ASSESSMENT OF FOETAL LUNG MATURITY BY STUDY OF BIOCHEMICAL PARAMETERS IN AMNIOTIC FLUID

by

DEVINDRABEN S. SHAH
SANDHYA B. MAHIDA
P. B. SHAH
and
N. B. VASAVADA

SUMMARY

In present series 3 parameters have been studied. The main purpose is to find out the specificity of a simpler test. O.D. test at 650nm is a rapid and inexpensive way to determine the foetal maturity with 98% accuracy. In this study, maximum false negative results of 36% were in O.D. test while FS-50% gave 24% false negative against 10% that of L/S ratio after 32 weeks of gestational age.

Introduction

The newborn infant is a foetus removed from complete dependance on the maternal organism in an aqueous environment to a relatively independent status in air. Successful transmission from intra to extra uterine life is dependent on the readjustment of a great number of functions to be accomplished more or less immediately during and following delivery. Amongst these adaptive processes, the most dramatic changes are those occurring in the circulatory and respiratory systems. The readjustment processes occurring in these two systems are initially linked together and are interdependent. At birth, the inactive foetal lungs have to take over the functions of the placenta as an effective gas-exchanger to ascertain the survival in new environment.

Respiratory problems present the major threats to the survival of the new born infant, (Driscoll and Smith, 1962) which is the major cause of perinatal mortality.

A great deal of interest and research is found during recent years for assessment of foetal lung maturity. The presence of surfactants in amniotic fluid provides reliable means of measuring the degree of pulmonary maturity in foetus. In order to make correct decision in cases of high-risk pregnancy, measurement of Lecithin/Sphingomyelin ratio (L/S ratio) in amniotic fluid (AF) provides an accurate means for assessment of foetal lung maturity (Gluck *et al*, 1971). It is difficult to establish an accurate laboratory procedure for determination of L/S ratio in a smaller obstetric unit. Now, simpler tests like shake test and measurement of optical

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density of amniotic fluid at 640 nm which give immediate and accurate correlation with L/S ratio are available (Sherr G. *et al*, 1979; Spellacy *et al*, 1975).

The present study has been carried out to correlate the L/S ratio determination in AF by

1. FS –50% test (Shake test).
2. O.D. measurement at 640 nm.
3. L/S ratio chemical estimation by thin layer chromatography (One dimensional).

All these three parameters are evaluated with clinical observation and outcome of babies in relation to their respiratory function.

Material and Methods

The study was carried out at Govt. Medical College and New Civil Hospital, Surat. Out of 96 cases, 90 clear samples were studied for analysis of L/S ratio.

The cases of normal pregnancy as well as patients with B.O.H., where there was a question of foetal maturity were selected. Duration of pregnancy was expressed in weeks by clinical examination like L.M.P., fundal height and from abdominal examination. The patient was throughly examined to know the position of foetus before amniocentesis. Written consent was taken. Transabdominal route during antenatal period and transvaginal route during labour was chosen for amniocentesis. About 10-15 ml. of amniotic fluid was collected in a clean test tube. The fluids having blood, meconium vaginal contamination were excluded. In 4 cases there was a dry tap and in 2 fluid was blood stained. All the patients were observed for 48 hours after amniocentesis. They were also followed till delivery for any complications.

The samples were analysed within two hours of its collection in Biochemistry Department. L/S ratio was estimated by three different methods.

1. Foam Stability—50 per cent test (FS-50% test, Shake test, Sherr G. *et al*, 1979).

One mililitre of uncentrifuged AF was added to 1 ml. of 100% (Absolute) ethanol in a dry Kahn's tube of 5 cm × 1.3 cm in size. The tube was stoppered and vigorously shaken for 30 seconds. The presence of an uninterrupted ring of bubbles around the entire meniscus was interpreted as positive, anything less than that was considered negative.

2. Measurement of optical density at 640 nm (O.D. 640 nm) (Spellacy *et al*, 1975).

AF was centrifuged at 3000 r.p.m. for 10 minutes. The absorbance of surfactant was determined at 640 nf against distilled water on E-Litz photometer.

3. Estimation of Lecithin and Sphingomyelin by thin layer chromatography (TLC) (Gluck *et al*, 1971).

L/S ratio more than 2 or equal to 2 indicates mature foetal lungs.

L/S ratio less than 2 indicates immature foetal lungs.

Repeat amniocentesis was performed in cases during labour and only shake test was performed. New-borns were examined for respiratory function during first 24 hours.

Results

In the present study 90 samples of AF were analysed and all three tests were performed in 72 samples, while F.S. 50% test + optical density 640 nm were performed in all 90 cases as shown in Table I. Repeat amniocentesis was performed in 74 patients at the time of labour and only F.S. 50% test was carried out with those 74 samples (Table I).

TABLE I

Methods Used for Determination of L/S Ratio

Sr. No.	Methods used	No. of cases
1.	F.S. 50% test + O.D. + T.L.C.	72
2.	F.S. 50% test + O.D.	90
3.	F.S. 50% test (at repeat amniocentesis)	74

As the concentration of phospholipids increases after 24 weeks of pregnancy, most of the patients (85%) are selected during third trimester of pregnancy. It is seen from Table II that all the samples showed negative shake test, with O.D. less than 0.15 below 24 weeks of pregnancy. With increase in gestational age L/S ratio rises, shake test become positive and O.D. greater than 0.15 in majority of cases.

Correlating the FS-50 test with neonatal pulmonary status in Table III shows the predictive value of L/S ratio with FS 50% test. On repeating shake test at time of labour out of 74 cases, 71 showed positive shake test and none of them developed H M D. In one patient at 39 weeks gestation eventhough the shake test was negative the newborn did not develop H M D.

It has been observed that birth weight of the newborn has definite relation with the development of R D S. The results are tabulated in Table IV. Out of 74 cases babies weighing more than 2.0 Kg. none of them developed HMD and only 2 cases with low birth weight (Less than 1.9 Kg.) developed HMD with negative shake test.

Complications

No major complication like infection, premature, labour, injury to the foetus

TABLE II

Comparision of Three Tests at Various Gestations at First Amniocentesis

Gestational age in weeks	No. of Pts.	FS 50 test		O.D. 640 nm		L/S ratio	
		Positive	Negative	More than 0.15	Less than 0.15	More than 2	Less than 2
16-24	6	0	6	0	6	-	-
25-32	20	1	19	2	18	1	7
33-36	44	31	13	24	20	39	5
37-40	20	18	2	17	5	19	1

TABLE III

Relation of Shake Test and Neonatal Pulmonary Status

Gestational age in weeks during delivery	No. of cases	FS 50 test		Neonatal pulmonary status	
		Positive	Negative	Normal	R D S
		P	N	N	R
33-36	6	4	2	4	2
37-40	64	63	1	64	0
More than 40	4	4	0	4	0
Total	74	71	3	72	2

TABLE IV
Relation of Birth Weight, Shake Test and Neonatal Pulmonary Status

Birth Wt. Kg.	No. of cases	Shake test		H M D	
		Positive	Negative	Yes	No.
1.5-1.9	6	4	2	2	4
2.0-2,5	55	54	1	0	55
More than 2.5	13	13	0	0	13

etc. have been observed on account of technique of amniocentesis. There were only two bloody taps and four dry taps which are minor complications.

Discussion

It is very well known that the presence of surfactant in AF has a direct relation with normal respiratory function in the newborn. Therefore, an attempt has been made in this study to evaluate foetal lung maturity by three different methods. FS 50% test, O.D. measurements at 640 nm and L/S ratio by TLC.

In majority of cases surfactants have started appearing after 32 weeks of gestation. Out of 64 cases from 33 to 40 weeks of gestation, 76% showed the shake test positive, 64% showed O.D. more than 0.15 and L/S ratio was more than 2 in 90% of cases.

The present study also revealed that positive shake test correlated well with mature L/S ratio and none of the newborns developed HMD.

Clements *et al* (1972) have shown that appreciable titre of surfactant appears at 33 weeks of gestation, but the time of appearance of surfactant may be even as early as 25 weeks. Two cases in present series show the presence of surfactant before 32 weeks. Reddy *et al* (1978) and Bhusan and Mirchandani (1978) have observed the positive shake test of 35 to 36 weeks of gestation.

On comparison of shake test and neonatal pulmonary status (Table III), 96% of cases above 33 weeks of gestation showed positive shake test, and none of them developed HMD, while 40% of cases having negative shake test from which, only 2.7% developed HMD.

This is in close agreement with the observation of Ranjana Jain and Vijay (1982), Hobel *et al* (1972), Reddy *et al* (1978) and Bhusan and Mirchandani (1978). Our observation leads to conclusion that the pulmonary status of the newborn has definite relationship with shake test. Bhagwanani *et al* (1973), and Fisher and Sutherland (1973) have also stressed clinical reliability of the shake test to the development of RDS.

In addition to assessment of foetal lung maturity shake test can also be used as a good indicator to predict the foetal weight.

Results of shake test have been compared with birth weight and incidence of RDS in newborn in Table IV. In present study it is found that RDS occured in newborn weighing less than 2.0 Kg. with negative shake test. Newborns weighing 2 Kg. or more did not develop RDS. Donald *et al* (1973) observed that low birth weight babies suffered more frequently from RDS than babies weighing 2.5 Kg. or more.

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