THE TAP TEST — AN ACCURATE FIRST-LINE TEST FOR FETAL LUNG MATURITY TESTING

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Objective. To determine the accuracy of near-patient and laboratory-based fetal lung maturity tests in predicting the need for neonatal ventilation.

Design. A prospective descriptive study.

Subjects. One hundred high-risk obstetric patients where confirmation of fetal lung maturity would initiate delivery.

Methods. Fetal weight estimation, placental maturity grading, and amniocentesis were performed. The investigators examined the amniotic fluid visually, and performed the tap test and shake test. Laboratory technicians estimated the lecithin-sphingomyelin (L/S) ratio, determined the presence of a phosphatidyl glycerol (PG) band on gel electrophoresis, and the optical density at 650 nm. Neonates delivered within 1 week of amniocentesis were included in the analysis. The primary end-point was the ability of the lung maturity tests to predict the need for neonatal ventilation.

Results. Twelve of 100 neonates required ventilation. The tap test and optical density (OD) shift at 650 nm predicted the need for neonatal ventilation with the greatest accuracy.

Conclusion. The tap test is a rapid, easy and accurate predictor of the need for neonatal ventilation. The OD shift at 650 nm is the laboratory-based test with the greatest accuracy in our setting.

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Preterm delivery of the medically compromised obstetric patient poses the risk of delivering an immature infant. This risk is difficult to quantify when uncertainty exists about the gestational age. One of the main contributors to the neonatal morbidity of the immature infant is hyaline membrane disease.[1] Fetal lung maturity testing, performed on amniotic fluid, can be used to avoid hyaline membrane disease. On the other hand, delivery can be initiated when fetal lung maturity is deemed likely.

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The estimation of the lecithin/sphingomyelin (L/S) ratio in amniotic fluid was described by Gluck and Kulovich[2] to confirm fetal lung maturity. It is still the mainstay of fetal lung maturity testing,[3] together with the presence of a phosphatidyl glycerol (PG) band on gel electrophoresis.[4] These tests are time consuming and costly, require extensive training to perform, and are not always readily available after hours. The main disadvantage, however, remains the high rate of false immature results.[5]

A near-patient test such as the shake test has the advantage that it can be done at the patient's bedside. Decisions about delivery or expectant management can therefore be made without undue delay. The shake test is also widely promoted as a bedside test of fetal lung maturity.[6,7] Unfortunately, the shake test has a high rate of false immature results. A false immature result is safe from the fetal or neonatal point of view, but puts the mother at unnecessary risk. Different studies[8-11] have shown that the tap test is as good a predictor of maturity, but a better predictor of immaturity, than the shake test.[8-11] The present study was therefore undertaken to evaluate the ability of different near-patient and laboratory tests to predict fetal lung maturity.

PATIENTS AND METHODS

A prospective study was done on 100 high-risk obstetric patients. Patients qualified for inclusion in the study if uncertainty existed about the gestational age, if the estimated fetal weight was between 500 and 2 500 g, and if the patient's medical condition warranted elective delivery if fetal lung maturity was predicted. Patients were excluded from the study in cases of diabetes mellitus or multiple pregnancy, where the patient was younger than 18 years, where informed consent could not be obtained, and where the delay between amniocentesis and delivery was longer than 1 week. The L/S ratio was taken as the gold standard and the results of the other tests were not made available to the clinicians. Results of the L/S ratio normally take 12 - 18 hours to be made available to the managing clinicians.

The patient's age, indication for elective delivery, estimation of gestational age and estimated fetal weight were noted. Betamethasone was given to promote fetal lung maturity at a dose of 12 mg, repeated after 12 - 24 hours. The amniocentesis was done during the time when the corticosteroids were given. Detailed obstetric ultrasound was undertaken, including placental maturity grading[12] and fetal weight estimation.[13,14]

A Toshiba Sonolayer SSA-250 (Tecmed, Halfway House, South Africa) with a 3.5 MHz curvilinear probe was used. When grade III maturity changes were seen in all areas of the placenta, it was recorded as mature. When no grade III changes were seen, the placental maturity grading was recorded as immature, and when it was seen in part of the

placenta, it was recorded as inconclusive.[12] After obtaining informed consent, amniocentesis was done by the managing registrar or consultant under sterile conditions using a 20G spinal needle under sonographic guidance. Contamination of the amniotic fluid, as well as any complications of the procedure were noted. Amniotic fluid samples contaminated with blood, meconium or mucus were centrifuged at 400 g for 5 minutes before further testing. Three millilitres of amniotic fluid were reserved for near-patient testing. The remainder of a volume of 10 ml was dispatched to the laboratory for determination of L/S ratio, PG band and difference in optical density at 650 nm.

Near-patient testing of the amniotic fluid included visual assessment, the shake test and the tap test. If it was impossible to read newsprint through a 12 mm diameter glass tube filled with amniotic fluid, the visual assessment was deemed mature. Clear fluid was recorded as immature, and doubtful cases as inconclusive.[15]

To perform the shake test, 1 ml of amniotic fluid was placed in an 85 × 12 mm test tube together with 1 ml 95% ethanol (1:1 solution). In another test tube, 0.5 ml of amniotic fluid and 0.5 ml 0.9% saline were placed together with 1 ml 95% ethanol (1:2 solution). Both tubes were shaken for 15 seconds, and placed in an upright rack for 15 minutes. Persistence of a 360° ring of bubbles at the air-liquid interface in the 1:2 solution was interpreted to predict maturity. Clumping of bubbles that would create a 360° ring if spread out was also considered mature. If no bubbles persisted in either solution, the test was considered immature. If the 1:1 solution was considered mature and the 1:2 solution immature, the test was recorded as being inconclusive.

To perform the tap test, one drop of 6N hydrochloric acid was added to 1 ml of amniotic fluid in an 85 × 12 mm glass test tube. Diethyl ether (1.5 ml) was added to form a layer on top of the amniotic fluid. The test tube was tapped briskly three or four times to form 200 - 300 bubbles in the ether layer. If more than five bubbles remained in the ether layer after 10 minutes, the test was considered immature. If five or less bubbles remained in the ether layer after 10 minutes, the test was considered mature.[8]

In the laboratory the phospholipids were extracted using a chloroform-methanol mixture and then separated by two-dimensional thin-layer chromatography. The results were compared with control solutions of L/S standard 2:1 and PG standard.[16] The optic density at 650 nm was assessed spectrophotometrically.[17]

After delivery, the time interval from amniocentesis to delivery was noted. The need for neonatal ventilation, as well as the indication for ventilation, were also noted.

Epi-info version 6.1 (WHO 1993) was used to calculate the sensitivity, specificity, positive and negative predictive value and 95% confidence intervals of the different tests.

The ethics committee of the University of Pretoria approved the study.

Results

One hundred and eleven patients qualified for inclusion in the trial. Two patients did not provide informed consent. Nine patients delivered more than 1 week after amniocentesis, and were excluded from analysis. All 9 had an L/S ratio less than 2 and hence were not delivered. The indications for the amniocentesis were proteinuric hypertension (N = 55), fetal growth impairment (N = 15), preterm labour (N = 11), preterm ruptured membranes (N = 4), antepartum haemorrhage (N = 7), poor obstetric history (N = 6) and 11 other indications. Complications of amniocentesis included 5 patients with an unsuccessful first attempt at amniocentesis, and 5 bloody taps. Fourteen samples were contaminated with meconium. In 3 patients ruptured membranes were confirmed within 24 hours of amniocentesis. All of these patients delivered within the week. Ten neonates required ventilation for hyaline membrane disease, 1 for congenital pneumonia, and 1 for asphyxia neonatorum. Two neonates were not ventilated because of a shortage of ventilators in the neonatal intensive care unit, and a poor prognosis owing to extreme multi-organ immaturity. The predictive value of the different tests with regard to the need for ventilation is depicted in Table I.

Discussion

Accurate prediction of fetal lung maturity is crucial in the management of high-risk obstetric patients, especially those with unsure gestational age.

This investigation shows that the tap test is an easy and reliable bedside test for fetal lung maturity, with better predictive values for ventilation than the shake test and conventional laboratory tests. These results are supported by other data from the literature (Table II).

The lower predictive value for immaturity in this study can be explained on three grounds. In this study, there was a longer amniocentesis-delivery interval, corticosteroids for fetal lung maturation were not withheld, and the final end-point used, namely neonatal ventilation, was more strict than the diagnostic category of hyaline membrane disease, the spectrum of which includes mild disease.

In line with the proposal of a cascade system of fetal lung maturity testing by Garite and Freeman,[18] the tap test can surely be instituted as first-line testing. However, strict adherence to the test description is necessary.

A test predicting maturity can initiate delivery even in a secondary hospital, while a test predicting immaturity should initiate immediate referral of the patient to a tertiary centre, where expectant management could be considered. If delivery

Table I. Accuracy of different tests in predicting the need for neonatal ventilation (%, (% range), *N*)

Test	Sensitivity	Specificity	Predictive value for ventilation	Predictive value for absence of ventilation
Placental	80	11	8	86
maturity	(30-99)	(5-23)	(3-19)	(42-99)
grading	4/5	6/54	4/52	6/7
Visual	83	45	17	95
assessment	(51-97)	(34-56)	(9-30)	(82-99)
	10/12	39/87	10/58	39/41
Shake test	100	10	13	100
	(70-100)	(5-19)	(7-23)	(63-100)
	12/12	9/87	12/90	9/9
Tap test	92	84	44	99
	(60-99)	(74-91)	(25-65)	(92-100)
	11/12	73/87	11/25	73/74
Optical density	100	79	46	100
	(66-100)	(66-88)	(25-67)	(90-100)
	10/10	45/57	10/22	45/45
L/S ratio	100	55	23	100
	(68-100)	(44-66)	(13-38)	(90-100)
	11/11	45/82	11/48	45/45
Phosphatidyl glycerol	100	59	25	100
	(68-100)	(48-70)	(14-40)	(91-100)
	11/11	48/81	11/44	48/48

L/S = lecithin- sphingomyelin.

Table II. Comparison of studies on the tap test with respect to their predictive values for the endpoints used

Tap test	Predictive value of immature test (%)	Predictive value of mature test (%)	End-point
Socol[8]	67	97	Hyaline membrane disease
Guidozzi and Gobetz[9]	63	91	Correlation with L/S ratio
Rodriques-Macias[10]	98	100	Hyaline membrane disease
Kucuk[11]	68	95	Hyaline membrane disease
Pretoria	44	99	Neonatal ventilation

is indicated in the face of predicted neonatal pulmonary immaturity, the immediate availability of NICU facilities would offer the best chance of survival. This proposal could prevent unnecessary referrals, and on the other hand initiate early referral of patients where tertiary care is appropriate. In bigger centres with availability of spectrophotometers, an OD of 650 nm can safely be initiated as the second-line test to confirm immaturity. This is less costly and time consuming than the L/S and PG estimations. Only contaminated specimens and immature prediction using OD 650 nm would necessitate testing of the full phospholipid profile.

While fetal lung maturity testing in the First-World setting is not often indicated, accurate and easy fetal lung maturity tests in the developing world, where unsure gestational age is a common problem, is a valuable tool in the clinical management of high-risk obstetric patients.

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IMPACT OF URBANISATION ON SERUM LIPID PROFILES — THE THUSA SURVEY

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Objective. To examine the impact of urbanisation on lipid profiles of black South Africans, stratified for HIV status.

Design. Cross-sectional population-based survey.

Setting. North West province of South Africa.

Subjects. A representative sample of 1 854 apparently healthy volunteers aged ≥ 15 years, was recruited from 37 randomly selected sites throughout the province. Subjects were stratified into five urbanisation strata (S): S1 rural villages, S2 farms, S3 informal housing or 'squatter camps', S4 urban townships, and S5 surburban housing.

Outcome measures. Demographic, physical activity and dietary intake information was collected using validated and culture-sensitive questionnaires. Anthropometric measurements and lipid analyses were determined using standardised methodology.

Results. The results revealed significantly lower mean (95% confidence interval) total serum cholesterol (TC) levels in HIV-negative men in S1 - S4 compared with S5 (S1 3.91 (3.77 - 4.05) v. S5 4.79 (4.54 - 5.04) mmol/l). In HIV-negative women, TC levels were significantly lower in S1 - S3 than in S4 and S5 (S1 4.05 (3.94 - 4.17) v. S5 4.79 (4.59 - 5.00) mmol/l). The same trends were seen for serum low-density lipoprotein cholesterol (LDLC) and triglycerides and in HIV-positive subjects. Binary logistical analysis indicated that the main factor responsible for the increased TC levels seemed to be increased body mass index (BMI) due to decreased physical activity.

Conclusions. Serum lipid levels increased with urbanisation although they remained within levels recommended for

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