should always be looked with suspicion because chances of R.D.S. are quite high. L/S ratio though described by many as a rapid test takes few hours in yielding results. Hence, this test is not suitable as bedside procedure. A rapid test for surfactant (R.S.T.) in amniotic fluid has been introduced which can be performed within 15 minutes and can be done at bedside with minimum equipments and reagents.

The present study was undertaken to compare the result of this rapid surfactant test with L/S ratio by T.L.C. Thirty samples gave mature test with (positive in all dilution or undiluted, 0.75 and 0.5 diluted), 29 out of 30 newborns with mature test borns were perfectly normal. In 11 cases giving intermediate test (positive in all dilutions or 0.75 dilutions only) 9 new borns escaped respiratory distress but 2 died of R.D.S., 14 samples showed clearly immature test. 8 babies were free from R.D.S. In 3, varying degree of R.D.S. occurred. Two fetuses were stillborn showing immature R.S.T. and 1 neonate delivered at 32nd week with immature R.S.T. and died of neonatal septicaemia.

In case of mature R.S.T., a 100 per cent correlation was observed with L/S ratio, of immature group (R.S.T.) only 28.57 per cent cases also showed immature L/S ratio and 64.28 per cent cases gave mature L/S ratio. In intermediate group (R.S.T.), in 27.27 per cent immatures

L/S ratio was found. Here 72.72 per cent cases showed mature L/S ratio.

Boehm *et al* (1973) obtained corresponding to L/S pattern with an immature R.S.T. with 64.0 per cent accuracy. In comparison to L/S ratio, R.S.T. has been found to be a quicker, simpler and a bedside method while the former needs a well equipped laboratory. R.S.T., though a simple method, lags far behind than L/S ratio in prediction of fetal lung maturity. It has only proved its worth as a screening procedure, to be employed with limitations.

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References

1. Adams, F., Fujiwara, T., Emmanauilides, G. and Scudder, A.: J. Ped. **66**: 357, 1965.
2. Avery, M. E. and Mead, J.: Am. J. Dis. Child. **97**: 517, 1959.
3. Boehm, F. H., Srisupundit, S. and Ishii, T.: Obstet. & Gynec. **41**: 829, 1973.
4. Clements, J. A., Platzeker, A. C. G., Tierney, D. G., Hobel, C. J., Creasy, R. K. and Margolis, A. J.: New England J. of Med. **286**: 1077, 1972.
5. Edwards, J. and Baillie, P.: South African. J. of Med. **47**: 2070, 1973.
6. Gluck, L., Kulovich, M. V. and Borer, R. C.: Am. J. Obstet. & Gynaec. **109**: 440, 1971.
7. Hobbins, J. C., Brock, W., Speroff, L., Anderson, G. C. and Caldwell, B.: Obstet. & Gynec. **33**: 660, 1972.
8. Lipshaw, L. A., Weinberg, J. H. and Sherman, A. I.: Obstet. & Gynec. **40**: 93, 1973.
9. Morgan, T. E.: Intern. Med. **127**: 401, 1971.
10. Spellacy, W. N. and Buhi, W. C.: Obstet. & Gynec. **39**: 852, 1972.
11. Whitfield, C. R. and Sproule, W. B.: Lancet, **1**: 382, 1972.
12. Whitfield, C. R., Chan, W. H., Sproule, W. B. and Steward, A. D.: British Medicine. J. **2**: 85, 1972.