POSTNATAL EVALUATION OF LUNG MATURITY IN PREMATURES BY TDXFLM AND TAP TEST IN GASTRIC ASPIRATE

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SUMMARY :

Purpose: *To define the reliability of TDxFLM assay and tap test in gastric aspirates of premature babies in evaluating their pulmonary maturity and to reduce unnecessary use of surfactant therapy.* ***Methods:*** *Thirty three premature infants whose gestational ages were between 27-34 weeks (31.2 SD 1.9), and birth weights were between 820-2650 grams (1860 SD 470) were included in the study. Gastric aspirate was obtained from all babies within the first hour after birth, before they were fed. Gastric aspirate fluid of each infant was divided into 2 to be used in TDxFLM assay and tap test. The infants were also observed for clinical and radiological signs and symptoms of respiratory distress syndrome (RDS). Sensitivity, specificity, and predictive values for maturity and immaturity of both tests were determined by comparing the results of these tests to the absence or presence of the neonatal RDS.* ***Results:*** *Clinical and radiological findings of RDS were detected in 8 of 33 infants. The specificity, sensitivity and predictive values for maturity and immaturity for TDxFLM assay were calculated as 88%, 75%, 91.7 %, 66.7 %; and for Tap test as 88%, 62.5%, 88%, and 62.5% respectively. The difference between the two tests was not statistically significant.* ***Conclusion:*** *Premature infants with RDS risk can be evaluated easily and rapidly with lung maturity tests performed in gastric aspirate. Tap test and TDxFLM assay are both reliable for this purpose and may be used to predict the neonates with surfactant deficiency in clinical application of prophylactic surfactant therapy when the cost of therapy is a concern.*

Key Words: *Pulmonary Maturity, Tap Test, TDxFLM Assay, Respiratory Distress Syndrome.*

INTRODUCTION

Respiratory distress syndrome (RDS) continues to be one of the main causes of mortality and morbidity in premature infants (1) and an early postnatal evaluation of lung maturity may be important for early diagnosis and treatment of RDS. Since the 1950's when pulmonary immaturity and surfactant deficiency were reported as the cause of RDS, there have been many studies on surfactant replacement therapy (2-4). Surfactant application is an expensive procedure, so the patients who will receive prophylactic therapy should be defined carefully. Therefore there is an increasing need for a rapid and reliable test to predict the risk of RDS.

The standard test for fetal lung maturity has been the lecithin/sphingomyelin (L/S) ratio.

Recently it is being replaced in many centers by other tests which are easier to perform, such as Tap test (5, 6) and fetal lung maturity test implemented by TDx analyser (Abbott laboratories) called TDx FLM assay (7). These lung maturity tests are performed in amniotic fluid, but when amniocentesis has not been performed, alternative biological fluids such as oropharyngeal and gastric aspirates of neonates obtained soon after birth are shown to be appropriate for testing. (8, 9).

The purpose of this study was to define and compare the reliability of Tap test and TDx FLM assay in gastric aspirate to predict the RDS risk of premature babies and to avoid unnecessary prophylactic use of surfactant.

MATERIAL AND METHODS

Thirty three babies who were thought to carry the risk of RDS because of their gestational age and birth weights were included in this study. The gestational age of the babies ranged between 27-34 weeks and birth weights ranged between 820-2650 grams. Only the infants whose birth weights were appropriate for gestational age were included. Gestational age was determined by last menstrual date, ultrasound if available and Dubowitz examination (10). Babies who had intrauterine growth retardation, meconium in amniotic fluid, congenital malformation or infection such as sepsis or pneumonia were excluded.

All patients were followed by the attending neonatologist. None of the patients received prophylactic surfactant therapy before the signs and symptoms of RDS were observed. The diagnosis of RDS was based on standard clinical and radiologic findings which included chest retraction, grunting, cyanosis, oxygen supplementation for a minimum of 24 hours; a ground glass or reticulonodular appearance with air bronchograms on chest x-ray film and negative culture results (5, 7)

Assessment of lung maturity: Two milliliters of gastric aspirate was obtained by a nasogastric tube from all babies within the first hour after birth, before they were fed. Parental consent was obtained for the procedure. The aspirates did not contain meconium or blood. Gastric aspirate was divided into two parts to be used in tap test and TDxFLM assay. The tests were performed by the same biochemistry specialist who was unaware of the patients' clinical course.

TDxFLM assay: (Abbott laboratories) This test uses the automated capabilities of the TDx analyser to make all necessary reagent and sample additions, monitor the reaction and determine net fluorescence polarization values, and calculate the surfactant-to-albumin ratio on the basis of a six-point calibration curve (0, 10, 20, 40, 80 and 160 mg surfactant/albumin) (7). The TDxFLM assay was performed according to the instructions provided by the manufacturer. A volume of 0.5 ml of gastric aspirate was passed through a filter disk into a specially designed sample well for processing on the Abbott TDx analyser. The results were interpreted according to the manufacturer's recommendations which were as follows: If surfactant/albumin ratio was less than 50 mg/g, the babies lungs were considered immature; if it was between 50-69 mg/g it was considered as a transitional zone and if it was greater than 70 mg/g the lungs were thought to be mature.

Tap test: This test was performed as defined by Socol et al previously (5). 1 ml of gastric aspirate fluid was mixed with 1 drop of 6N hydrochloric acid (concentrated hydrochloric acid diluted 1:1) and then 1.5 ml of diethyl ether was added. The 16X150 mm test tube was briskly tapped three or four times, which creates an estimated 200-300 bubbles in the ether layer. In mature babies' gastric aspirate fluid bubbles quickly rise to the surface and break down; in immature babies the bubbles are stable or break down slowly. The test was read at 5th minute and cutoff for maturity was set at 5 bubbles. If no more than 5 bubbles persisted in the ether layer the test was considered mature.

For both tests sensitivity, specificity and predictive values for maturity and immaturity were determined by comparing the results of these tests to the absence or presence of neonatal RDS.

RESULTS

The gestational ages of the 33 babies ranged from 27 to 34 weeks (31.3 SD 1.9) and birth weights were between 820-2650 gr (1860 SD 470). Fifteen babies were female (45.5 %) and 18 were males (54.5%). Mean gestational ages and

Table 1: Mean gestational age and birth weight of the patients.

Group	No of patients	Mean gestational age (week)	Mean birth weight (gram)
RDS (-)	25	31.9 ± 1.509	1970 ± 400
RDS (+)	8	29.5 ± 2.070	1480 ± 500

mean birth weights of the neonates with and without RDS are presented in table 1. In 8 of these 33 babies (24.2%), clinical and radiological findings of RDS were observed. The results of tap test and TDxFLM assay in gastric aspirates of the patients are shown in table 2. Both tests predicted 22 infants as mature and they did not develop RDS. Three patients in the tap test group and 2 patients in TDxFLM assay developed RDS although the tests showed them as mature (false mature). Tap test predicted 8 infants as immature, 5 of which developed RDS. TDxFLM assay revealed 9 immature results and 6 of them had RDS. Sensitivity, specificity and predictive values for maturity and immaturity of both tests are shown in table 3. The specificity of both tests was 88%. Sensitivity and predictive value for maturity and immaturity for tap test were 62.5%, 88% and 62.5 % respectively. These values were 75%, 91.5% and 66.7% respectively for TDxFLM assay.

Table 2: Results of tap test and TDxFLM assay.

	True mature[a]	False mature[b]	True immature[c]	False immature[d]
Tap test	22	3	5	3

a. True mature: Predicted mature, no respiratory distress syndrome
b. False mature: Predicted mature, Respiratory distress syndrome
c. True immature: Predicted immature, respiratory distress syndrome
d. False immature: Predicted immature, no respiratory distress syndrome

Table 3: Comparison of TDxFLM assay and Tap test.

Test	Specificity (%)	Sensitivity (%)	Predictive value for maturity (%)	Predictive value for immaturity (%)
TDx FLM	88	75	91.7	66.7
Tap Test	88	62.5	88	62.5

$$\text{Sensitivity} = \frac{\text{correctly predicted immature}}{\text{all immature}}$$

$$\text{Specificity} = \frac{\text{correctly predicted mature}}{\text{all mature}}$$

$$\text{Predictive value for maturity} = \frac{\text{correctly predicted mature}}{\text{all predicted mature}}$$

$$\text{Predictive value for immaturity} = \frac{\text{correctly predicted immature}}{\text{all predicted immature}}$$

DISCUSSION

Although modern neonatal intensive care has considerably reduced the mortality and morbidity of infants born prematurely, RDS continues to be a major problem for them (11, 12). The survival of these infants is largely dependent on early application of appropriate and sophisticated care, such as surfactant therapy and ventilatory support when needed. Another approach in surfactant therapy is to administer it before the signs and symptoms of RDS develop (13). As it is an expensive procedure, its unnecessary use in premature babies with low risk of RDS must be avoided. Lung maturity tests enable the clinician

to make an early diagnosis and to provide the most appropriate treatment for the patient (14). L/S ratio in amniotic fluid and phospholipid profile are the most accepted tests used for this purpose (15,16). However as they were difficult and time consuming assays, more practical tests were looked for. Clements et al described ethanol shake test in 1972 (17), and Sokol et al described Tap test which has a similar principle (5, 16). It is a simple, rapid and inexpensive test depending on bubble stability which changes with the phospholipid content of the fluid (5). TDxFLM assay, an automated test that is based on fluorescein polarization method, can also be performed easily and rapidly (18-22), but it is more expensive than Tap test. These tests are usually performed in amniotic fluid, but as the fetus swallows amniotic fluid in utero they may also be carried out in gastric aspirate which is obtained soon after birth (8, 19, 20). It is an easy and reliable way for postnatal assessment of lung maturity. To our knowledge, in our study these two tests are performed together in gastric aspirates of premature babies for the first time and compared with each other.

In previous studies, Tap test and TDxFLM assay were reported to have high predictive values for lung maturity (2, 7, 22). In our study it was calculated as 88% and 91.7% for Tap test and TDx FLM assay respectively. It is an important finding as it helps to not use surfactant in the babies who were predicted to be mature by these tests, thus reducing the treatment costs. For our study this means those tests helped us to save the cost of treatment in 22 babies who would otherwise have received prophylactic surfactant therapy.. The results of our study are similar to other studies in terms of specificity as well (7, 22), which was 88% in both of our tests. Sensitivities of these tests were reported to be over 85 % in many studies (7, 22). In our study it was 62.5 % and 75 % for tap test and TDxFLM respectively. This difference might be related to the small number of patients who developed RDS in our study. Actually we only missed the opportunity to treat 6% (2 out of 33) and 9 % (3 out of 33) of patients before the symptoms had begun, relying on TDx FLM and Tap test respectively. When we compared sensitivity and predictive value for maturity and immaturity values, TDx FLM assay seemed to be superior to Tap test but the difference was not statistically significant.

We consider that these two tests are both reliable in assessment of lung maturity. As tap test is cheaper and easier to perform, it may be used as a bedside test. Both tests may be used to predict neonates with surfactant deficiency in clinical application of prophylactic surfactant therapy when the cost of therapy is a concern.

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