Improved Thin-Layer Chromatographic Determination of Phospholipids in Gastric Aspirate from Newborns, for Assessment of Lung Maturity

D. Serrano de la Cruz, E. Santillana, A. Mingo, G. Fuenmayor, A. Pantoja, and E. Fernández

This one-dimensional thin-layer chromatographic method is used for assay of phospholipids in the gastric aspirate of newborns. The solvent mixture (chloroform/hexane/methanol/glacial acetic acid/water, 12:7:4:3:0.3 by vol) completely resolves lecithin, sphingomyelin, phosphatidylglycerol, phosphatidylserine, phosphatidylethanolamine, and phosphatidylglycerol. The method is simple, precise, inexpensive, and rapid (chromatographic development takes less than 25 min) and gives high chromatographic resolution. We used this method to determine the lecithin/sphingomyelin densitometric ratio (L/S ratio) and the phosphatidylglycerol percentage in 200 samples of gastric aspirate and found an L/S ratio of 2.5 to be a satisfactory cutoff value for distinguishing fetal lung maturity and immaturity. We confirmed that the presence of phosphatidylglycerol excluded the possibility of respiratory distress.

Additional Keyphrases: respiratory distress syndrome • lecithin/sphingomyelin ratio • phosphatidylglycerol

Respiratory distress syndrome (RDS) results from surfactant deficiency in the pulmonary alveoli (1, 2). Surfactant decreases the surface tension, so that the alveoli tend to collapse. More than 80% of active surfactant consists of phospholipids, of which lecithin is present in the greatest concentration. Surfactant production is low during the first weeks of gestation, increasing progressively from the 26th week and then very rapidly from the 35th week until birth (3, 4).

In 1971, Gluck et al. (5) used the lecithin/sphingomyelin (L/S) ratio to predict the degree of fetal lung maturity; a value of >2 for this ratio in amniotic fluid is accepted as an index of maturity (6). Other authors have determined the L/S ratio in tracheal and pharyngeal (7) and gastric (8) aspirates from newborn infants.

RDS diagnosis is based on clinical (9) and radiological (10) criteria. However, these criteria are not always reliable, because they are also present with other diseases (for example, sepsis with β-hemolytic streptococcus pneumonia). Therefore, an accurate analytical test is necessary to confirm the diagnosis.

In recent years, different methods for determination of phospholipids (especially lecithin and sphingomyelin) have been studied. Most of these have been based on one-dimensional (11–14) or two-dimensional (15) chromatography. Other methods have involved the colorimetric estimation of phosphorus (16) or enzymatic techniques (17–19). Here we report an improved one-dimensional thin-layer chromatographic procedure for determining phospholipids in gastric aspirate fluid from newborns.

Materials and Methods

Clinical samples. Gastric aspirates from newborns were collected through a nasogastric tube within 2 h of birth and either analyzed immediately or stored at −20 °C until analysis. Samples containing blood (20) or meconium (21, 22) were excluded. The minimum sample volume used for this method is 1 mL.

Reagents. All reagents were of analytical-reagent grade. Standards. L-α-Lecithin, sphingomyelin, phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, and phosphatidylglycerol were from Sigma Chemical Co., St. Louis, MO. We prepared standards to contain 1 mg of each phospholipid per milliliter of a chloroform/hexane (equi-volume) mixture.

Controls. These were from Helena Labs., Beaumont, TX, with "mature," "immature," and "borderline" phospholipid contents (L/S ratios in the range from 1 to 4.2).

Chromatography solvent. We used chloroform/hexane/methanol/glacial acetic acid/distilled water (127/4/3/0.3, by vol), prepared freshly each day.

Thin-layer chromatography plates. Silica gel, 10 × 10 cm (60. F254; Merck, Darmstadt, F.R.G.).

Staining reagent. This solution contained cupric acetate (30 g/L) and phosphoric acid (80 mL/L).

Equipment. Centrifuge: Rotanta/P, Hettich; chromatography chamber, 21 × 5 × 11 cm; oven: Melag; densitometer: Cliniscan, Helena Labs.

Procedure. Mix 1 mL of gastric aspirate sample by inversion with 1 mL of isotonic saline solution (NaCl, 9 g/L) until completely homogenized. Centrifuge at 670 × g for 5 min to remove residual materials. Extract the phospholipids from the supernatant fluid with a mixture of 2 mL of methanol and 4 mL of chloroform, mixing for 1 min, then centrifuge (860 × g, 3 min). Filter the chloroform layer through 1 FS Whatman paper (Whatman Lab. Products, Clifton, NJ) and evaporate under a stream of nitrogen in a waterbath at 37 °C. Redissolve the dry residue in 50 μL of an equi-volume mixture of chloroform/methanol, and apply 3 μL of this to the thin-layer chromatography plate. Place the plate in the chamber (already equilibrated with 26.3 mL of solvent for 15 min) and develop until the solvent front is 1 cm from the top of the plate. Dry the plate under a stream of air, immerse it in the cupric acetate/phosphoric acid reagent for 15 s, then dry it again to remove excess solution. Heat the plate in an oven at 180 °C for 8 min to make the phospholipid spots visible.

Scan the developed chromatogram by transmission densitometry, using a 525 nm filter, to determine the relative amounts of each phospholipid. Calculate the L/S ratio by dividing the area of the lecithin by that of the sphingomyelin.

Run known mixtures of the six phospholipid standards (from Sigma) in parallel on the same plate beside the unknown mixtures.

Results

Figure 1 shows a typical chromatogram of standards and patients’ samples.

---

Department of Biochemistry, Hospital Virgen de la Salud, Toledo, Spain.

1 Nonstandard abbreviations: L/S ratio, lecithin/sphingomyelin ratio; PG, phosphatidylglycerol; RDS, respiratory distress syndrome.

Received September 2, 1986; accepted January 13, 1988.
mothers) had respiratory disorders: transient tachypnea, which developed 1 h after birth. The L/S ratio ranged from 2.5 to 3 and the mean PG value was 7%.

Group III consisted of 31 children, who presented with clinical and radiological symptoms of RDS. Their L/S ratio was <2.5, and PG was absent in all cases. Twenty-eight of them developed favorably after treatment and, after five or six days, their L/S ratios exceeded 2.5. The three other children did not survive; evidence of hyaline membrane disease was found in each. All the children in group III were born to mothers whose pregnancy had been complicated by toxemia, Rh isoimmunization, insulin-dependent diabetes, or placental insufficiency.

Discussion

In our laboratory this method is used for the routine assessment of pulmonary maturity by the measurement of phospholipids, principally the L/S ratio and the PG content, both in gastric aspirate and in amniotic fluid. We add saline to the gastric aspirate to increase the sample volume, which facilitates separation of the two extraction phases. For analysis of amniotic fluid, 2-mL specimens are obtained, so no saline is added.

We observed that the incidence of RDS is accompanied by low L/S ratios and is more frequent after short periods of gestation. In our hospital every newborn infant with an L/S ratio of <2.5 developed RDS, and within this group of infants there was a direct relationship between the lowest L/S ratios and the severity of the disease. Therefore we consider this method useful for deciding the prognosis of patients with RDS. We confirmed the evidence of others (23, 24) that indicates the importance of PG in the diagnosis of RDS.

Our method is specific, precise, requires only small sample volumes, and is quick: the results are obtained in less than 50 min after the sample is received in the laboratory. The chromatographic separation is excellent. For these reasons and because of its simplicity, this method can be used for routine analysis in any laboratory.

References


Table 1. Day-to-Day Precision in Three Control Samples

<table>
<thead>
<tr>
<th>L/S</th>
<th>Stated value</th>
<th>Stated range</th>
<th>Measured mean (±SD)</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mature</td>
<td>3.3</td>
<td>2.4–4.2</td>
<td>3.5 ± 0.5</td>
<td>14.28</td>
</tr>
<tr>
<td>Immature</td>
<td>2.1</td>
<td>1.8–2.4</td>
<td>2.1 ± 0.2</td>
<td>9.5</td>
</tr>
<tr>
<td>Borderline</td>
<td>1.2</td>
<td>1.0–1.4</td>
<td>1.1 ± 0.2</td>
<td>18.18</td>
</tr>
<tr>
<td>n = 30 each.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. L/S Ratio, %PG, Birthweight, Gestational Age, and Incidence of RDS in 200 Samples of Gastric Aspirate

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>L/S</th>
<th>%PG</th>
<th>Birthweight, g</th>
<th>Gestation, weeks</th>
<th>No. with RDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>145</td>
<td>&gt;3</td>
<td>11</td>
<td>3250</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(3.2–14)</td>
<td></td>
<td></td>
<td>(2345–4363)</td>
<td>(36–40)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>24</td>
<td>2.5–3</td>
<td>7</td>
<td>2128</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(1.4–12)</td>
<td></td>
<td></td>
<td>(1740–3427)</td>
<td>(33–40)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>31</td>
<td>&lt;2.5</td>
<td>Absent</td>
<td>1537</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>(0.8–2.2)</td>
<td></td>
<td></td>
<td>(1125–2130)</td>
<td>(29–36)</td>
<td></td>
</tr>
</tbody>
</table>


