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Lamellar Bodies Count (LBC) as a Predictor of Fetal Lung Maturity in Preterm Premature Rupture of Membranes Compared to Neonatal Assessment

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Abstract

Background: Respiratory distress syndrome (RDS) is a major cause of neonatal morbidity and mortality, affecting approximately 1% of all live births and 10% of all preterm infants. Lamellar bodies represent a storage form of pulmonary surfactant within Type II pneumocytes, secretion of which increases with advancing gestational age, thus enabling prediction of the degree of FLM. Preterm premature rupture of membranes (PPROM) complicates approximately 1/3 of all preterm births. Birth within 1 week is the most likely outcome for any patient with PPROM in the absence of adjunctive treatments. Respiratory distress has been reported to be the most common complication of preterm birth. Sepsis, intraventricular haemorrhage, and necrotizing enterocolitis also are associated with prematurity, but these are less common near to term. **Objective:** To assess the efficacy of the amniotic fluid lamellar body counting from a vaginal pool in predicting fetal lung maturity in women with preterm premature rupture of membranes. **Methods:** This study was conducted at Ain Shams University Maternity Hospital in the emergency ward from January 2019 to September 2019. It included 106 women with singleton pregnancies, gestational age from 28 - 36 weeks with preterm premature rupture of membranes. This study is designed to assess the efficacy of the amniotic fluid lamellar body counting (LBC) from a vaginal pool in predicting fetal lung maturity in women with preterm premature rupture of membranes. **Results:** The current study revealed a highly significant increase in the lamellar body count in cases giving birth to neonates without RDS compared to that cases giving birth to neonates with RDS. Also, no statistically significant difference between LBC and age, parity and number of previous miscarriages

in the mother was found. Gestational age at delivery was significantly lower among cases with respiratory distress. Steroid administration was significantly less frequent among cases with respiratory distress. However, lamellar bodies had high diagnostic performance in the prediction of respiratory distress. **Conclusion:** Lamellar body count (LBC) is an effective, safe, easy, and cost-effective method to assess fetal lung maturity (FLM). It does not need a highly equipped laboratory or specially trained personnel, it just needs the conventional blood count analyzer. Measurement of LBC is now replacing the conventional Lecithin/Sphyngomyelin L/S ratio. LBC cut-off value of $\leq 42.5 \times 10^3/\mu L$ can be used safely to decide fetal lung maturity with sensitivity of 95.7% and specificity of 97.6%.

Keywords

Fetal Lung Maturity, Lamellar Bodies Count, Preterm Premature Rupture of Membranes, Respiratory Distress Syndrome

1. Introduction

Preterm premature rupture of membranes (PPROM) defines spontaneous rupture of the fetal membranes before completed 37 weeks' gestation and before labor onset [1].

Preterm premature rupture of membranes may occur for a variety of risk factors. The infection has been shown to be commonly associated with preterm PROM, especially at earlier gestational ages. A prior history of preterm PROM is a major risk factor for preterm PROM or preterm labor in a subsequent pregnancy. Additional risk factors associated with preterm PROM are similar to those associated with spontaneous preterm birth and include short cervical length, second-trimester and third-trimester bleeding, low body mass index, low socioeconomic status, cigarette smoking, and illicit drug use. Although each of these risk factors is associated with preterm PROM, it often occurs in the absence of recognized risk factors or an obvious cause [1].

Several studies have been done to ascertain the incidence of infection-induced premature membrane rupture. Bacterial cultures of amnionic fluid support a role for infection in a significant proportion. A review of 18 studies comprising almost 1500 women with PPROM found that in a third, bacteria were isolated from amnionic fluid [2] [3]. Accordingly, high-risk women have been given prophylactic antimicrobial treatment to prevent premature rupture of membranes [2] [3].

Neonatal respiratory distress syndrome (RDS) is a disorder due to pulmonary immaturity with high mortality characterized by low levels of pulmonary surfactant. Gestational age determines risk based on the concentration of pulmonary surfactant, *i.e.* as gestation progresses the concentration of pulmonary surfactant increases. As a result, newborns delivered at less than 28 weeks have

more than 60% risk of RDS, whereas those delivered at more than 34 weeks have less than 5% risk of RDS [4]. In situations where gestational age alone is not sufficient to determine RDS risk and preterm delivery is medically needed, amniotic fluid analysis can be performed to determine pulmonary surfactant concentration [4].

Laboratory assessment of fetal lung maturity assists obstetricians in estimating the risk of respiratory distress syndrome (RDS) when premature delivery of an infant is being considered [5]. There are common methods such as the lecithin-sphingomyelin ratio, phosphatidylglycerol measurement, and surfactant-albumin ratio. All of these tests have excellent negative predictive values but poor positive predictive values, *i.e.* they are great at confirming maturity but poor at confirming immaturity [4]. Most of them are either complex, expensive, or with low diagnostic efficiency [6].

Surfactant is stored in the form of lamellar bodies. They are secreted into alveolar space and passed into amniotic fluid where they can be found. The similarity of lamellar body size to platelet size permits the use of a standard automated hematologic cell counter to estimate the number of lamellar bodies in amniotic fluid [7]. Lamellar bodies are essentially small packages of lung surfactant that are found in intracellular storage granules in lung cells or pneumocytes. The lamellar bodies are released (exocytosed) and unfold to form a surfactant monolayer in the alveolar space. Surfactants and lamellar bodies are released into the amniotic fluid due to fetal breathing movements beginning around 28 to 32 weeks of fetal development, with levels increasing exponentially as the fetus matures. The risk of respiratory distress syndrome due to insufficient surfactant levels is significant during gestational weeks 32 to 36, and a more accurate assessment of that risk is facilitated by measurement of surfactant phospholipid ratios or, as has recently been shown, by lamellar body counts (LBC) [5].

Amniotic fluid lamellar body counts performed on cell-counting equipment available in most clinical laboratories is a simple, rapid, inexpensive, and the most practical antenatal method for the efficient evaluation of fetal lung maturity and prediction of neonatal RDS [8]. This study is designed to assess the efficacy of the amniotic fluid lamellar body counting (LBC) from a vaginal pool in predicting fetal lung maturity in women with preterm premature rupture of membranes.

2. Subjects and Methods

This study was conducted at the emergency ward of Ain Shams University Maternity Hospital from January 2019 to September 2019.

The study design was prospective cohort study.

The primary outcome measure is the accuracy of the LBC in vaginal fluid for prediction of fetal lung maturity. A previous study reported that the LBC had a sensitivity of approximately 92% and a specificity of 100% [9]. The incidence of RDS in the study of Salim *et al.* [9] was 17%.

Accuracy of a Diagnostic test for PPROM controlled blind comparison to a gold standard: Neonatal assessment and Lamellar Bodies Count in amniotic fluid.

A sample size of 106 women would yield 18 (17%) patients whose babies would develop RDS and 88 (83%) patients whose babies would have mature lungs.

This calculation used a two-sided binominal test with a confidence level of 99% (Type I error, 0.01) and assumed that the incidence of RDS in the study population is 17%.

Pregnant women with preterm premature rupture of membranes, singleton pregnancies, gestational age from 28 - 36 weeks and the fetus is alive with regular heart beats by ultrasound and no fetal distress were included in the study. While patients with gestational age 37 wks or more, uncertain gestational age, oligohydramnios before preterm premature rupture of membranes, infants with major congenital or chromosomal abnormalities, amniotic fluid samples containing blood or meconium, diabetes mellitus (diabetic mother) and presence of chorioamnionitis were excluded from the study.

Amniotic fluid samples were collected by a sterile speculum inserted in the posterior fornix of the vagina.

Samples containing 2 mL of amniotic fluid was transported to the clinical laboratory in a test tube and analyzed according to an established protocol.

Lamellar bodies count (LBC) will be estimated in uncentrifugated amniotic fluid samples using a hematological analyzer and its platelet channel.

A protocol and interpretive guideline have been published by a consensus panel [10].

LBC (counts/uL)	Interpretation
0 - 15,000	Immature
15,000 - 50,000	Indeterminate
>50,000	Mature

Diagnosis of RDS is based on the presence of the following items:

- Physical signs (nasal flaring, chest retractions, grunting and tachypnea).
- Supplementary oxygen requirement longer than 24 hours.
- Radiographic findings (reticulogranular opacification of lung fields with superimposed air bronchogram).

All women participating were subjected to the following counseling about all the steps of the study and the procedure was fully explained to them, informed consent was obtained, careful history taking regarding personal, last menstrual period, obstetric, medical, surgical histories and history of present pregnancy, complete physical examination to exclude any disorders that may interfere with the results, investigations such as Ultrasound, total leucocytic count and random blood sugar to ensure that they comply with the inclusion and exclusion criteria, gestational age was calculated from the last menstrual period and confirmed by

ultrasound measurement during the first trimester of pregnancy, amniotic fluid samples were collected from the posterior vaginal fornix following a sterile speculum inserted in the vagina and samples containing 2 mL of amniotic fluid was transported to the clinical laboratory in a test tube and analyzed according to an established protocol. A protocol and interpretive guideline have been published by a consensus panel [10].

Lamellar bodies count (LBC) was estimated in uncentrifuged amniotic fluid samples using a hematological analyzer and its platelet channel.

Special recommendations and indications in some cases

Vaginal pool specimens containing obvious mucus wasn't processed for lamellar body counts. Beyond potentially interfering with the cell counter, mucus also artificially increases the lamellar body count and decreases the lecithin-sphingomyelin ratio. A vaginal pool specimen that was obtained from patients with ruptured membranes who have "free flowing" fluid and do not have obvious mucus was processed for lamellar body counts [10].

The effect of meconium on lamellar body counts had been evaluated. Meconium has a marginal impact on lamellar body counts, increasing the count by a modest 5000/ml. Even heavy meconium staining does not increase the count greatly. Further, the presence of meconium may provide a compelling reason to move toward delivery, irrespective of lung maturity status. Clinical judgment had been exercised when the lamellar body count was borderline mature in the setting of meconium-stained amniotic fluid [10].

The effect of contamination of AF with whole blood is biphasic. The lamellar body count was initially increased because the platelets in the blood are counted as lamellar bodies. This effect, however, is relatively small. Indeed, even addition of enough blood to produce an AF hematocrit of 2% (which, incidentally, is rare) lead to only a 5% increase in lamellar body counts, which for only approximately 20 minutes after introduction of blood. Over the next 2 hours, however, the pro-coagulant activity of AF causes coagulation, which traps both platelets and lamellar bodies and lead to a decrease in lamellar body counts. Because of the potential effect of contamination of AF with blood on lamellar body counts, the responsible physician was notified if the AF hematocrit exceeds 1%. This most commonly would lead to a falsely decreased lamellar body count [10].

3. Statistical Analysis

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 28.0, IBM Corp., Chicago, USA, 2021. Quantitative data tested for normality using Shapiro-Wilk test, then if normally distributed described as mean ± SD (standard deviation) as well as minimum and maximum of the range, then compared using independent t-test, while if not normally distributed described as Median (1st - 3rd Interquartiles) then compared using Mann-Whitney test. Qualitative

data described as number and percentage and compared using Chi-square test. ROC curve was used to evaluate the performance of different tests differentiate between certain groups, the cut point was selected based on its highet Youden's Index among all levels. The level of significance was taken at p-value < 0.050 was significant, otherwise was non-significant.

4. Results

In the current study the lamellar body count (LBC) was used in prediction of fetal lung maturity. The study included 106 pregnant women with singleton normal pregnancies between 28 and 36 weeks of gestation; amniotic fluid sample was collected for each woman for performance of lamellar body count. After delivery, neonates were assessed clinically for evidence of respiratory distress syndrome (RDS). Neonatal respiratory distress syndrome was diagnosed in 23 neonates. These neonates with diagnosis of prenatal lung immaturity had significantly younger gestational age.

Table 1 shows that more than half of the studied cases had steroids before current delivery.

A protocol and interpretive guideline have been published by a consensus panel [10].

Table 2 shows that less than three quarters of the studied cases had **mature lung**.

Table 3 shows that **ceasrean delivery** was in more than one third of the studied cases.

Table 4 shows that **respiratory distress** was in less than one quarter of the studied cases.

Table 5 shows that **gestational age** at delivery was significantly lower among cases with respiratory distress. Steroids intake was significantly less frequent among cases with respiratory distress.

Table 6 shows that **birth weight** was significantly lower among cases with respiratory distress. **Cesarean delivery** was significantly more frequents among cases with respiratory distress.

Table 1. Age, body mass index, parity, getational age and steroids intake of studied cases.

		Mean ± SD	Range
Age (years)		27.3 ± 5.0	18.0 - 40.0
BMI (kg/m^2)		28.5 ± 2.8	24.1 - 36.1
GA (weeks)		33.6 ± 2.1	28.0 - 36.0
		N	**%**
Parity	**Nulli**	26	24.5
	Multi	80	75.5
Steroids Intake		58	54.7

Total = 106; BMI: Body mass index; GA: Gestational age.

Table 2. Lamellar bodies ($\times 10^3/\mu L$) of studied cases.

		Median (1st - 3rd IQ)	Range
Level		101.0 (44.8 - 135.0)	7.0 - 224.0
		N	%
Grade	0.0 - 15.0 Immature	5	4.7
	15.0 - 50.0 Intermediate	25	23.6
	>50.0 Mature	76	71.7

Total = 106; IQ: Inter-quartiles.

Table 3. Delivery outcome among studied cases.

		N	%
Mode of Delivery	Vaginal	66	62.3
	Cesarean	40	37.7
		Mean ± SD	Range
Birth Weight (kg)		2.4 ± 0.4	1.2 - 3.1

Total = 106.

Table 4. Respiratory distress among studied cases.

	N	%
Respiratory Distress	23	21.7
No Respiratory Distress	83	78.3

Total = 106.

Table 5. Comparison according to respiratory distress (RD) regarding age, BMI, parity, gestational age and steroids intake.

		RD (N = 23)	No RD (N = 83)	P-value
Age (years)		27.8 ± 5.0	27.1 ± 5.0	^0.588
BMI (kg/m^2)		28.9 ± 2.8	28.4 ± 2.8	^0.463
Parity	Nulli	5 (21.7%)	21 (25.3%)	#0.725
	Multi	18 (78.3%)	62 (74.7%)	
GA (weeks)		30.7 ± 2.3	34.4 ± 1.2	**^<0.001***
Steroids intake		5 (21.7%)	53 (63.9%)	**#<0.001***

BMI: Body mass index; GA: Gestational age; ^Independent t-test; #Chi-square test; *Significant.

Table 7 shows that **lamellar bodies** were significantly lower among cases with respiratory distress.

Table 8 shows that **lamellar bodies** were significantly higher in cases that were given steroids than those who did not receive steroids.

ROC curve was used to evaluate the performance of different tests differentiate

between certain groups, the cut point was selected based on its highet Youden's Index among all levels. The level of significance was taken at p-value < 0.050 was significant, otherwise was non-significant.

Table 9 shows that **lamellar bodies** had high diagnostic performance **in prediction of respiratory distress**.

Table 10 shows that lamellar bodies' cutoff point ≤ 42.5 × 10^3/μL had high characteristics in prediction of respiratory distress.

Table 6. Comparison according to respiratory distress (RD) regarding delivery outcome.

		RD (N = 23)	No RD (N = 83)	P-value
Mode of Delivery	Vaginal	9 (39.1%)	57 (68.7%)	#0.010*
	Cesarean	14 (60.9%)	26 (31.3%)	
Birth Weight (kg)		1.9 ± 0.5	2.6 ± 0.3	^<0.001*

^Independent t-test; #Chi-square test; *Significant.

Table 7. Comparison according to respiratory distress (RD) regarding lamellar bodies (×10^3/μL).

		RD (N = 23)	No RD (N = 83)	P-value
Level, Median (1st - 3rd IQ)		23.0 (17.0 - 36.0)	122.0 (79.0 - 144.0)	^<0.001*
Grade	0.0 - 15.0 Immature	5 (21.7%)	0 (0.0%)	#<0.001*
	15.0 - 50.0 Intermediate	17 (73.9%)	8 (9.6%)	
	>50.0 Mature	1 (4.3%)	75 (90.4%)	

IQ: Inter-quartiles; ^Mann-Whitney test; #Chi-square test; *Significant.

Table 8. Comparison according to steroid intake regarding lamellar bodies (×10^3/μL).

	Steroids (N = 58)	No steroids (N = 48)	P#
Median (1st - 3rd IQ)	121.5 (78.0 - 141.0)	58.0 (33.5 - 130.0)	0.001*

IQ: Inter-quartiles; #Mann-Whitney test; *Significant.

Table 9. Performance of lamellar bodies in prediction of respiratory distress.

AUC	SE	P	95% CI	Cutoff
0.993	0.006	<0.001*	0.000 - 1.000	≤42.5 × 10^3

AUC: Area under curve; SE: Standard error; CI: Confidence interval; *Significant.

Table 10. Diagnostic characteristics of lamellar bodies cutoff point ≤ 42.5 × 10^3/μL in prediction of respiratory distress.

Characteristics	Value	95% CI
Sensitivity	95.7%	78.1% - 99.9%
Specificity	97.6%	91.6% - 99.7%
Diagnostic Accuracy (DA)	97.2%	92.0% - 99.4%

Continued

Youden's Index	93.2%	84.3% - 102.2%
Positive Predictive Value (PPV)	91.7%	73.0% - 99.0%
Negative Predictive Value (NPV)	98.8%	93.4% - 100.0%
Positive Likelihood Ratio (LR+)	39.70	10.07 - 156.51
Negative Likelihood Ratio (LR−)	0.04	0.01 - 0.30
Diagnostic Odds Ratio (DOR)	891.00	77.18 - 10286.75

CI: Confidence interval.

5. Discussion

In the current study, the lamellar body count (LBC) was used in prediction of fetal lung maturity. The study included 106 pregnant women with singleton normal pregnancies between 28 and 36 + 6 completed weeks of gestation; amniotic fluid sample was collected for each woman for performance of lamellar body count.

After delivery, neonates were assessed clinically for evidence of respiratory distress syndrome RDS. RDS was diagnosed in 23 neonates. These neonates with diagnosis of prenatal lung immaturity had significantly younger gestational age.

The current study revealed a significant increase in the lamellar body count with a median of (79.0 - 144.0) cases giving birth to neonates without RDS compared to (17.0 - 36.0) in cases giving birth to neonates with RDS. This result was similar to the findings of Štimac *et al.* [8] who stated that antenatal amniotic fluid LBC method was able to differentiate between the neonates without RDS and the neonates who are expected to develop moderate and/or severe forms of acute RDS. And hence, based on their results, more severe forms of RDS were accompanied by lower median LBC.

The present study revealed no statistically significant difference between LBC, age of the mother, parity of the mother and number of previous abortions.

On the contrary, this finding was in disagreement to the results reported by Rimar *et al.* [11] who found that the incidence of RDS in newborns born after week 32 of gestation did not significantly change. What did change are the causes. They attributed this to some leading causes of RDS (e.g. sepsis, the influence of which diminished due to better prenatal care).

The present study showed a significant correlation between levels of LBC and fetal lung maturity using $42.5 \times 10^3/\mu L$ as a cut-off point for LBC; as it is can be considered a good predictor for fetal lung maturity with sensitivity 95.7% and specificity 97.6%. This finding was in accordance with Zarean *et al.* [12] who indicated that lamellar body counting test has a high positive predictive value with a good sensitivity, specificity and negative predictive value.

Zarean *et al.* [12] had conducted a study with One hundred and twenty-eight amniotic samples and 131 infants evaluated. The means of maternal and gestational ages were 28.12 ± 3.84 years and 32.56 ± 2.72 weeks, respectively. The mean of lamellar body was 31,266 ± 15,831 μl in matured lung infants compared

to 63,081 ± 16,966 µl in immature lung infants ($p < 0.001$). The optimal cut-off point was evaluated as 47,500 µl in predicted pulmonary maturity with sensitivity of 85.1%, specificity of 91.2%, positive predictive value of 92.6% and negative predictive value of 82.5%.

Besnard *et al.* [13] reported that an LBC of 32,000/µL guaranteed fetal lung maturity. They showed that the performance of the LBC in the prediction of RDS is equal to the L/S ratio. In their meta-analysis, they concluded that LBC may be considered as the test of first choice in the assessment of fetal lung maturity.

KulKarni *and* Jayamma [14] found that Among 50 cases, LBC was <30,000/µl in 15 cases between 30,000 - 35,000/µl in five cases and >35,000/µl in 30 cases. Those who developed RDS had LBC between 3000 - 28,000/µl. Sensitivity and specificity of LBC to predict RDS with cut-off values of 30,000/µl was 100% and 97.2% respectively.

Asem *et al.* [15] agreed with the recent study in that the measurement of LBC can be used in detecting fetal lung maturity and predicting neonatal respiratory distress, but concluded that the measurement of main pulmonary artery (MPA) acceleration time (AT)/ejection time (ET) ratio could predict the same but more sensitive, specific, and less invasive.

6. Conclusion

Lamellar body count (LBC) is an effective, safe, easy, and cost-effective method to detect fetal lung maturity (FLM). It does not need a highly equipped laboratory or specially trained personnel, it just needs the conventional blood count analyzer. Measurement of LBC is now replacing the conventional Lecithin/Sphyngomyelin L/S ratio. LBC cut-off value of $\leq 42.5 \times 10^3/\mu L$ can be used safely to decide fetal lung maturity with sensitivity of 95.7% and specificity of 97.6%.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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