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Original Research Article

Lamellar body count as a predictor of neonatal respiratory distress syndrome in preterm premature rupture of membranes

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ABSTRACT

Background: Lamellar bodies are present in amniotic fluid and their quantity increases with increased gestational age. Preterm premature rupture of the membranes (P-PROM) is one of the most common complications of pregnancy and is a major cause of preterm deliveries and thus the important cause of RDS. Fetal pulmonary maturity can be assessed by direct or indirect measurement of surfactant phospholipids secreted by the fetal lungs into amniotic fluid. Lamellar body count (LBC) has been introduced as an alternative to other methods.
Methods: The study's prime aim is to establish LBC as a predictor of RDS in P-PROM. We included pregnant women with P-PROM and gestational age between 28 weeks and 37 weeks and singleton live pregnancy. The lamellar body counting from amniotic fluid was done with the use of a standard hematology cell counter, sysmex KX-21. There was statistically significant co-relation between lamellar body count and period of gestation (by applying ANOVA).
Results: Lamellar body counts were significantly less in cases of RDS as compared to non RDS cases.
Conclusions: LBC count was selected among all other tests because the test can be performed with equipment found in most clinical analysis laboratories and is reliable in predicting fetal lung maturity.

Keywords: Amniotic fluid, Fetal lung maturity, Lamellar body count, Neonatal respiratory distress syndrome, P-PROM, Sysmex KX-21

INTRODUCTION

Lamellar bodies are concentrically layered packages of phospholipids. Type II pneumocytes, a representing form of surfactant is the source of production of lamellar bodies. They are present in amniotic fluid and their increase is directly proportional to the period of gestation.[1,2] Size of lamellar bodies is similar to that of platelets, therefore these can be counted by using platelet channel of automated hematologic cell counter.[3,4] Inadequate amount of pulmonary surfactant is the primary cause of respiratory distress syndrome (RDS) that affects 1% of all live births but affects 10% to 15% of all infants with a birth weight less than 2500g.[5-8] It typically affects infants <35 weeks gestational age (GA) but may affect older infants who have delayed lung maturation. Preterm premature rupture of the membranes (P-PROM) is one of the most common complications of pregnancy and is one of the major causes of preterm deliveries and thus the important cause of RDS.[9]

The American college of obstetricians and gynecologists (ACOG) recommends that obstetricians to confirm fetal pulmonary maturity prior to elective delivery less than 39 weeks of gestation.[5,6]

Fetal pulmonary maturity can be assessed by direct or indirect measurement of surfactant phospholipids secreted by the fetal lungs into amniotic fluid. These tests can be broadly categorized into two groups:

- Biochemical tests, including lecithin/sphingomyelin ratio (L/S ratio), phosphatidylglycerol (PG) and surfactant-to-albumin ratio
- Biophysical test, including Foam stability index (FSI) and Lamellar body counts (LBC), which tests the surface-active effects of these bioactive phospholipids.[10]

Although L/S ratio is highly sensitive in predicting the development of RDS, it is labor intensive, time consuming, prone to subjective interpretation and technique dependent. It is expensive and not universally available.[10]

As, fetal lung maturity tests are not readily available in developing countries, except in research settings. Lamellar body counts may be able to fulfill the need for a reliable yet rapid, simple, less technique dependent and inexpensive method of assessing fetal lung maturity in the hospital set up.

The present study is planned to test the validity of LBC in amniotic fluid for predicting fetal lung maturity among women with P-PROM, to find the cut off value of LBC to indicate pulmonary maturity and immaturity and to correlate the severity of RDS of LBC. Further this test may also be extended to situations when faced with dilemma to electively terminate pregnancy before term and also an area of fetal therapy may utilize fetal lung maturity assessment using LBC.

METHODS

T All pregnant women with 28 to 37 weeks of gestation, singleton live pregnancy with preterm premature rupture of membrane who went into spontaneous labour were included in the study. A detailed clinical history was taken which was followed by a complete clinical examination. Gestational age was confirmed by last menstrual period (LMP) and by performing abdominal sonography. USG also confirmed the viability, placental localization, any gross congenital abnormality, amount of liquor and fetal well-being. CTG (cardiotocography) was also conducted on all the participating women. Females with a history of leaking per vaginum for >48 hours, multiple gestation, inaccurate gestational age, cesarean deliveries, clinical chorioamnionitis, pre-eclampsia, diabetes mellitus, with history of administration of antenatal corticosteroids, non-reassuring fetal heart and malpresentations were excluded. Also, newborns with respiratory distress due to meconium aspiration syndrome and neonates with transient tachypnoea/ congenital pneumonia were excluded.

A sterile Sim's speculum was inserted in the vagina to retract down the posterior vaginal wall and 3 ml of amniotic fluid was collected in a sterile container and was immediately taken to the laboratory. The lamellar body counting was done with the use of a standard hematology cell counter, sysmex KX-21 in the department of pathology. The sample was processed without centrifugation as described in a consensus protocol.[11] Amniotic samples were mixed by inversion for a minimum of two minutes to ensure re-suspension of lamellar bodies and the lamellar bodies were then enumerated using the CBC mode of the analyzer. The results from the platelet channel were recorded as the LBC.

Complete clinical examination of new born for the features suggestive of respiratory distress syndrome (RDS) was done by a neonatologist and APGAR score at 1 and 5 minutes was performed. Respiratory distress, if present was classified as mild, moderate and severe according to the severity of disease by Silverman scoring system. RDS (respiratory distress syndrome) was defined by the presence of two or more of the following criteria; Clinically (Nasal flaring, grunting, retractions, tachypnoea), radiological appearance (opacification/ground glass appearance) of Hyaline membrane disease and administration of oxygen for more than 24 hours.

Statistical analysis

The statistical analysis included the correlation between LBC and period of gestation was obtained by applying Spearman Rho test, ANOVA and Kruskal-Wallis test. Correlation between LBC and RDS was obtained by applying Pearson test. The study groups were compared with each other in respect to age and parity by applying ANOVA.

RESULTS

This cross-sectional study was conducted in the Department of Pathology in collaboration with the Department of Obstetrics and Gynecology in a tertiary care hospital.

A total of 120 pregnant women, who came with preterm premature rupture of membranes with singleton pregnancy, meeting all the inclusion and exclusion criteria were enrolled for the study. The subjects were distributed into three groups based on the period of gestation, as:

- Group 1 included 40 women with period of gestation between 28 weeks to 31.6 weeks
- Group 2 included 40 women with period of gestation between 32 weeks to 34.6 weeks
- Group 3 included 40 women with period of gestation between 35 weeks to 37 complete weeks.

The mean age of group 1 was 23.223±3.340 years, of group 2 was 24.33±3.460 years and that of group 3 was 23.78±2.722 years. Maximum women were in the age group of 20 – 25 years in all the groups. The three groups were comparable with each other in respect to age (ANOVA, p=0.308).

Majority of the women in all the three groups were nulliparous, 42.5% in group 1, 47.5% in group 2 and 47.5% in group 3 but there was no statistically significant difference among the parity between three groups (ANOVA, p=0.710).

The lamellar body count ranged from $9x10^3$ to $373x10^3$/mcl with mean value of $74.975±78.003x10^3$/mcl. The mean value of LBC was lower in group 1 as compared to group 2 and 3 ($22.7x10^3$/mcl±$10.272x10^3$, $48.9x10^3$/mcl±$19.061x10^3$, $153x10^3$/mcl±$35.153x10^3$ group 1, 2 and 3 respectively).

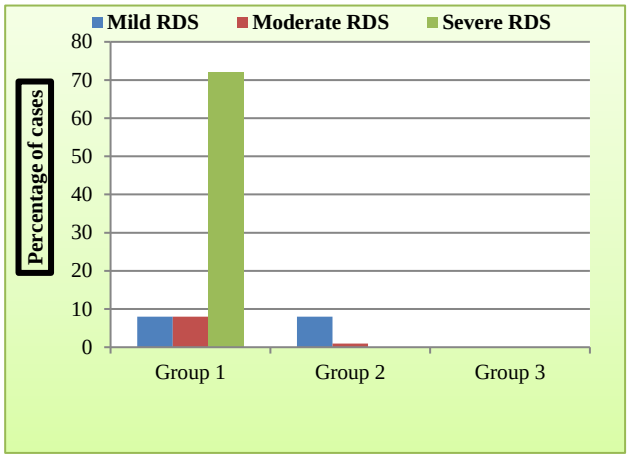

Figure 1: Correlation between RDS period of gestation.

Out of 120 cases, 25 (20.8%) belonged to the category of RDS. In group 1, there were 88%(22/40) RDS cases, in group 2, there were 12% (3/40) of RDS cases and there were no RDS cases in group 3. There were 18 (72%) cases of severe RDS, 3 (12%) cases of moderate RDS and 4 (16%) cases of mild RDS. In group 1, all cases (N=40) had LBC value of $<50x10^3$/mcl whereas RDS cases (22/40) had LBC $<40x10^3$/mcl. On the other hand, in group 2 and 3, all cases had LBC value above $20x10^3$/mcl and $40x10^3$/mcl respectively. Mean LBC in cases of RDS and Non RDS cases was 16.44±7.211 for RDS cases and 90.33±80.861 for Non RDS cases. LBC in cases with severe, moderate and mild RDS were between $9x10^3$/mcl and $25x10^3$/mcl, $18x10^3$/mcl and $30x10^3$/mcl and $22x10^3$/mcl and $31x10^3$/mcl.

Severity of RDS also decreased with increase in gestation (Figure 1). A co-relation was done between lamellar body count and period of gestation, which was statistically significant (The correlation coefficient r= 0.897, Spearman Rho, p-value was <0.001).

Table 1: Correlation between various cut offs of lamellar body counts and respiratory distress syndrome cases.

LBC cut off (counts$x10^3$/mcl)	Total no. of cases (n)	No. of cases (RDS)	No. of cases (Non RDS)
≤10	4	4	0
≤15	17	17	0
≤20	20	19	1
≤25	22	21	1
≤30	31	24	7
≤35	43	25	18
>35	77	0	77

Table 2: Sensitivity, specificity, negative predictive value and positive predictive value at various cut offs of LBC.

LBC (counts x 103/mcl)	True positive	False positive	True negative	False negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
≤10	4	0	95	21	16	100	100	81.89
≤15	17	0	95	8	68	100	100	92.23
≤20	19	1	94	6	76	98.4	95	94
≤25	21	1	94	4	84	98.94	95.45	95.91
≤30	24	7	88	1	96	95.63	77.41	98.87
≤35	25	18	77	0	100	81.05	58.13	100
>35	25	18	77	0	100	81.05	58.13	100

Table 3: Compiled comparison of studies of lamellar body count with present study.

Manufacturer	Author		LBC cut off values	No. of RDS cases	Sensitivity	Specificity	PPV	NPV
Beckman Coulter (Gens)	Haymond		<50,000/mcl	12/184	92	60	14	99
Sysmex	K800	Bahasadri	<45,000/mcl	23/104	99	98	77	99
	XE 2100	Karcher	<30,000/mcl	13/220	85	75	18	99
Cell Dyn	Hernandez R		<79,000/mcl	39/264	92	71	36	98
Sysmex KX 21	Present study		35,000/mcl	25/120	100	81.05	58.13	100

Determination of optimal cut off values of lamellar body counts to indicate fetal lung maturity and immaturity was done by plotting a receiver operator curve (ROC) which came out to be $31x10^3$/mcl. However, on establishing a correlation between various cut offs of LBC and RDS two cut offs were taken i.e. $>35x10^3$/mcl (to indicate fetal lung maturity) and $<15x10^3$/mcl (to indicate fetal lung immaturity) (Table 1).

Sensitivity, specificity, positive and negative predictive values of LBC at various cut off values were calculated as shown in Table 2.

DISCUSSION

Fetal pulmonary maturity can be estimated by direct or indirect measurement of surfactant phospholipids secreted by the fetal lungs into amniotic fluid.

The purpose of the study was to test the validity of a non-invasive method for predicting fetal lung maturity among women with P-PROM and therefore the presence/absence of RDS. To do so, we performed a lamellar bodies (LB) count in amniotic fluid by three-part hematology analyzer and calculated a cutoff for lamellar body counts which indicate fetal lung maturity or immaturity. Furthermore, such counting had been reported as reliably predicting fetal lung maturity and is simple, rapid and inexpensive test.[12]

The validity of amniotic fluid collected vaginally to predict fetal lung maturity has been reported previously. Studies evaluated the performance of the TDx/TDxFLM II assay on amniotic fluid specimens collected vaginally from women with P-PROM showed that mature results predicted the absence of RDS with a high degree of accuracy.[10] However, while extracting amniotic fluid from the vaginal pool, the possible presence of vaginal mucus may virtually increase the number of lamellar bodies.[13] Special precaution was taken while collecting amniotic fluid to prevent its contamination by vaginal mucus.

In the present study, lamellar body counts were significantly less in cases of RDS as compared to non RDS cases which signifies that LBC is the indicator of lung maturity. This corresponds with the study performed by Greenspoon JS, Rosen DJD et al.[14]

A co-relation was done between lamellar body count and period of gestation, which was statistically significant. Fakhoury et al also performed a regression analysis plotting LBC against fetal gestational age and observed that LBC is best represented as an exponential curvilinear function of gestational age.[1]

As it is a relatively new test, most authors have recommended that each laboratory should establish its own cut-offs. We calculated the cut-off value by plotting the ROC and maturity criteria was set above $31x10^3$/mcl.

Various cut-off values of LBC's were considered and correlation between various cut-offs was done with the presence or absence of RDS cases at those values (Table 1). Out of various cut-off values, two values indicating fetal lung maturity (no RDS cases) and immaturity (no non-RDS cases) were considered as lower ($<15x10^3$/mcl) and upper cut-off ($>35x10^3$/mcl).

At maturity level $>35x10^3$/mcl sensitivity and specificity remained high (100% and 81.05%) b and at maturity level $<15x10^3$/mcl specificity and positive predictive value came up to 100% but sensitivity dropped to 64% (Table 2).

Values between $15x10^3$/mcl and $35x10^3$/mcl indicate intermediate risk to develop RDS. Our results of LBC differ slightly from those of other investigators. Dubin recommended thresholds of $26x10^3$/mcl and $40x10^3$/mcl on centrifuged and uncentrifuged samples respectively.[15] In addition, he centrifuged samples at 500xg for 5 minutes which resulted in 40% lower values. Bowie et al recommended a threshold of 19,000/mcl on centrifuged samples.[16] The difference observed between the decision threshold can be attributed to different centrifugation protocols. In present study uncentrifuged samples were used as per recommendations by Neerhof.[13] Moreover, at LBC cut-off of $<15x10^3$/mcl, RDS was present in all the 16 (100%) cases. At LBC value between $15x10^3$ – $35x10^3$/mcl, there were 9 cases of RDS and 77 cases of Non-RDS. At LBC levels $>35x10^3$/mcl, there was no case of RDS and there were 77 (100%) cases of Non-RDS. Our results are in agreement with study by Abd El Aal et al. He observed RDS in all 15 (100%) cases at LBC cut-off of $15x10^3$/mcl. At LBC value between $16x10^3$ to $49x10^3$/mcl, there were 5 (36%) RDS cases and 9 (64%) Non-RDS cases.[17]

In our study clinical outcome i.e. RDS is used as an outcome of LBC performance. In other studies, LBC threshold values were determined in comparison with the predictions of L/S, PG and FSI.[18,19]

We performed the lamellar body count on Sysmex three-part hematology analyzer. On comparing the results with other hematology analysers, it was found that cut off values varied from 30 $x10^3$/mcl to 79,000 $x10^3$/mcl (table 3) insisting on the fact that all laboratories need to calculate their own cut offs. Sensitivity and specificity of Sysmex analysers was found to be high which was also found by Szallasi et al.[20] They found that Sysmex XE-2100 showed best concordance. This could be explained due to the difference in underlying mechanism used to count lamellar bodies. The ADVIA 120 identifies the platelets on basis of their volume and refractive index.[21] Platelet counts in ADVIA 120 include platelets with volumes up to 60 fl and exclude other similar sized particles. Thus, LBC count on ADVIA 120 is the calculated value of sum of all platelet sized particles. Coulter identifies the platelets based on their volume. Sysmex differs from Coulter in that the former

simultaneously detects conventional (direct current) and radiofrequency impedance, which reflects the intracellular changes.[22] To enhance the accuracy of particle counting, Cell-Dyn 3500 combines optical scatter and impedance.[23] Therefore, it is evident from various studies that different hematology analysers count lamellar bodies differently. So, it is necessary to establish analyzer specific LBC clinical decision limits that could then be confirmed by outcome based studies.

We selected, LBC count among all other tests for assessing fetal lung maturity because the test can be performed with equipment found in most clinical analysis laboratories. Furthermore, such counting had been reported as reliably predicting fetal lung maturity and is simple, rapid, non-invasive, less labor intensive, less time consuming, not prone to subjective interpretation, and not technique dependent. Other tests to assess fetal lung maturity are not routinely available in India except in research settings. Present study was conducted on a very basic hematology analyzer i.e. KX 21, which is available even in small laboratories. Moreover, it has an advantage of objectivity and precision when compared with other tests such as L/S ratio or lung lipid profile. The aim of our study was to search for evidence that allowed the evaluation of this test's performance, majorly in clinical practice.

However, more number of studies are needed so that the LBC on three-part hematology analyzer can be used as a routine test and the controls can be made available to increase the validity of the test in routine use.

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