EVALUATION AND COMPARISON OF "MODIFIED SHAKE TEST" AND NILE BLUE SULPHATE STAINING TEST

by

RAJEE IYER,*

and

MURARI NANAVETI**

Predication of the gestational age and fetal lung maturity is essential when delivery is contemplated before term to prevent both the delivery of a premature infant as well as respiratory distress syndrome in the new born. This assumes significant clinical importance especially when details of the last menstrual period is unavailable because of conception occurring during lactational amenorrhoea coupled with patients coming late in pregnancy for ante-natal registration or because of irregularity in menstrual cycles.

Lecithin/Sphingomyelin ratio and chemical estimation of Lecithin (Gluck *et al* 1971, Bhagivanani *et al* 1972) have been reported for assessing the fetal surfactant status and lung maturity. Clements *et al* (1972) described a simple and rapid test for the measurements of amniotic fluid surfactant referred to as the "shake test".

There has been considerable disagreement as to the accuracy of the Clements shake test in predicting lung maturity, especially in relation to a high incidence of false negative results in comparative studies of shake test and Lecithin in amniotic fluid (Bhagivanani *et al* 1973; Fischer 1973).

Edwards and Baillie (1973) described a modification of the above shake test. In the present study, a modification of the shake test, differing from Edwards and Baillie (1973) has been used. In view of the simplicity involved in performing the modified shake test and Nile Blue sulphate test a prospective study was undertaken, initially to simultaneously evaluate and compare both our modified shake test and the Nile Blue sulphate test in assessing the lung maturity as well as the gestational age. Later the findings were used as diagnostic parameters prior to induction of labour.

Material and Methods

For the initial study of evaluation and comparison of the tests, 65 singleton and 2 twin pregnancies were studied. Uncontaminated amniotic fluid was obtained at amniocentesis after 36 weeks of gestation from normal pregnancies and at caesarean sections. Amniotic fluid was also obtained by the transvaginal route by artificial rupture of membranes in patients who were admitted with spontaneous onset of labour. In the latter part of the study, amniotic fluid obtained from 29 patients at amniocentesis was analysed prior to induction of labour. Table I depicts the clinical details of the patients.

*Honorary Assistant Consultant Obstetrician and Gynaecologist.

**House Officer, Nowrosjee Wadia Maternity Hosiptal, Parel, Bombay 400 012, India.

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TABLE I
Clinical Details of the 96 Cases Studied

Clinical Details	No. of Cases
For Evaluation—	
Normal Pregnancies (Includes Mechanical Complications)	67
For Diagnosis—	
Prior to Induction of Labour	29
Post Maturity	11
Severe Rhesus Iso-immunisation	1
Previous Stillbirth	1
Pre-eclamptic Toxaemia	1
Bad Obstetric History	3
Intra-uterine growth Retardation	1
Prior to Elective Caesarean Section (for Cephalo-pelvic Disproportion, previous Vesico-vaginal Fistula Repair, Toxaemia, Bad Obstetric History)	11
TOTAL	96

Both the modified shake and the Nile Blue sulphate staining tests were performed immediately after collection. The latter test was performed as described in the previous study (Iyer 1979). The modified shake test was performed as follows. The tube containing the sample was gently inverted to obtain a uniform suspension. In clean glass tubes measuring 14 x 100 mm, volumes of 1.0, 0.75, 0.50, 0.25 and 0.20 ml of liquor are pipetted and labelled as Tube 1—1:1 dilution; Tube 2—1:1.3 dilution; Tube 3—1:2 dilution; Tube 4—1:4 dilution; Tube 5—1;5 dilution respectively. Volumes of 0.25, 0.50, 0.75, 0.80 ml of 0.9% saline was added to the above tubes, followed by the final addition of 1 ml of absolute 100% ethanol. All the tubes were mechanically shaken by hand for 15 seconds and placed on a rack. After 15 minutes, the tubes were viewed against sunlight and the presence of a continuous ring of stable bubbles around the meniscus was recorded as positive for that tube. The results were recorded as follows:

A positive test—presence of stable bubbles upto 1:2 dilution, or more i.e. in 1st, 2nd or 3rd tubes, or 4th or 5th tube as well.

An intermediate test—presence of stable bubbles up to 1:1.3 dilution, i.e. in 1st and 2nd tube.

A negative test—absence of bubbles beyond a dilution of 1:1, i.e. in 1st tube.

All babies born were assessed for maturity by a paediatrician and close observation kept for respiratory difficulties as cyanotic attacks and respiratory distress syndrome.
syndrome.

In the diagnostic group, the tests were carried out after amniocentesis. When the shake test was intermediate or negative, induction of labour was deferred and the tests performed after either a week or ten days depending on the urgency of the clinical situation. These babies were also assessed by a paediatrician post-natally.

Results

In Figure 1 results of both the modified shake test and the Nile Blue sulphate tests have been plotted against the weeks of gestation.

Modified Positive Shake Tests

From the scatter of results, it is clear that after 36.5 weeks of gestation, majority of the samples gave an orange cell count over 10% with positive shake test. None of the babies from this group had any respiratory problems.

When gestation below 36.5 weeks, i.e. between 33 and 36.5 weeks is considered, an orange cell count in the range of 2-9% was associated with a positive shake test.

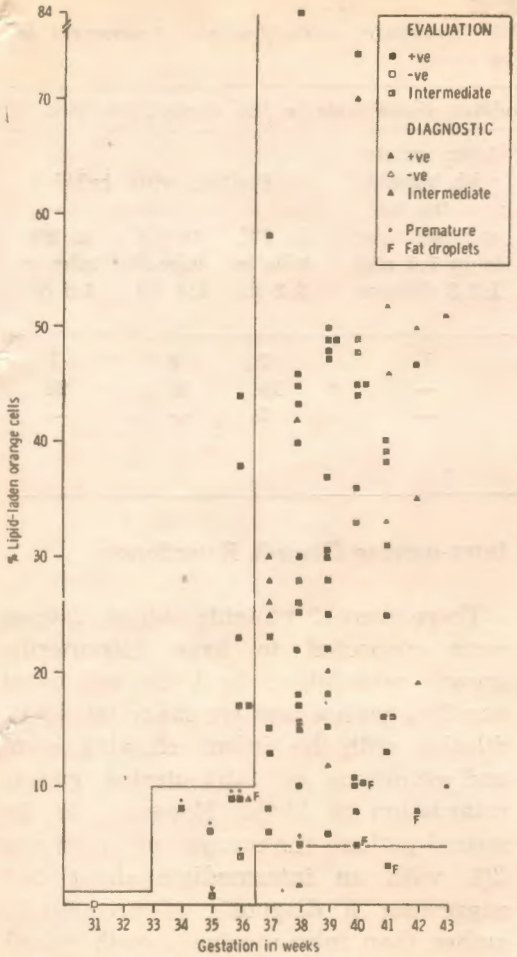

Fig. 1.

Although none of the infants had respiratory difficulties at birth, there were premature infants. Six out of 7 were premature at birth from this group.

Intermediate Shake Tests

In the entire study including the diagnostic group as well, there were 5 intermediate shake test results, 1 from the evaluation group and 4 from the diagnostic group. The patient from the evaluation group was admitted in premature labour. The shake test was intermediate with an orange cell count of 4%. She delivered a premature baby weighing 1.6 Kg. The baby had few transient cyanotic attacks in the first 6 hours, but later settled down and was discharged home after a month and a half. The remaining 4 intermediate shake tests were obtained in the diagnostic group, tests being performed prior to induction of labour. The orange cell count in these 4 cases varied between 2% and 12%. In all the patients induction was deferred. Tests were repeated after 8 and 12 days respectively. When the shake tests became positive, at least in 1:2 dilution, labour was induced followed by the birth of mature babies with an uneventful neonatal period.

Analysis of Shake Tests in the Various Dilutions

The individual shake tests in various dilutions correlated with postnatal clinical assessment of infants are presented in Table II. When the modified shake test was positive in the first three tubes, i.e. including 1:2 dilution, there were 7 out of 17 babies who were premature, although none of these babies had any respiratory difficulties. When the test was positive in the first four tubes including 1:4 dilution, only 2 out of 24 babies were premature at birth and 1 had respiratory difficulties. However, when all the five tubes had positive results including 1:5 dilution there was 1 infant born premature out of 23 infants. Again no respiratory problems were encountered.

Figure 2 correlates the orange cell count and shake tests. It is evident that a positive modified shake test is invariably associated with a count over 5%, although the majority had counts over 10%, the range being 10%-50%.

TABLE II

Results of Modified Shake Tests in Individual Tube Correlated with Postnatal Assessment in 67 Evaluation Cases

Maturity	Total Number of Samples in Each Group	Results of modified shake tests in the various dilutions					
		Negative with no bubbles	Negative with no bubbles in 1st tube ie 1:1 dil	Intermediate with bubbles in the 1st and 2nd tube ie in 1:1 and 1:1.3 dilution	Positive with bubbles		
					in 3rd tube ie 1:2 dil	in 4th tube ie 1:4 dil	in 5th tube ie 1:5 dil
Premature	11	1	—	1	7	2	1
Mature	55	—	—	—	10	22	22
Intra-uterine Growth Retardation	1	—	—	—	1	—	—

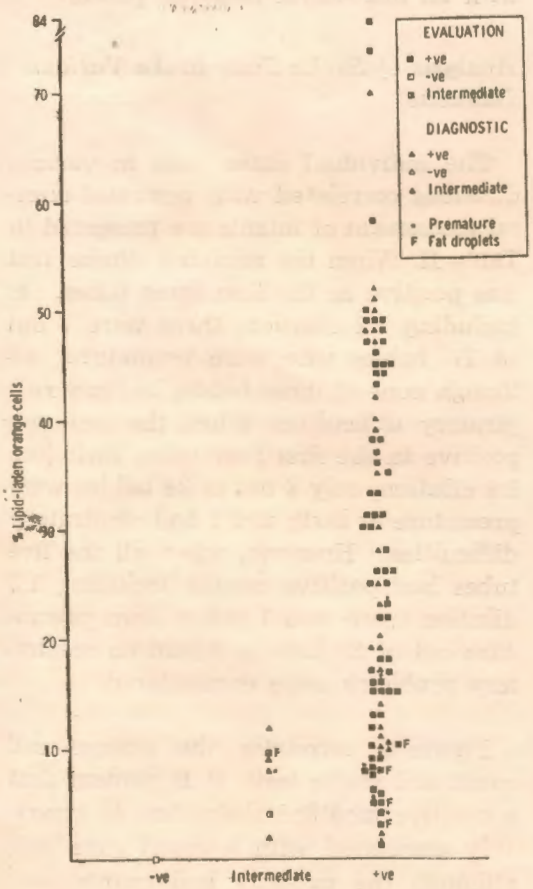

Fig. 2.

Intra-uterine Growth Retardation

There were 2 patients whose fetuses were suspected to have intrauterine growth retardation. In 1 the cell count was 17% with a positive shake test in 1:2 dilution with the infant showing signs and symptoms of intra-uterine growth retardation at birth. However, in the second patient, the orange cell count was 2% with an intermediate shake test, suggesting a diagnosis of prematurity rather than intra-uterine growth retardation from our earlier finding (Iyer, 1979). The pregnancy was allowed to continue, with the patient delivering a mature infant after four and a half weeks.

Discussion

Both the Nile Blue sulphate staining and the shake tests have the common denominators of being essentially simple, inexpensive and both can be carried out by resident staffs as a side room procedure. When only the Nile Blue sulphate test was used (Iyer, 1979), it was found that no range could be given for post-mature pregnancies, as even those past

term gave a count in the 30-50% range, as opposed to the English figures of over 50% suggesting postmaturity (Brosens and Gordon, 1966).

Since lipid laden orange cell count only reflects the skin maturity we decided to combine both the shake test and Nile Blue sulphate test for assessing maturity. In our modification except for the use of 100% absolute ethanol, no other steps have been modified as opposed to modification of Edwards and Baillie (1973) where in addition to the use of 100% ethanol, no dilution with normal saline is done and results are given a scoring. In our modification of the Clements test (1972), the personal error involved in scoring when different members of the resident staff perform the tests has been eliminated, in addition to incorporating 100% ethanol, whereby an 0.50 ethanol volume fraction is achieved, which seems critical and modifies the potential for stable foam formation as apposed to the 0.475 final ethanol volume fraction in the original Clements shake test as suggested by Statland *et al*, 1978.

When a positive shake test in either all the dilutions, i.e. till 1:5 dilution or until 1:4 dilution (4th tube) with a count in the range of 10%-30% is obtained, one can confidently predict that the baby will be both mature and have no respiratory problems in the neonatal period. Even when the shake test is positive until 1:2 dilution (i.e. first 3 tubes) with a mature cell count of over 10% the same clinical expectation as above can be predicted. However, when the shake test is positive until 1:2 (i.e. first 3 tubes) but with count below 9% and not associated with fat globules, then although there may be no respiratory difficulties, the chances of the newborn infant being premature are high.

In the intermediate result group, irrespective of the orange cell count, it would suggest the fetus of being premature and any obstetric interference is better deferred, if the clinical situation should permit such a delay.

However, since even this present modification using 100% ethanol giving a 0.50 ethanol volume fraction gives only a qualitative information, in cases where an intermediate or a negative shake test result is obtained, and the clinical situation demands a more quantitative assessment, then either chemical estimations of Palmitic acid as suggested by Maclennan *et al* (1975) or total phospholipid assay (Fairbrother *et al*, 1975) may be necessary, which will be helpful in these cases where the clinical risk of a continuing pregnancy has to be decided.

Using both the tests the ambiguity in some cases of intermediate and positive results in 1:2 dilution is resumed and labour may be induced. However, if the test results in intermediate or negative in association with a low orange cell count, the chances of the baby being premature are more likely than growth retarded, should these be clinically suspected pregnancy should be allowed to continue, especially when the details of the last menstrual period are unavailable.

Summary

Results of simultaneous Nile Blue sulphaste staining and a "modified shake test" on uncontaminated liquor amnii samples are presented. Both the tests were compared and evaluated for their accuracy in predicting the gestational age and fetal lung maturity. Initially 67 normal pregnancies were studied and later these tests were performed prior to induction of labour in 29 pregnancies. Detailed analysis of our modified shake test in the various dilutions is presented. When the shake test was positive in all

dilutions the mean orange cell count was over 12%, the range being between 10%-35%. This correlated with a clinical maturity of over 38 weeks. These infants had no respiratory difficulties in the neonatal period. When the modified shake test was positive upto and including the 1:2 dilution, the orange cell count was over 10%, with a mature baby having no respiratory difficulties. However, when the orange cell count was low with this 1:2 dilution more babies were premature at birth. In this group of cases, when the orange cell count was between 2%-9%, associated with plenty of fat globules and droplets (6-8 per low power field) irrespective of the dilution, no respiratory distress was encountered.

Intermediate shake tests either with low or normal orange cell count warrants delay when obstetrical interference is contemplated. In these circumstances further biochemical measurements may be necessary. Gestation of 33 weeks or less were invariably associated with a negative shake test and a zero orange cell count. Such babies are prone to R.D.S. Both tests were valuable in differentiating the intra-uterine growth retarded fetus from a premature one.

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