AMNIOTIC FLUID LECITHIN : SPHINGOMYELIN RATIO AS INDEX FOR PREDICTION OF IDIOPATHIC RESPIRATORY DISTRESS SYNDROME

by

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Abstract

Lecithin : Sphingomyelin ratio of patients in normal and abnormal pregnancy have been studied by precise methods of quantitation. The results indicate that the numerical values of the ratio of 1.50 or above predict that Idiopathic Respiratory Distress Syndrome (IRDS) would not occur. Values of 1.00 or below suggest danger of IRDS after delivery.

Introduction

Idiopathic Respiratory Distress Syndrome (IRDS) occurs due to foetal lung immaturity at birth. Incidence of IRDS as reported from Western countries is 10-16% of babies whose weight at birth is below 2.5 kilograms (Gairdner, 1965). In India, there is a great variation in the reported incidence of IRDS. (Webb *et al*, 1962; Bajpai *et al*, 1966; Surainder *et al*, 1971). In our hospital IRDS occurs in 5% of pre-term babies and the mortality is 60-70%. This condition is therefore an important cause of mortality in pre-term babies in our environment.

When an obstetrician contemplates induction of labour in cases of high-risk pregnancies, like intrauterine growth retardation, diabetes mellitus or rhesus incompatibility, it is helpful to know whether the baby's lungs have matured; so that, there is no risk of the baby dying subsequently of IRDS. This can be done by the determination of the Lecithin/Sphingomyelin ratio of the mother's amniotic fluid collected by amniocentesis (Gluck *et al*, 1971). It is also essential to establish the normal figures of L/S ratio for the relevant weeks of gestation before using this test for clinical assessment. Apart from this, the L/S ratio at which IRDS occurs in Indian babies needs to be clearly defined.

Material and Methods

The amniotic fluid was collected by amniotomy during labour and at the time of caesarean section. A number of these samples had to be discarded due to their being contaminated with blood or meconium. The gestation was estimated by calculation from the last menstrual

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period and confirmed by examination of the neonate according to Dubowitz scoring (Dubowitz *et al*, 1970). Detailed medical and obstetrical history of each patient was taken.

The presence of IRDS in these babies was looked for and diagnosed by the following criteria: (A) appearance of respiratory distress within 4 hours after birth, (B) presence of respiratory rate more than 60 per minute, (C) presence of intercostal and subcostal recession lasting for more than 24 hours, and (D) presence of grunting.

When a baby had similar signs and symptoms but tachypnoea lasted for less than 24 hours the case was termed as transient tachypnoea.

It was also considered necessary that the quantitation of these phospholipids be carried out by strictly quantitative techniques and not by visualizing the density of the coloured spots or of charred spots as done by other workers. This would put the ratio levels at different weeks of gestation on a more accurate basis. Thus the method of Ways and Hanahan (1964) was followed for the extraction of the amniotic fluid using methanol : chloroform mixture (1:2, V:V). The extract was washed free of contaminants by the method described by Folch *et al* (1957). The residue of the combined lipid extract, obtained after evaporation in vacuo was taken in a measured volume of chloroform. Total phospholipids were estimated directly in measured aliquots of this. Differential phospholipids were isolated by thin layer chromatography on silica gel G., using chloroform : Methanol : 25 per cent ammonia (65:25:4) as the solvent system. The spots of separated phospholipid fractions (mainly of lecithin and sphingomyelin) were identified by comparison with authentic standards run on the same TLC plates and visualized by iodine vapours, scraped and quantitated for lipid phosphorous.

Results

The L/S ratios at different periods of gestation in normal pregnancies are given in Table I. As is evident, there was a progressive increase in the levels of L/S ratio with increase in gestational period. From the 28th to the 34th weeks of gestation the ratio increased from 1.09 to 1.40. At 35 to 36 weeks of gestation the value was 1.50 and at 37 to 38 weeks of gesta-

TABLE I
L/S Ratio at Different Periods of Gestation

S. No.	Weeks of Gestation	No. of Cases	L/S Ratio	
			Range	Mean ± S.D.
1.	28 weeks and less	3	0.60-1.50	1.09 ± 0.45
2.	29-30 weeks	2	—	1.10
3.	31-32 weeks	1	—	1.50
4.	33-34 weeks	3	0.70-1.00	1.40 ± 0.62
5.	35-36 weeks	12	0.60-2.40	1.45 ± 0.53
6.	37-38 weeks	15	0.50-4-10	1.97 ± 1.06
7.	39-40 weeks	26	0.70-6.30	2.60 ± 1.27
8.	More than 41 weeks	5	2.00-3.60	2.50 ± 0.70

TABLE II
L/S Ratio in Abnormal Pregnancy

S. No.	Weeks of gestation	L/S Ratio	L/S Ratio at that period of gestation	Clinical Comments
1.	28	2.00	1.09 ± 0.45	Mother had severe diarrhoea
2.	31	2.00	1.50	Mother had infective hepatitis
3.	34	0.67	1.40 ± 0.62	Mother had severe Rh incompatibility
4.	36	3.70	1.45 ± 0.53	Mother had typhoid
5.	37	3.10	1.97 ± 1.06	Mother had uteric colic
6.	32	4.00	1.50	Severe PET
7.	33	2.70	1.40 ± 0.62	Severe eclampsia

tion it was 1.97. It further increased to 2.60 at 40 weeks of gestation.

Although a progressive increase in the mean values of L/S ratio was found with increase in gestation period, there was a wide variation in the actual values of L/S ratio at a particular period.

The L/S ratio in some abnormal pregnancies is given in Table II. It shows that in cases of severe pre-eclamptic toxemia (PET), eclampsia, intrauterine infection, infective hepatitis, the L/S ratio was increased, while in a case of Rh incompatibility the L/S ratio was decreased.

The incidence of IRDS with different values of L/S ratios is shown in Table III. Cases in which babies were still-birth or had died immediately after birth (due to severe congenital abnormalities or severe asphyxia neonatum) were excluded. It can be seen that with ratios below 1.00, 2 out of 3 babies had IRDS. When the ratio exceeded 1.50 none of the babies had IRDS. In cases which gave ratios between 1.00 and 1.50 out of 11 babies only 1 got IRDS and 1 had transient tachypnoea.

TABLE III
Neonatal Outcome

L/S Ratio	No. of Cases	No. of IRDS
<1.0	3	2
1.0-1.5	11	1+1*
1.6-2.0	8	None
>2.0	35	None

* Case with transient tachypnoea.

Discussion

The normal synthesis of surfactant determines the ability of an infant to maintain normal respiration. The surfactant reduces the surface tension and thus enables the alveoli to maintain residual volume during expiration and permits adequate expansion. Inadequate synthesis of surfactant or its faulty secretion into alveolar surface by the immature lung produces IRDS. The surfactant is mainly composed of lecithin (66%). The other components are phosphatidyl ethanolamine, acylglycerol, phosphatidyl serine, lysolecithin, sphingomyelin, fatty

acids, protein and cholesterol (Holton, 1977). Since lung fluid is actively secreted into the amniotic fluid, the level of phospholipids in the amniotic fluid reflects that present in the lungs. There is a great variation in literature regarding the reported values of total phospholipids or lecithin at different periods of gestation (Bhagwanani, 1972; Nelson, 1972). This may be because of variation in the total volume of amniotic fluid in individual subjects. Gluck *et al* (1971) reported that the level of sphingomyelin parallels that of lecithin upto 35 weeks of gestation; after which it gradually decreases, while lecithin shows a sharp increase. They suggested that determining the ratio of lecithin to sphingomyelin would be useful for eliminating the problem of differing volumes of amniotic fluid. Its numerical value would thus be predictive of the presence or absence of IRDS. According to them, when the ratio of lecithin to sphingomyelin exceeds 2.00 the baby is unlikely to have IRDS.

Our values (Fig. 1) of L/S ratio compare well with those of Gluck *et al* (1973) till 34 weeks of gestation, but, thereafter they are lower but similar to those of Rodrigues *et al* (1979). Gluck *et al* obtained L/S ratio of 2.00 at 35 weeks of gestation. In our cases, values of 2.00 were reached at full term (i.e. 37 to 38 weeks of gestation). As is evident from the graph, our results are very much more comparable to those of Schirar *et al* (1975). The latter have also reported the L/S ratio of 2.00 at 37 to 38 weeks of gestation.

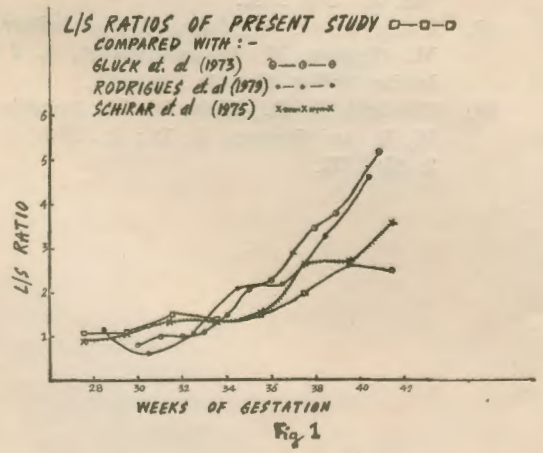

Fig 1

A wide variation (Table I) in the actual values of L/S ratio is observed at a particular period of gestation. These have also been reported by Whitfield *et al* (1972) and Schirar *et al* (1975). The reason for this is not very clear. Biezenski (1973) has proposed that since the concentration of sphingomyelin decreases at term, inability of the body's mechanism to remove this sphingomyelin may cause a low value of L/S ratio at full term. Other causative factors may also be present.

Our observations on the L/S ratio in cases of some abnormal pregnancies (Table II) are similar to those observed by Gluck *et al* (1973).

It has been reported by most workers that IRDS does not occur when the L/S ratio in amniotic fluid is more than 2.00. However, the incidence, of IRDS with L/S ratio below 2.00 varies greatly in literature. (Gluck *et al*, 1971. Spellacy *et al*, 1972; Schirar *et al*, 1975; Rodrigues *et al*, 1979).

In the present study, it was observed that no IRDS occurred when the L/S ratio was above 1.5. This has also been reported by Merkus *et al* (1973). In the present study we can predict the state of pulmonary maturity or lack of it when the ratio rises above 1.50 or gets lowered below 1.00. With intermediate results this test is not very helpful to the clinicians, as, in some of these cases delay of delivery may affect the fetus adversely. Therefore, it is felt, that other biochemi-

cal tests need to be performed to predict pulmonary maturity more accurately in this intermediate range. Tests like ratio of palmitic acid to stearic acid (Schirar *et al* 1975) may help. The determination of the percentage of lecithin to total phospholipid as well as the estimation of the level of saturated phosphatidyl choline (SPC) would also contribute in this regard as observed by Biezenski *et al* (1973) and Torday *et al* (1979). It is claimed by these workers that these tests are more contributive to the assessment of foetal lung maturity. The present study will be extended to the substantiation of this aspect as well.

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References

1. Bajpai, P. C., Kutty, D., Rajgopalan, K. C. and Wahi, K. M.: Indian Pediat. 3: 83, 1966.
2. Bhagwanani, S. G., Fahmy, D. and Turnbull, A.C.: Lancet. 1: 159, 1972.
3. Biezenski, J. J.: Obstet. Gynec. 41: 825, 1973.
4. Dubowitz, K. M., Dubowitz, V. and Goldberg, C.: J. Pediat. 77: 1, 1970.
5. Folch, J., Lees, M & Sloane Stainley, G. H.: Clin. Chim. Acta. 2: 25, 1957.
6. Gairdner, D.: Recent advances in Pediatrics, Ed. 3, London 1965, J & A Churchill Ltd., P 55.
7. Gluck, L., Kulovich, M. V., Borer, R. C., Brenner, P. H., Anderson, G. G. and Spellacy. W. N.: Am. J. Obstet. Gynec. 109: 440, 1971.
8. Gluck, L. and Kulovich, M. V.: Am. J. Obstet. Gynec. 115: 539, 1973.
9. Holton, J. B.: "Diagnostic Tests on Amniotic fluid", Essays Med. Biochem. 3, Biochemical Society Book Depot, London 1977 p. 75-107 (Editors V. Marks and C. N. Hales).
10. Merkus, J. M. W. M., Kook, H. C. L. V., Merkus, F. W. H. M. Verhoeven, A. G. J. and Beysons, A. J. M. M.: Am. J. Obstet. Gynec. 115: 859, 1973.
11. Nelson. G. H.: Am. J. Obstet. Gynec. 112: 827, 1972.
12. Rodrigues, C., Dhall, K., Bhakoo, O. N., Kumar, M.: Indian J. Med. Red. 70: 762, 1979.
13. Schirar, A., Vielh, J. P., Alcindor, L. G. and Gautray, J. P.: Am. J. Obstet. Gynec . 121: 653, 1975.
14. Spellacy, W. N., Buhi, W. C.: Obstet. Gynec. 39: 852, 1972.
15. Surainder, Y. A., Karan, S., Rao, A. D. and Marar, S. A.: Indian Pediat. 8: 759, 1971.
16. Torday, J., Carson, L. and Lawson, E. E.: N. Engl. J. Med. 301: 1013, 1979.
17. Ways, P. and Hanahan, D. J.: J. Lipid Res. 5: 318, 1964.
18. Webb, J. K. S., John, T. J., Jadhav, M., Graham, M. D. and Walter, A. J.: Indian Pediat. Soc. 1: 193, 1962.
19. Whitfield, C. R., Chan, W. H., Sproule, W. B. and Stewart, A. D.: Br. Med. J. 2: 85: 1972.