Amnionic Fluid Lamellar Body Counts as a Predictor of Fetal Lung Maturity

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Summary: **Predicting maturity of fetal lung is important and there is a need for a test which is rapid, feasible and available in majority of centres.**

The study was designed to evaluate the lamellar body count (LBC) by Coulter Counter and compare it with shake bubble test (SBT). LBC and SBT were done on 75 amnionic fluid samples in women with gestational age ≥ 28 weeks.

Prevalence of RDS was 17% (13/75). LBC ranged from 24,000 - 4,89,000/µl and had a linear relationship with gestational age. The cut off level of LBC at 35,000/µl was considered to be optimum. Sensitivity and negative predictive value of LBC at 35,000/µl and SBT were comparable; 76.9/76.9% and 95/95.3% respectively whereas with respect to SBT; 98.3/95.5% and 90.9/71.4% respectively. In the limited literature available, LBC has been observed to be superior to L:S ration too.

To conclude, LBC is a rapid, reliable and simple test to assess fetal lung maturity.

Introduction

Predicting maturity of fetal lung is extremely important in many obstetric situations as Respiratory Distress Syndrome (RDS) caused by surfactant deficiency remains one of the leading causes of neonatal morbidity and mortality. Though the survival rate of infants experiencing RDS has improved due to technological advances in neonatal care, the cost of such care is quite high and is not widely available. Hence amnionic fluid tests that assess fetal lung maturity(FLM) have become virtually indispensable in the management of complicated pregnancies. The various laboratory tests using amnionic fluid for predicting FLM include L : S ratio phosphatidylglycerol, lamellar body phospholipid, shake bubble test, surface tension and OD 650.

All these tests measure some aspect of surfactant contained in amniotic fluid. Amongst these test. L : S ratio is accepted as the 'Standard' test for FLM but being expensive, laborious and time consuming, its availability in small to medium sized clinical laboratories is limited. Shake bubble test (SBT) though simple, rapid and inexpensive, lacks surety when the result is intermediate.

Hence there is a need for a test for FLM which is simple, cheap, reliable, rapid and feasible throughout 24 hours of the day.

Pulmonary surfactant is synthesised by type-11 granular pneumocytes and packaged as Lamellar Bodies (LB) that are 1-5µm in diameter, approximately the size of a platelet. These surfactant storage granules contain phospholipid, cholesterol and several surfactant specific proteins. LB first appear in the cytoplasm of fetal pneumocytes between 20-24 wks. gestation and progressively increase in number with advancing gestation. These are continuously secreted into the fetal alveoli. Exudation of fluid into the bronchial tree along with fetal breathing movements carry these lamellar bodies into the amnionic

fluid. Thus, lamellar body counts (LBC) are a potential direct determinants of FLM. They can be easily and rapidly quantified in any clinical laboratory setting that has a facility of a Coulter Counter of any automated particle counter for platelet quantification and also does not demand specialised training.

Very few studies have been done in the words using this parameter for fetal lung maturity.

Hence this study was designed to evaluate lamellar body counts in amnionic fluid as a predictor of fetal lung maturity and compare it with shake bubble test.

Patients & Methods.

Amnionic fluid analysis for lamellar body counts was carried out in 85 pregnant women admitted in the Department of Obstetrics & Gynecology, University College of Medical Science and Guru Teg Bahadur Hospital, Delhi during the period of September, 1994 to February, 1995. The study subjects selected for the study were:

(1) Women who required confirmation of FLM before elective induction of labour as (a) not sure of dates; (b) conceived in lactational amenorrhoea; (c) with irregular cycles and (d) with complications of pregnancy like diabetes mellitus, fetal growth retardation, Rh isoimmunization, PIH etc. In these women, amnionic fluid was obtained by ultrasonographically guided amniocentesis after obtaining an informed consent. Patients with multiple pregnancy choriamnionitis, heart disease, severe anaemia and hydramnios were excluded.

(2) Women who reported in active labor with intact forewaters at gestational age of 28 wks or more. Amnionic fluid was collected by aspirating the forewaters. Patients with chorioamnionitis were excluded.

Amnionic fluid containated with blood and /or meconium was discarded.

With the amnionic fluid so obtained, SBT and LBC were done. Shake bubble test was done as described by Clements et al (1972). Lamellar body counting was done using standard hematology cell counter, Coulter Counter (T 890). The equipment was set for size parameters used for platelet counts between 2 and 20 fl in volume. The samples were either analysed immediately after collection or stored at 4°C and were processed within 3 days. The tubes were covered with parafilm inverted 5 times and were then centrifuged for 3 min. at 500 xg. The supernatant was then pipetted into a new test tube and placed on a tube rocked for 2 minutes. The aspiration tip of Coulter Counter was then wiped clean with a gauze pad and 50% bleach. Isoton-III, a balanced electrolyte solution was then aspirated at least once into the instrument to prime it and to purge any remaining blood that has been left on the tip from previous analysis. The well mixed amnionic fluid sample was then aspirated into the Coulter Counter. This instrument required a minimum of 125/µl of amnionic fluid.

The patients were followed up till delivery. All neonates were assessed for maturity based on Dubowitz etal's Criteria (1970) and patients were kept in the hospital for a minimum of 3 days after delivery for observation of the neonate for development of RDS, which was defined as tachypnoea, grunting, hypoxia. characteristic chest radiograph, need for O_2 by intubation or hood for 24 hrs. within first 72 hrs. of life.

Observations and Results

85 pregnant women in the age group of 19-35 years were recruited for the study. The characteristics of study subjects are shown in Table 1. 78 samples were obtained by vaginal route whereas 7 were obtained by transabdominal route. The indications for amniocentesis were fetal growth retardation (4), uncertain dates (2) & PIH (1). 10 patients were excluded as the liquor obtained was

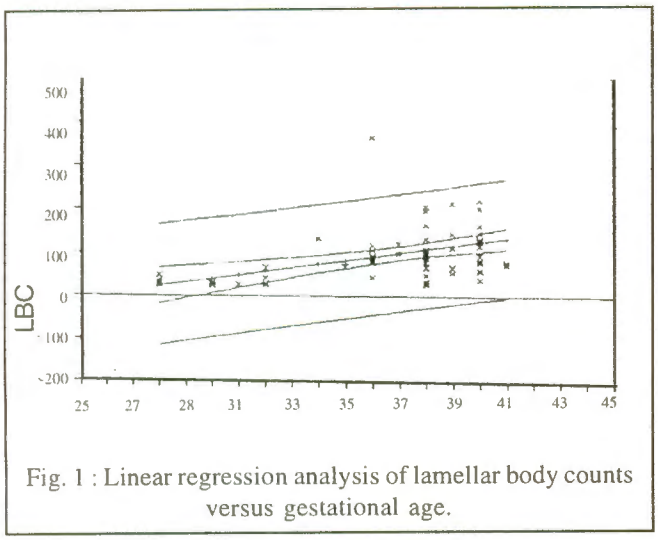

Fig. 1 : Linear regression analysis of lamellar body counts versus gestational age.

contamined by either blood or meconium. Hence 75 women were included in the final study. Of the 75 subject, 62 women knew the date of last menstruation whereas 13 patients were with unknown dates. Of the 62 subjects with definite dates, the gestational age was ≤ 34 weeks in 11 women (17.7%) and > 34 wks. in 51 women (81.3%). 61 test samples (81.33%) showed a positive shake bubble test while results were intermediate in 6(8%) and negative in 8(10.67%). The lemellar body counts in the study ranged from 24,000-4,98,000/µl.

Table1:

Characteristics of study subjects

Study Subjects	No*	%
Term normal cases	50	47.6
Patient not sure of dates	13	12.4
Premature rupture of membranes	13	12.4
Fetal growth retardation	5	4.8
Previous caesarean section	4	3.8
Preeclampsia	3	2.8
Breech presentation	2	1.9
Rh sensitization	2	1.9
Gestational diabetes	2	1.9
Chronic hypertension	1	0.9

(*Many subjects had more than one disorder, therefore the sum exceeds 75.)

On plotting the LBC against period of gestation the relationship was seen to be a linear one with a correlation coefficient of 0.39(p<.000) Fg. 1. The birth weight of the newborn varied from 1000-3500g with the mean of 2370±560g. The maturity of the newborns as assessed by Dubowitz(etal 1970) score varied from 28-40 wks. 14 babies (18.7%) were less than 34 weeks while 61 (81.3%) were 34 weeks or above. 22 babies were shifted to nursery because of birth asphyxia, prematurity, low birth weight and the rest 53 babies were kept with the mother. Out of the 22babies transferred to nursery 13 developed RDS. So the prevalence of RDS in the study population was 17% (13/75). All the babies with RDS had Tachypnoea., hypoxia and acidosis. X-ray chest was bilaterally hazy in

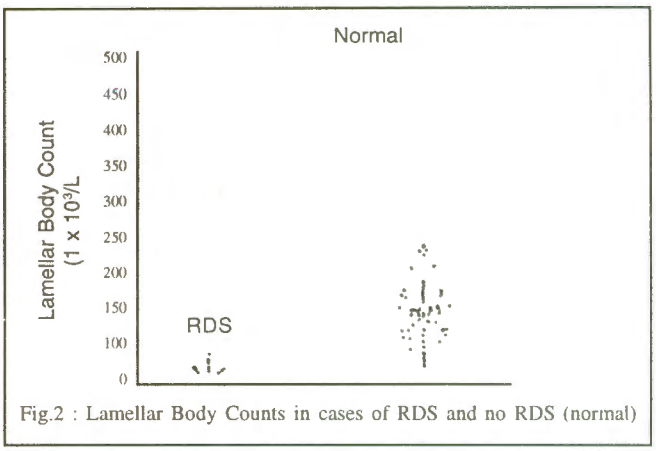

Fig.2 : Lamellar Body Counts in cases of RDS and no RDS (normal)

7 whereas normal in 6 babies. All the neonates who developed RDS received O_2 by ventilator, intravenous fluids and intravenous antibiotics. Unfortunately, all the 13 neonates died within 2-10 days despite good care.

As shown in Table 2, with 61 positive SBT results only 3 babies had RDS. With 8 negative results, 6 had RDS whereas with 6 intermediate results, 2 babies were normal while 4 developed RDS. All the new borns with maturity ≤ 32 weeks (17) had RDS while out of 63 with maturity > 32 weekIs only 1 had RDS. Out of 9 babies with birth weight ≤ 1.5kg. 8 developed RDS whereas with birth weight > 1.5kg. (66) only 5 developed RDS.

Table 2:

SBT, Maturity, and Birth weight in relation to RDS

	RDS	
	-	+
Shake bubble test results		
Positive (+)	58	3
Intermediate (±)	2	4
Negative (-)	2	6
Newborn maturity		
≤32 wks	-	12
>32wks	62	1
Birth weight		
≤ 1.5kg	1	8
>1.5kg	61	5

The lamellar counts varied from 24,000-49,000/µl in neonates who developed RDS with the mean of 31,800/µl (Fig.2.). The clinical utility of LBC at different cut-off levels was calculated (Table 4) and it was seen that cut-off level of 35,000/µl out of 11 neonates with LBC ≤ 35,00/µl. 10 developed RDS and out of 64 neonates with LBC > 35,000/µl only 3 developed RDS.

Table 3.

Birth Weight, Maturity, SBT and LBC in newborns with RDS.

S.No.	Birth weight (Kg)	Maturity (Weeks)	SBT	LBC x 10^3/µl
1.	1.2	32	-	49
2.	1.6	30	+	31
3.	1.25	35	+	27
4.	1.5	32	-	29
5.	1.6	30	±	38
6.	1.6	32	-	28
7.	1.2	30	-	26
8.	1.7	32	+	43
9.	1.5	30	±	29
10.	1.3	30	-	24
11.	1.5	30	±	26
12.	1.0	28	-	32
13.	1.7	32	±	31

Table 4:

The Clinical utility of Lamellar body counts at various cut-off levels.

LBCx 10^3/µl	No. of cases	RDS	pValue	Sensitivity (%)	Specificity (%)	+PV (%)	-PV (%)
≤30	7	7	<.0001	53.8	100	100	93.9
> 30	68	6					
≤ 35	11	10	<.0001	76.9	98.3	90.9	95.3
> 35	64	3					
≤ 40	14	11	<.001	84.6	95.2	78.5	96.7
> 40	61	2					
≤ 45	16	12	<.001	92.3	93.5	75.0	98.3
> 45	59	1					

From Table 5, it is evident that the sensitivity and negative predictive value of LBC and SBT are comparable whereas LBC seems to be superior to SBT in its specificity and positive predictive value.

Discussion

Fetal maturity is a complex state that involves many organ systems. In uncomplicated pregnancies fetal gestational maturity, somatic maturity and placental maturity seem to proceed at a rate similar to that of pulmonary maturity. However. in pathologic cases, the time at which fetal pulmonary maturity is reached deviates from that at which it occurs in normal pregnancy. Both accelerated and delayed lung maturity are possible in different clinical states and hence establishment of fetal pulmonary maturity before elective termination of pregnancy is mandatory. Quantification of lamellar bodies was first done by Dubin 1989, who showed a lamellar body number density of 40,000/µl in uncentrifuged and 26,000/µl in centrifuged samples to be highly concordant which established measures of fetal lung maturity.

Table 5:

Sensitivity, Specificity, +ve and -ve Predictive values of SBT and LBC at 35,000/µl

Parameters in Amnionic fluid	Sensitivity (%)	Specificity (%)	+pv(%)	-PV(%)
SBT	76..9	93.5	71.4	95.0
LBC	76.9	98.3	90.9	95.3

(Intermediate SBT taken as negative)

Table 6:

The Comparison of various tests for Fetal Lung Maturity.

Author	Methods	Sensitivity(%)	Specificity (%)	+PV(%)	-PV(%)
Pearlman et al	FSI > 46	83	84		
1991	LBC > 19,000	95	83		
Ashwood et al	L:S ratio≤2.0	83	84	35	98
1993	LBC≤55,000	100	59	24	100
Fakhoury et al	Phophatidyl				
1994	glycerol	100	100		
	L:S ratio < 2	75	100		
	LBC< 30,000	100	100		
Present Study	SBT	76.9	95.3	71.4	95
1994-95	LBC≤35,000	76.9	98.3	90.9	95.3

The lamellar body counts have ranged from 3800-1,60,000/µl in the various studies (Ashwood et al, 1990; Bowie et al, 1991 and Pearlman et at, 1991). Though gestation specific lamellar body counts are not available, a linear relationship between lamellar body counts and gestational age has been shown by Ashwood et al, 1993 and Fakhoury et al,1994. The present study showed a lamellar body count by Coulter Counter range between 24,000 and 4,89,000/µl. The high upper limit is due to high lamellar body counts of 2 samples 4,89,000 and 3,63,000/µl. The current study also showed a linear relationship between LBC and gestational age (Fig.1).

Thirteen neonates developed RDS and the lamellar body counts ranged between 24,000 and 49,000/µl in these cases. None of the neonates having lamellar body counts > 49,000/µl developed RDS. Ashwood et al, 1993 in his series stated that no case of RDS developed with the lamellar body counts more than 49,000/µl. The birth weight of neonates who developed RDS ranged between 1.0 kg and 1.7 kg. with 8 neonates with birth weight ≤ 1.5 kg. Twelve neonates with RDS had maturity ≤ 34 weeks while one neonate was of 35 weeks. Shake bubble test was negative in 6 cases of RDS, intermediate in 4 cases and found to be positive in 3 cases (Table 2). Lamellar body counts in the 3 cases of RDS with positive shake bubble test were 31,000, 27,000 and 43,000/µl respectively (table 3).

As depicted in Table 4, it is clear that there is statistically significant difference in the development of RDS with the lamellar body cut-off point taken as 30,000, 35,000,40,000 or 45000/µl. However, with increasing cut-off limits. The sensitivity of the test increases only at the cost of specificity and positive predictive value.

From the data available in our study of limited number of amnionic fluid samples, a cut-off limit of 35,000/µl would seem to offer the best clinical utility of the test with the sensiticity of 76.9%, specificity of 98.3%, positive predictive value of 90.9% and a negative predictive value of 95.3%. The lamellar body count at 35,000/µl cut-of showed a false positivity in one case and a false negativity in three cases. Shake bubble test showed a false positivity in 2 cases and a false negativity in 3 cases giving a sensitivity of 76.9% specificity of 93.5%, positive predictive value of 71.4% and negative predictive value of 95%.Thus both the tests individually predicted RDS in 10 out 13 neonates. However, had the two tests been combined, successful prediction of RDS would have been

possible in 12 out of 13 cases (sensitivity 92.3%). Thus these two tests which can be carried out easily in most of the centres are complimentary to each other.

Dubin et al 1989, recommended decision thresholds of 26,000/µl on centrifuged samples. (500g x 5 min) and 40,000/µl on uncentrifuged samples. Bowie et al 1991; recommended a threshold of 30,000/µl using a centrifugation protocol of 1000g x 5 minutes. Our study has a centrifugation protocol of 500g x 3 min which is similar to that of Ashwood et al, 1990 and Pearlman et al, 1991. Though the lamellar body count cut-off in the series by Ashwood et al 1990 was 18,000/µl and that of Pearlman et al 1991 was 19,000/µl. we observed 35,000/µl as the cut-off point more optimum.

Table 7:

Comparison of Methodology of LBC, SBT and L:S ratio

Methodology	LBC	SBT	LS ratio
Simplicity	Simple	Simple	Difficult
Duration	5 min	15 min	2-3 hours
Quantity of Amnionic fluid required	0.4 ml	2.25ml	3 ml
Observer bias	-	+	+
Accessibility	Readily accessible	Readily accessible	Available in selected hospitals
Specialised training	Not required	Not Required	Required
Cost	Inexpensive	Inexpensive	Expensive
Performance on meconium or blood contaminated liquor	Seems feasible	Not feasible	Not feasible

The effect of blood and meconium contamination on lamellar body count remains to be resolved. Ashwood et al 1990 reported decrease in LBC by 22% with 1% whole blood contamination, while Dubin 1989 showed no significant effect on addition of whole blood. Further work is needed to clarify and quantify the effect of blood contamination on the LBC, since it will be useful in cases of blood stained liquor. Dubin 1989 observed non-interference in LBC in meconium stained liquor which is again very beneficial as till now all the tests available are adversely affected by the presence of meconium in the liquor.

As per Table 6, LBC appears to be superior to all the other tests except phosphatidylglycerol with which it is comparable.

Thus our study, along with a few limited suggest that lamellar body count is a simple, rapid, reliable and practical assay, available in most of the centres and throughout 24 hrs. of the day. Further evaluation of this test in a larger population is recommended.

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