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Evaluation of Amniotic Fluid Elastolytic Activity: Can it be a Method of Fetal Lung Maturity Assessment? A Comparison with Gluck's L/S Test

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Abstract. Amniotic fluid elastolytic activity was assessed in a group of 120 women who delivered preterm infants and in 35 women who delivered at term. Amniotic fluid elastolytic activity decreases as pregnancy progresses. The lecithin-to-sphingomyelin (L/S) ratio in women's amniotic fluid was determined by the method developed by Gluck and associates [6] and elastolytic activity by that developed by Mehdi and associates. A significant negative correlation was found between the amniotic fluid L/S ratio and amniotic fluid elastolytic activity ($r = -0.932$; $p < 0.001$).

The border value of elastolytic activity that indicates lung maturity (L/S ratio equal to or greater than 2) is 2.01 ± 0.05 mmol/min ml. In the amniotic elastolytic activity test, it is the value that differentiates mature from immature lungs. The amniotic fluid elastolytic activity test is characterized by high sensitivity (91.43%) and specificity (91.67%), high positive prognostic value (76.19%) and low negative prognostic value (2.65%). The test parameters do not therefore differ greatly from those of the Gluck test. Moreover, the amniotic fluid elastolytic activity test is cheaper and takes less time to perform.

Key words: Amniotic fluid elastolytic activity, Fetal lung maturity assessment, Gluck's L/S test, Respiratory distress syndrome

INTRODUCTION

Most investigations of the early diagnosis of respiratory distress syndrome (RDS) in neonates have focused on surfactant lipid components [3, 6, 8]. However, the etiology of respiratory distress in premature infants consists not only in surfactant deficiency, but also in altered connective tissue metabolism [5, 7, 9].

Normal connective tissue structure is indispensable for the proper functioning of the air passages and the alveoli, proper lung fluid clearance and epithelial as well as endothelial barriers. Elasticity of lung tissue is determined by the tissue protein – namely elastin [4]. Desmosine and isodesmosine are elastin-type amino acids. The quantities of the amino acids in amniotic fluid change according to lung maturity [11, 12]. Elastine-indicatory amino acids enter the amniotic fluid as the result of elastin being degraded by elastase. The purpose of our study was to establish the value of amniotic fluid elastolytic-activity determination in the assessment of fetal lung maturity.

MATERIALS AND METHODS

Study group

The study included 155 pregnant women between 29 and 40 weeks' gestation. They were admitted to the Department of Obstetrics and Gynaecology at the Silesian Medical School of Medicine, Zabrze, Poland, between February 1990 and December 1992. Of the women, 14 delivered their infants between the 29th and 30th week of pregnancy, 108 between the 31st and 35th week, and 33 between the 36th and 40th week. Their mean age was 23.3 years, (range 18-33 years, SD 3.0). Among these women, 56 were multiparas and 99 were primigravidas. Pregnant women with hypertension, corticosteroid treatment, diabetes, Rh serologic incompatibility and intrauterine growth retardation (IUGR) were excluded from the study. Repeated ultrasound scans were carried out in each woman in order to evaluate all biometrical indices of the fetus (age, mass and general condition).

Amniotic fluid

In 125 women, amniotic fluid was collected during premature rupture of the amniotic sac (up to 12 hours after the rupture). After colposcope insertion and cervix visualization, amniotic fluid was collected directly with a syringe, thus avoiding contact with vaginal secretions. In the remaining 30 women, amniotic fluid was obtained by amniocentesis during the delivery.

L/S ratio in amniotic fluid

Amniotic fluid lipids were extracted according to the method first described in the early 1970s [6] and subsequently modified [1]. A 4 ml sample of amniotic fluid was centrifuged at 800 × g for 10 minutes at a temperature of 4°C. Next, 2 ml of methanol were added to 2 ml of the supernatant and mixed. Following that, 4 ml of chloroform were added, and the whole mixture was vortexed for 5 minutes and then centrifuged. The chloroform layer was transferred to a dry vial and evaporated under lowered pressure. 1 ml of acetone (–20° C) was added to the residue, and the mixture placed in a fridge for 10 minutes. The acetone solution was centrifuged (800 × g at a temperature –10° C 10 minutes). The supernatant was carefully poured out and the sediment once again dissolved in 0.5 ml chloroform-methanol (2:1) solution at room temperature.

An intraseries variability rate of $v = 7.3\%$ was calculated for $n = 10$ samples of mean lecithin concentration (12.4 μg/ml) and mean sphingomyelin concentration (4.3 μg/ml) while an interseries variability rate of $v = 9.1\%$ was calculated for the four study series.

Elastolytic activity determination

Amniotic fluid elastolytic activity was determined by a more recently developed method [10]. N-methoxysuccynyl -trialanine -p-nitroanilide (Serva, Heidelberg, Germany) was used as a specific substrate. It was dissolved in DMSO at a concentration of 20 mmol/1. The reaction mixture was prepared in a 0.1 M HEPES buffer with 0.5 mmol/1 NaCl and 0.1% Brij-35 detergent. The final concentration of the substrate in the reaction mixture was 0.2 mmol/1. 4 ml of the substrate buffer solution were heated in a spectrophotometer cuvette to a temperature of 37°C, and then 2 ml of amniotic fluid were added (the beginning of the reaction). After a 15-minute incubation period, a reading was taken, at a wavelength of 410 nm. The enzyme activity was expressed as the amount of moles of the substrate decomposed in 1 s by 1 ml of amniotic fluid (nkat/ml).

Mathematical analysis of the results of biochemical investigation of lung maturity

Sensitivity, specificity and prognostic values were calculated according to the method described by a team of researchers in the mid-1980s [2]. The calculation proceeded as follows:

$$\text{Sensitivity} = \frac{a}{a+b} \qquad \text{Specificity} = \frac{d}{c+d}$$

$$\text{Positive prognostic value} = \frac{a}{a+c}$$

$$\text{Negative prognostic value} = \frac{b}{b+d}$$

Where

a = neonates with immature lungs and infant respiratory distress syndrome (IRDS)
b = neonates with mature lungs and IRDS
c = neonates with immature lungs and without IRDS
d = neonates with mature lungs and without IRDS.

Statistical analysis of the results

After its applicability had been confirmed by the Shapiro-Wilk test, Student's t test was used to compare the results between different groups. Correlation coefficients and statistical significance of the correlations were calculated by Pearson's standard method. The calculations were performed using a PC/AT microcomputer running the STATGRAPHIC programme, Version 2.5 (Statistical Graphic Corp. Inc., Rockville, MD).

RESULTS

Amniotic fluid samples taken from 155 normal gravidas between the 25th and 40th weeks of gestation were examined. Fig. 1 shows the results of this activity at the various gestational ages. The linear decrease in elastase activity is observed after 29 weeks' gestation, and elastase activity reaches a value of 2.63 ± 0.07 nkat/ml (n = 79) at 29-40 weeks' gestation. The modified version of the Gluck test was carried out in all women examined, in order to determine the lecithin-to-sphingomyelin ratio in their amniotic fluid. The value of 2 was accepted as the border value of the L/S ratio, differentiating between mature and immature lungs. The L/S test revealed mature lungs in 112 neonates (72.25%). Respiratory distress syndrome (RDS) was subsequently found in two neonates

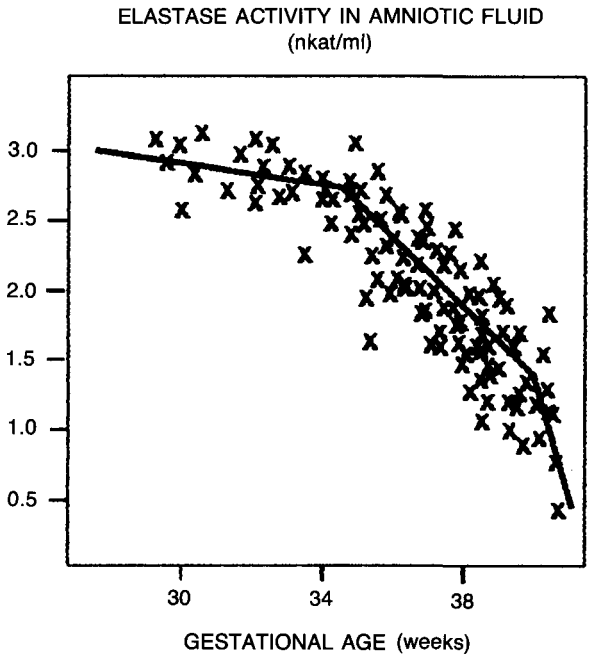

Fig. 1. Amniotic fluid elastase activity at various gestational ages.

Table 1 - Results of the L/S tests according to Gluck et al. in the amniotic fluid of 155 parturient women in relation to RDS occurence

L/S test result	Respiratory distress syndrome (No. of newborns = 155)	
	Present	Absent
<2.0 (immature)	33 (76.74%)	10 (33.26%)
>2.0 (mature)	2 (1.78%)	110 (98.22%)

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of the group (1.78%), however. The L/S test indicated mature lungs in 43 neonates (27.75%), RDS was diagnosed in 33 of these infants (76.74%). No respiratory distress was found in the remaining 10 (33.26%). From these results, the following were then calculated for the L/S test: sensitivity 94.29%, specificity 91.74%, positive prognostic value 76.74%, and negative prognostic value 1.76%.

The results of the L/S test were analysed with reference to birthweight and fetal age. The correlation between the L/S test and neonatal weight is $r = 0.899$ and its statistical significance $p < 0.002$. Amniotic fluid elastolytic activity in fluids with an L/S ratio indicating immature lungs is significantly higher when compared to fluids with an L/S ratio indicative of mature lungs (Table 2). The results were also analysed with respect to elastolytic activity and the development of RDS (Table 3). Elastolytic activity is significantly higher in those groups with than in those without RDS.

A significant negative correlation was found between L/S ratio and amniotic fluid elastolytic activity ($r = -0.932$; $p < 0.001$). The value read from the curve of elastolytic activity where the L/S ratio is equal to 2 (the border L/S value for lung maturation) is 2.01 ± 0.05 mmol/min ml. As can be seen in Fig. 2, lung maturation, as reflected by the increased L/S ratios, is accompanied by a significant decrease in elastase activity. A correlation coefficient of $r = -0.891$ ($p < 0.02$) between L/S and elastase activity was calculated on the basis of the results shown in Fig. 2.

Elastolytic activity and RDS development were analysed (Table 3). For the purpose of testing, the value of 2.01 mmol/min. ml was taken as the border value differentiating

Table 2 - Amniotic fluid elastolytic activity in relation to the L/S test results

L/S ratio	Below 2.0 (n = 43)	Equal to or greater than 2.0 (n = 112)
Elastolytic activity[a]	2.27 ± 0.14*	1.68 ± 0.15

Key: n = the number of newborns.
* = statistical significance at $p < 0.01$ level
[a] = Elastolytic activity expressed as nkat/ml of amniotic fluid

Table 3 - The results of amniotic fluid elastolytic activity testing in 155 parturients according to the development of respiratory distress syndrome (RDS)

Elastolytic activity*	Respiratory distress syndrome	
	Present	Absent
Below 2.01 (mature)	2 (1.44%)	110 (72.25%)
Equal to or greater than 2.01 (immature)	33 (76.74%)	10 (23.26%)

* The borderline value of elastolytic activity differentiating between mature and immature lungs was calculated on the basis of Gluck et al.'s L/S test.

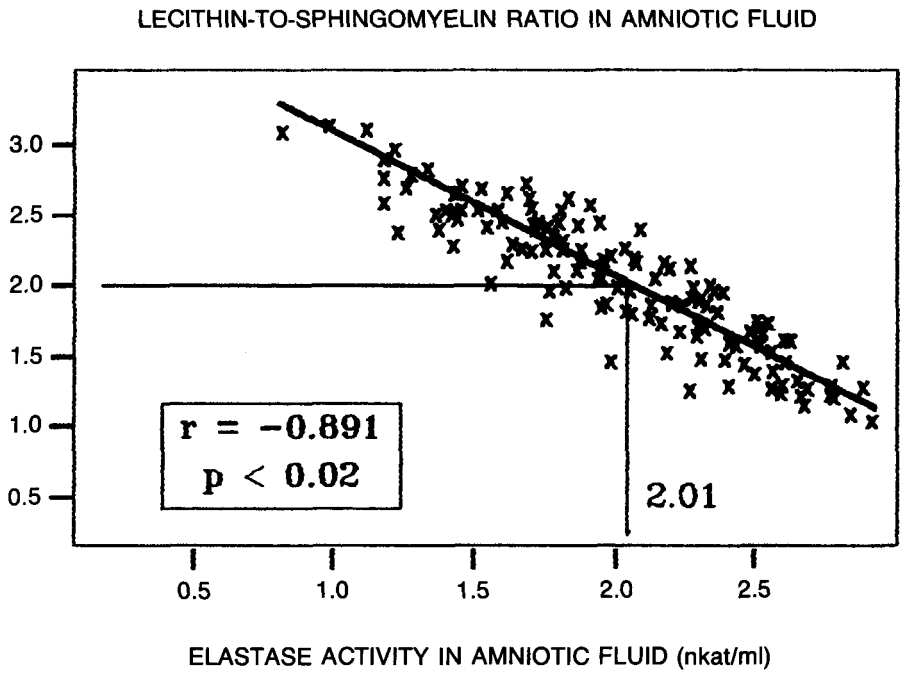

Fig. 2. Correlation between elastase activity and lecithin-to-sphingomyelin ratio in amniotic fluid.

mature from immature lungs. Values lower than this evidenced lung maturity. 112 neonates were found to have mature lungs (72.75%) according to this criterion. RDS was observed in 2 neonates (1.44%) of the group. The test revealed lung immaturity in 43 neonates (27.75%), and in 33 (76.74%) RDS was subsequently found, while in 10 (23.26%) it was not. The following values were calculated from the amniotic elastolytic activity test: sensitivity 91.43%, specificity 91.67%, positive prognostic value 76.19%, and negative prognostic value 2.65%.

In addition, the correlation between birthweight and amniotic fluid elastolytic activity and between gestational age and amniotic fluid elastolytic activity was determined at $r = -0.877$ (statistical significance $p < 0.002$) and $r = -0.788$ (statistical significance $p < 0.005$) respectively. The parameters of the elastolytic activity test are similar to the corresponding parameters of the Gluck test.

DISCUSSION

The incidence of RDS significantly influences the mortality rate among preterm infants. The degree of fetal respiratory system maturity is a vital factor in the pathophysiology of hyaline membrane syndrome, atalectasis and pneumonia, the basic causes of RDS [7, 13]. The problem is complex, because the lungs are made up of over forty different

cell types joined by connective tissue. Lung maturity is the result of the maturity of numerous separate systems, among them the vascular and biochemical systems.

The Gluck L/S test remains the 'golden standard', and for many investigators is still the point of reference. At the beginning of the 1980s, a team of researchers found products of elastin degradation in amniotic fluid: desmosine and isodesmosine. The concentration of amino acids decreased during the course of pregnancy. In evaluating a test, it is important to pay attention to its sensitivity and specificity, as well as to its positive and negative prognostic values. The amniotic elastolytic activity method of fetal lung maturity assessment takes less time to perform than the L/S test, and its sensitivity as well as its specificity values do not differ greatly from those yielded by Gluck et al's L/S test. Its positive prognostic value is a little lower, and therefore the test should be applied as the second in tests cascade.

The amniotic elastolytic activity test requires further investigation, and should also be carried out in women with diabetes and Rh-system immunisation. Also, the usefulness of the test in amniotic fluid soiled with meconium, haemoglobin or colonised bacteria ought to be determined.

CONCLUSIONS

The interdependence between amniotic fluid elastolytic activity and the amniotic fluid L/S ratio proves simultaneous maturation of fetal lung connective tissue and fetal lung surfactant. The sensitivity and specificity of the amniotic fluid elastolytic activity test is comparable to that of the Gluck test. It also takes less time to perform.

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