Ultrasonographic evaluation of fetal lung histogram versus lamellar body count in the prediction of fetal lung maturity

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Abstract

Objective: *The current study aims to compare the ultrasonographic evaluation of fetal lung Gray-level histogram width (GLHW) ratio with an amniotic fluid lamellar body count (LBC) in the prediction of fetal lung maturity.*

Methods: *A prospective cohort study was conducted at a tertiary University Hospital in the period between May 1, 2017 and March 31, 2018. The study included pregnant women with a single fetus at ≥37 weeks of gestation scheduled for delivery by elective cesarean section (CS). Ultrasound evaluation was performed for assessment of the fetal lung to liver GLHW ratio to predict lung maturity. Lamellar body count was determined from an amniotic fluid sample obtained via amniotomy during CS. The lamellar body count for this sample was measured using a hematology analyzer. These data were further compared to Apgar scores at 1 and 5 minutes after delivery to assess the condition of the newborn immediately after birth, the degree of respiratory distress syndrome (RDS) and the need for resuscitation.*

Results: *One hundred twenty women and their neonates were included in the study. There was a statistically significant decrease in the levels of both GLHW and LBC among those neonates that showed distressed respiration after Apgar testing as compared with those who did not show similar distress, with p-value <0.001. The Receiver Operating Characteristic Curve (ROC) for LBC levels in the prediction of respiratory distress shows the best cutoff point for LBC was found at ≤20214/μL with a sensitivity of 100.0%, specificity of 75.47% and area under the curve (AUC) of 88.4%. The ROC curve for GLWH levels in the prediction of respiratory distress shows the best cutoff point for GLWH was found at ≤0.93 with sensitivity of 100.0%, specificity of 84.91% and AUC of 97.1%.*

Conclusions: *Ultrasonographic evaluation of GLHW of the fetal lung and liver is a non-invasive, inexpensive and time-efficient test for prediction of fetal lung maturity that has higher sensitivity and specificity.*

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Introduction

Fetal lung maturity (FLM) testing has played an important role in predicting the risk of respiratory distress syndrome (RDS) in preterm infants.[1] Over the past decade, however, FLM test utilization has decreased by about 60%. This trend can be attributed to advances in obstetric care (e.g., use of corticosteroids), as well as improved neonatal care (e.g., use of surfactant). Additionally, given improved neonatal care, there is less reluctance to delay delivery until fetal lung maturity has been determined.[2] However, RDS remains a major newborn complication, especially in hospitals where high-risk obstetric services are limited. Therefore, it is important that rapid laboratory evaluation of FLM be available and that physicians are aware of the features of commonly used FLM tests for optimal interpretation of results.[3]

In recent years, fetal lung maturity has been indirectly assessed based on ultrasonographic fetal lung morphology[4], fetal respiratory movement or visualization of fetal nasal respiratory flow by Doppler[5], ripening of the placental image[6], and Doppler blood flow waveform.[7] In contrast, gray-level histogram (GLH) is an ultrasonic tissue characterization which is used for quantification of sonographic echogenicity where GLH is measurable, and its value is stable to changes in mode gain, the depth of the imaging subjects and in various scanner models.[8] The advantage of this quantitative method is its ease of performance and the possibility for better analysis of tissue echogenicity since it does not rely exclusively on visual assessments, which may differ from examiner to examiner.[9]

To assess the FLM, several methods can be used to determine the relative concentration of surfactant-active phospholipids in the amniotic fluid. Currently, the gold standard for the determination of FLM is the evaluation of phospholipids (i.e., measurement of lecithin/sphingomyelin ratio and quantification of phosphatidylglycerol) in amniotic fluid samples by thin-layer chromatography. These tests are, however, time-consuming and not continuously available at most hospitals. A rapid test that does not require any reagents is the quantification of the number of lamellar bodies in the amniotic fluid. Lamellar bodies are lamellated phospholipids that represent a storage form of surfactant.[10] For this purpose, a consensus lamellar body count (LBC) protocol is published[11] for which an LBC cutoff value of 50,000/μL is used to suggest lung maturity, depending on the hematology analyzer used.

Based on these findings, the current study aims to compare the ultrasonographic evaluation of fetal lung Gray-level histogram width (GLHW) ratio with an amniotic fluid lamellar body count in the prediction of fetal lung maturity.

Materials and methods

Study type, setting, and duration

The current prospective cohort study was conducted at a tertiary University Hospital in the period between May 1, 2017, and March 31, 2018. The Institutional Ethical Review Board approved the study protocol. All women who participated in the study signed a written informed consent form before inclusion.

Study participants

All eligible women attending the outpatient clinic for antenatal care were approached to participate in the study. We included pregnant women with a single fetus at ≥37 weeks of gestation, as calculated based on reliable dates, who were scheduled for delivery by elective cesarean section (CS). We excluded women with unreliable dates, multiple pregnancy and any medical disease with pregnancy. Additionally, women with congenital fetal anomalies, oligohydramnios and premature rupture of membranes were excluded.

Recruitment

The recruited women were assessed through both a detailed medical history and clinical examination. Transabdominal B-mode images of the fetal lung and liver were obtained from all participants using 2.5- or 3-MHz ultrasound (Voluson 730 pro GE ultrasound machine, USA). Longitudinal or cross-sectional planes and a 1 × 1-cm region of interest (ROI), from which the rib and its shadow were excluded, were placed on the images. The computer programming of the ultrasound device provided a graphical representation of the number of pixels associated with each gray-level present in the image. The GLHW represents the width of the gray-level histogram. In the current study, a pixel number in the ROI of 1000 or more was used.

The fetal lung to liver GLHW ratio was studied in the same study population as a predictor of immature fetal lung (Figure 1). The threshold for predicting the maturity of fetal lung tissue was 0.94. Thus, the lung was predicted to be mature when GLHW ratios were 0.94 or more and was predicted to be immature when GLHW ratios are less than 0.94.[12]

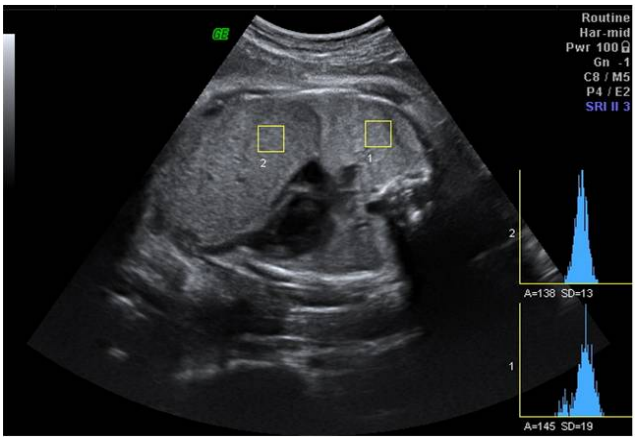

Figure 1:B-mode images and the grey-level histograms of the fetal lung (1) and liver (2), recorded in a single photograph in 38 weeks of pregnancy (mature lung).

Amniotic fluid sample

An amniotic fluid sample was obtained via amniotomy during CS. Amniotic fluid samples were collected in conventional blood picture tubes. The collected

specimens were sent to the laboratory to measure lamellar body count using a hematology analyzer.[11] The amniotic fluid sample was mixed by inverting the capped sample container five times. The fluid was then transferred to a clean test tube, and the sample was inspected. Fluids containing obvious mucus or meconium were not processed during the lamellar body count. The test tube was next placed on a tube rocker for two minutes. The platelet channel was flushed. The specimen was subsequently processed through a cell counter, and the platelet channel was recorded as the lamellar body count. The threshold used for estimation of fetal lung maturity was as follows: <15,000/μL was considered immature, 15,000-50,000/μL considered transitional and >50,000/μL was considered mature.

Follow up

RDS in newborns is a syndrome in premature infants caused by developmental insufficiency of pulmonary surfactant production and structural immaturity in the lungs. For the current study, the best determiner of the accuracy of both ultrasonographic evaluation of a fetal lung GLHW ratio and amniotic fluid lamellar body count as predictors of fetal lung maturity is to compare prenatal findings with actual post-partum lung development. To this end, Apgar scores at 1 and 5 minutes after delivery by CS were collected from the newborn infants of study participants. These scores were used to assess the condition of the newborn immediately after birth, the degree of RDS and the need for resuscitation.

Statistical analysis

The data collected was entered into a Microsoft Access database and then analyzed using the Statistical Package for Social Science (SPSS Inc., Chicago, version 22). Quantitative data was presented in terms of mean ± SD and compared using a Student's t-test. Qualitative variables were presented as frequency and percentage. A chi-square test was used for comparison between groups. The sensitivity and specificity of GLH and LBC were calculated for the prediction of respiratory distress and lung immaturity. For analysis, comparative values for $p < 0.05$ were considered to be significant.

Results

One hundred thirty women were approached to participate in the study. Ten women were excluded as they did not meet the inclusion criteria. The remaining 120 women were assessed for demographic characteristics. The mean age of included women was 27.68±4.65(20-32 years) with mean gestation of 38.68±0.65 weeks.

Table 1 shows the mean GLHW of lungs (34.28±0.31) and livers (33.62±0.79) of the infants studied group. There was a statistically significant difference between both figures ($p<0.001$). The mean ratio between the lung and liver GLHW was (1.02±0.05). The mean LBC in the study participants was 36673.6±14919.1/μL.

Table 1: Grey level histogram width GLHW (%) of lung, liver and their ratio

n	Fetal lung GLHW % Mean ± SD	Fetal liver GLHW % Mean ± SD	Ratio fetal lung/liver Mean ± SD	t-test	p-value
120	34.28±0.31	33.62±0.79	1.02±0.05	8.519	<0.001*

* Significant P≤ 0.05
GLHW; Grey level histogram width

After delivery, 14 neonates developed RDS and consequently were classified as having immature lungs. At five minutes Apgar scores were determined, nine neonates with Apgar scores of 6–7 had mild respiratory distress with tachypnea and working ala nasi while 9 neonates with Apgar scores of 4–5 developed moderate respiratory distress with intercostal¸ subcostal¸ suprasternal retractions (Table 2).

Table (3) shows that there was a statistically significant decrease in the level of GLHW and LBC in the distressed group of neonates compared with the non-distressed group, with p-value <0.001.

Table 2: Distribution of distressed neonates according to degree of distress by Apgar score

Degree of distress	No. of cases	Lamellar Body Count per µL (Mean)	GLHW ratio (%) of lung and liver ratio (Mean)
Mild	9 (64.3%)	17089.35	0.92
Moderate	5 (35.7%)	13156.69	0.90
Severe	0	_	_

GLHW; Grey level histogram width

Table 3: Comparison between distressed group and non-distressed group regarding the level of GLHW and LBC

		Distressed (n =14)	Non-Distressed (n=106)	Independent t-test t	p-value
GLHW %	Mean±SD	0.91±0.01	0.97±0.04	4.876	<0.001*
	Range	0.9–0.93	0.9–1.1		
LBC/µL	Mean±SD	13079.71±2802.96	42053.96±13966.87	7.714	<0.001*
	Range	11198–20214	13254–55632		

**Significant P ≤0.05*
GLHW; Grey level histogram width, LBC; lamellar body count

Figure 2 shows the Receiver Operating Characteristic Curve (ROC) for LBC levels in the prediction of respiratory distress and consequent lung immaturity. The best cutoff point for LBC was found at ≤20214/µL with a

sensitivity of 100.0%, specificity of 75.47% and area under the curve (AUC) of 88.4%. Figure 3 shows the ROC curve for GLWH levels in the prediction of respiratory distress. The best cutoff point for GLWH was found at ≤0.93 with a sensitivity of 100.0%, specificity of 84.91% and AUC of 97.1%.

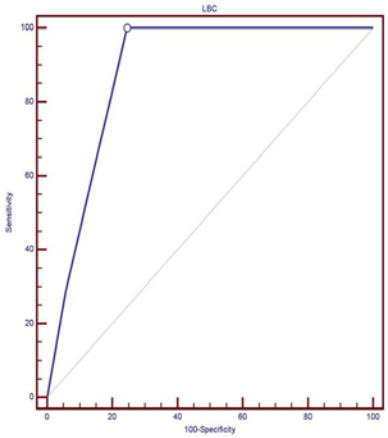

Figure 2: Receiver operating characteristic curve (ROC) for LBC in the prediction of lung immaturity

Discussion

Optimal timing of delivery is a critical determinant of perinatal outcome. Thus, knowledge of factors such as lung maturity status can assist obstetricians in making the best decisions about when delivery should occur, especially when complications of pregnancy indicate greater possibility of premature birth.[13]

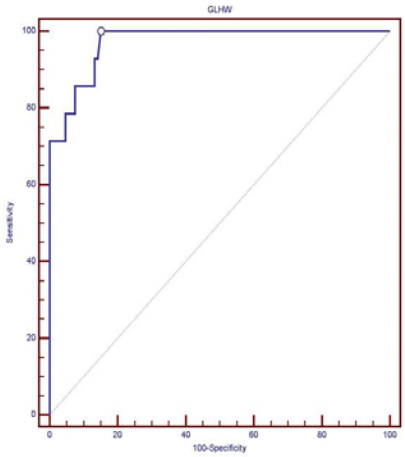

Figure 3: Receiver operating characteristic curve (ROC) for GLWH levels in the prediction of lung immaturity

Respiratory distress syndrome affects about 1% of newborn infants and is the leading cause of death in babies who are born prematurely.[14] The risk of RDS rises with increasing prematurity. As many as 60% of babies born before 29 weeks of gestation may be affected by RDS, but babies born at full term rarely develop this condition. Maternal risk factors for preterm birth include low maternal weight, previous preterm birth, poor antenatal care, periodontal disease and poverty.[15]

LBC is a direct measurement that can be used to determine fetal lung maturity based on surfactant production by type II pneumocyte cells within fetal lungs. Lamellar bodies produced by type II pneumocytes in fetal alveolus and secreted to the amniotic fluid during respiratory movement are similar to platelets.[16]

Ultrasonographic evaluation of fetal lung morphology, fetal respiratory movement,

fetal nasal respiratory flow by Doppler[17-19] and grading of placental maturity are also helpful in estimation of fetal lung maturity.

In the current study, the mean GLHW of lungs was of 34.28±0.31% and the liver was 33.62±0.79%. The mean ratio between lung and liver GLHW was 1.2±0.05. The mean LBC was 36673.6±14919.1/μL. Progressive increase of GLHW of the lung was previously reported by Maeda et al., 1999.[20] They found the GLHW value of fetal lungs was significantly lower at 24–29 weeks of pregnancy than at 30–38 weeks. There were also significant differences at 24–26 versus 27–29 weeks and versus 36–38 weeks, respectively.

In a previous study by Maeda et al., 2002, a single GLHW value for fetal lung of <31% was indicative of fetal lung immaturity with a sensitivity of 72.7% and specificity of 85.9%.[8] However, further analysis revealed that the ratio between the fetal lung and liver GLHW provided more stable and precise results for assessing fetal lung immaturity. This was because the fetal liver was stable in the studies on mean gray level and fetal liver GLHW was stable during pregnancy.[20] In the current study, comparison of ratios of lung/liver GLWH levels showed that the best correlation cutoff for the prediction of respiratory distress at delivery was found at ≤0.93, with a sensitivity of 100.0%, specificity of 84.91% and area under the curve (AUC) of 97.1%.

In the current study, the LBC in neonates who develop RDS (13079.71±2802.96/μL) was significantly lower than in normal neonates (42053.96±13966.87/μL), where $p<0.001$. In contrast, LBC ≤20214/μL was predictive of RDS with a sensitivity of 100% and specificity of 75.47%. Ashwood et al., who used a BC hematology analyzer to rule out RBS, recommended an LBC maturity cutoff of >55,000/μL, for which they found 100% sensitivity and 59% specificity.[21] Similarly, Lee et al. reported 100% sensitivity and 73% specificity using a maturity cutoff of LBC >50,000/μL.[22] These two studies both had large patient populations (247 and 170, respectively), and both used centrifuged amniocentesis and vaginal pool specimens.

Using these and other studies primarily relying on results from a BC hematology analyzer, a 2001 consensus protocol established optimal LBC cutoffs for determination of fetal lung maturity.[11] An LBC of >50,000/μL in uncentrifuged samples was suggested as the cutoff for maturity while lungs with values under 15,000/μL were considered immature. Lamellar body counts between these two values were considered indeterminate and further testing, using alternative biochemical methods, was recommended.[11]

A recent study of 313 patients with singleton 24–41 week pregnancies measured a cutoff value of ≥20000/μL that had sensitivity, specificity, and positive and negative predictive values useful for determining mature fetal lungs of 96%, 88%, 45.6%, and 99.5%, respectively.[23]

The findings of the current study suggest that both GLHW ratio study and

LBC offer more objective methods for determining the maturity of fetal lungs than traditional ultrasound studies and that GLHW, in particular, offers a non-invasive, inexpensive, efficient test with slightly higher specificity. As such, these methods could be used as either primary or supportive techniques to help obstetricians determine optimal delivery dates for patients undergoing elective CS.

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