

AMNIOTIC FLUID LAMELLAR BODY COUNT FOR PREDICTION OF FOETAL LUNG MATURITYAruna Ratikrinda¹, Saroja Adapa²¹Assistant Professor, Department of Obstetrics & Gynaecology, Government Maternity Hospital, OMC Hyderabad.²Assistant Professor, Department of Obstetrics & Gynaecology, Government Maternity Hospital, OMC Hyderabad.**ABSTRACT****INTRODUCTION**

Predicting maturity of the foetal lung is extremely important in many obstetric situations as respiratory distress syndrome caused by surfactant deficiency remains one of the leading causes of neonatal morbidity and mortality. The need for predicting the foetal lung maturity by means of an accurate test which is done rapidly and available in majority of centres was the objective for this study.

AIM

To find out the optimum lamellar body count which correlates with foetal lung maturity and to compare the lamellar body count with shake bubble test.

MATERIAL AND METHODS

This prospective study was designed to evaluate the lamellar body count by standard haematology cell counter that is coulter counter and compare it with shake bubble test. Both the tests were done on 100 amniotic fluid samples in women with gestational age more than 28 weeks. Patients recruited were those who were sure of their last menstrual period (LMP), who were in active labour, and likely to deliver within 72 hours of collection of sample. Amniotic fluid samples obtained by transabdominal amniocentesis or by aspirating the forewaters per vaginum. Both shake bubble test and lamellar body count were done on the sample.

RESULTS

Lamellar body counts ranged from 10,000-2,43,000/microlitre and had a linear relationship with gestational age. The cut-off level of 30,000/microlitre was considered to be optimum to predict foetal lung maturity. Sensitivity and negative predictive value of lamellar body count at 30,000/microlitre and shake bubble test were comparable at 91.7%/91.7% and 97.3%/96.6% respectively whereas with respect to specificity and positive predictive value, lamellar body count was superior to shake bubble test at 93.4%/73.7% and 81.5%/50%.

CONCLUSION

Lamellar body count is a rapid, inexpensive, simple and more reliable test to assess foetal lung maturity.

KEYWORDS

Lamellar Body Count, Shake Bubble Test, Amniotic Fluid, Respiratory Distress Syndrome, Foetal Lung Maturity, Pneumocytes.

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INTRODUCTION: Predicting maturity of the foetal lung is extremely important in many obstetric situations. As respiratory distress syndrome caused by surfactant deficiency remains one of the leading causes of neonatal morbidity and mortality.

Identification of various indices of functional foetal lung maturity is of considerable importance to the obstetrician in the perinatal management of high risk pregnancies, more so in the developing countries where most of the patients are unaware of their last menstrual period, for those who conceive during lactational amenorrhea and for women with irregular periods.

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All the available laboratory tests using amniotic fluid for predicting foetal lung maturity measure some aspect of surfactant contained in the amniotic fluid. Amongst these tests, lecithin to sphingomyelin ratio is accepted as the standard test for foetal lung maturity 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 predicting foetal lung maturity which is simple, cheap, reliable, rapid and feasible throughout 24 hours of the day,¹ foetal respiratory system and the surfactant.

There are more than 40 cell types in the lung, but surfactant is formed specifically in the type-II pneumocytes that line the alveoli. The type -II cells are characterised by multi-vesicular bodies, the cellular progenitors of the lamellar bodies in which surfactant is assembled and stored. Ultimately, lamellar bodies are secreted from the lung.

During late foetal life at a time when the alveolus is characterised by a water to tissue interphase, the intact lamellar bodies are swept into the amniotic fluid during foetal respiratory like movements i.e. foetal breathing. Thus, the appearance of surfactant in the amniotic fluid heralds the commencement of biochemical maturation of foetal lungs at least with respect to surfactant synthesis.

At birth with the first breath, an air to tissue interphase is produced in the lung alveolus. This permits surfactant to "uncoil" from the lamellar bodies and the surface tension lowering material, then spreads to line the alveolus and thereby prevents alveolar collapse during expiration. Lamellar bodies first appear in the cytoplasm of foetal pneumocytes between 20-24 weeks' gestation and progressively increase in number with advancing gestation.

AIM:

1. To find out the optimum lamellar body count which correlates with foetal lung maturity.
2. To compare the lamellar body count with shake bubble test.

MATERIAL AND METHODS: 114 pregnant women who were admitted to Government Maternity Hospital, Sultan Bazar, Hyderabad during the period May 2014 to April 2015 were recruited for the study. The patients recruited were those who were definite about the date of last menstrual period, with singleton pregnancy and those with normal amount of liquor. Patients recruited were those in active labour or likely to deliver within 72 hours of collection of sample with a gestational age ranged from 28 weeks to term which was determined accurately by the clinical methods and ultrasonographically, prior to including them for the study. 14 patients were excluded from the study as 5 of them delivered after 72 hours of collection of the sample and the other 9 amniotic fluid specimens were contaminated by either blood or meconium. Hence 100 women were included in the study. The patients were selected irrespective of any medical or obstetric disorders complicating pregnancy.

Amniotic fluid specimens were obtained either by² transabdominal amniocentesis or by aspirating the forewaters per vaginum or during caesarean section. Shake bubble test was done immediately with the same sample as described by³ Clements et al and the samples were either analysed immediately for lamellar body count or stored at 4 degrees centigrade and were processed within 72 hours. Lamellar Body Count – was done using the standard haematology cell counter, Coulter counter (660). The equipment was set for size parameters used for platelet count between 2 and 20 fl in volume. One mL amniotic fluid was taken into a test tube (75 X 12 mm). The tubes were covered with parafilm inverted 5 times and were then centrifuged for 3 minutes at 2000 rpm. The supernatant is then pipetted into a new test tube and placed on a tube-rack for 2 minutes. The aspiration tip of the counter was then wiped clean with a gauze pad and 50% bleach. Isotone III, a balanced electrolyte solution was then aspirated at least once into the instrument to prime it and to purge any blood that would have remained in the tip from the previous

analysis. The well-mixed amniotic fluid sample was then aspirated into the Coulter Counter. The instrument requires a minimum of 125 microlitre of amniotic fluid. Lamellar body count was read from automated electronic monitor of the Coulter Counter.

The patients were followed up till the delivery. All the neonates were assessed for maturity based on ⁴Dubowitz et al criteria. All the patients were kept in the hospital for a minimum of 3 days after the delivery, for the observation of the neonate, for development of respiratory distress syndrome. The babies with birth asphyxia, prematurity, low birth weight, etc. were shifted to nursery.

The diagnosis of respiratory distress syndrome (RDS) was based on rigid criteria.

1. The clinical triad of tachypnoea, expiratory grunt and inspiratory chest retraction.
2. Radiological evidence e.g. opacification.
3. Oxygen support required for 24 hours or more.

OBSERVATION AND RESULTS: The lamellar body counts in the 100 women ranged between 10,000 and 2,43,000/ microlitre. On plotting the lamellar body count against period of gestation, the relationship was seen to be a linear one with a correlation coefficient of 0.39 ($p < .011$). Out of the 100 babies delivered, 38 babies were shifted to nursery, because of birth asphyxia, prematurity, low birth weight and the rest 62 babies were kept with the mothers. Out of the 38 babies transferred to nursery, 24 babies developed respiratory distress syndrome. So the prevalence of respiratory distress syndrome in the study population was 24% (24/100). 18 neonates (75%) out of 24 died within 10 days despite good intensive neonatal care. Analysis of the data in newborns with respiratory distress syndrome is given in table 1.

Maturity, Birth weight, SBT, LBC in new-born with RDS:

Sl. No.	Maturity	Birth weight	Lamellar bodies X 10 /ul	SBT
1	34	2.2-	37	-- ve
2	35	2.1	32	+ve
3	34	1.7	30	+ve
4	37	3.4	28	--ve
5	34	2.1	28	-- ve
6	30	2.0	28	-- ve
7	35	1.8	28	-- ve
8	33	2.1	26	-- ve
9	30	2.3	24	-- ve
10	28	1.8	24	-- ve
11	33	2.2	21	-- ve
12	34	2.2	20	-- ve
13	30	2.3	20	-- ve
14	28	1.6	20	-- ve
15	32	2.4	18	-- ve
16	30	2.2	18	-- ve
17	30	2.3	18	-- ve
18	28	1.3	17	-- ve
19	28	1.7	17	-- ve
20	28	1.4	17	-- ve
21	30	1.7	13	-- ve
22	32	2.4	12	-- ve
23	28	1.1	10	-- ve
24	28	1.2	10	-- ve

Table 1: Analysis of data in newborns with RDS

Result of SBT	No. cases	No. of cases OF RDS
Positive	58	2
Intermediate	1	0
Negative	41	22
Table 2: Validity of shake bubble test in RDS cases		

Intermediate test is taken as negative.

2 babies developed RDS out of 58 positive results, 22 babies developed RDS with 42 negative test results giving the value of 91.7% sensitivity, 73.7% specificity, 50% positive predictive value and 96.6% negative predictive value.

LBC X 1000/microlit	No. of cases	RDS	p value	SN %	SP %	+vePv %	-VE PV %
≤29	25	21	<.001	87.5	94.7	84	96
>29	75	3					
≤ 30	27	22	<.001	91.70	93.4	81.5	97.3
>30	73	2					
≤ 31	29	22	<0.001	91.6	90.8	75.5	97.2
>31	71	2					
≤32	31	23	<0.001	95.8	89.5	74.2	98.6
>32	69	1					
≤33	34	23	<0.001	95.8	85.5	67.6	98
>33	66	1					
≤35	38	23	<0.001	95.8	80.3	60.5	98
>35	62	1					
Table 3: The clinical utility of lamellar body counts at various cut off levels							

Methodology	Lamellar bodies	SBT
Simplicity	Simple	Simple
Duration	5 min.	15 min.
Quantity of sample	0.4ml	2.25 ml
Accessibility	Readily accessible	Readily accessible
Specialised Training	Not required	Not required
Cost	Inexpensive	Inexpensive
Performance on meconium	seems	Not
Or blood contaminated	feasible	Feasible
Liquor		
Table 4: Comparison of Methodology of Lamellar Body count (LBC). Shake Bubble		

DISCUSSION: Lamellar bodies have long been recognised to have a direct relationship with foetal lung maturity. Previously, technical difficulties have made lamellar body assays impractical for foetal lung maturity assessment. Dubin successfully used a commercial cell counter to quantitate lamellar bodies in amniotic fluid.

The Coulter Counter is adjusted to measure platelet size between 2 and 20 fl, rejecting particles outside these limits. Because lamellar body size is in the range of 1 to 5 micrometres (1.28 to 6.4 fl). With most being near to 2 micrometres in diameter (2.56 fl). Hence, Coulter can be used to assay lamellar body counts.

The lamellar body counts have ranged from 3,800 – 1,60,000/microlitre in the various studies Ashwood et al,⁵ 1990: Bowie et al,⁶ 1991 & Pearlman et al 1991. Though the 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,⁷ 1993 & Fakhoury et al, 1994. Preeti Mittal & Associates showed a

lamellar body count range between 24,000-4,89,000/microlitre. The present study showed a lamellar body count ranging between 10,000 and 2,43,000/microlitre. 24 neonates developed respiratory distress syndrome and the lamellar body count ranged between 10,000 and 37,000/microlitre. None of the neonates having lamellar body count > 37000/microlitre developed respiratory distress syndrome. Ashwood et al, 1993 in his series, Preeti Mittal & associates stated that no case of respiratory distress syndrome developed with the lamellar body counts more than 49,000/microlitre.

It is clear that there is a statistically significant difference in the development of respiratory distress syndrome with the lamellar body count cut-off point taken as 30,000, 31,000, 33,000 and 35,000. However, with increasing cut-off limits, the sensitivity of the test increases only at the cost of specificity and positive predictive value Table 3.

Welsh⁸ & Colleague in their study (849 samples) reported that lamellar body count of at least 35,000/microlitre as a reliable predictor of foetal lung maturity Dubin et al⁹ 1989, recommended decision thresholds of 26,000/microlitre in centrifuged sample and 40,000/microlitre in uncentrifuged samples.

Bowie et al, 1991 recommended a threshold of 30,000/microlitre using a centrifugation protocol.

The lamellar body count cut-off in the series by Ashwood et al 1990 was 18,000/microlitre. Pearlman et al 1991 was 19000/microlitre, ¹⁰Preeti Mittal & Associates 35000/microlitre, the present study observed 30,000/microlitre as the cut-off point more optimum, which is correlating with the observations of Bowie et al 1991.

The present study on 100 amniotic fluid samples, a cut-off limit of 30,000/microlitre would seem to offer the best clinical utility of the test with the sensitivity of 91.7%, specificity of 93.4%. Positive predictive value of 81.5%, negative predictive value of 97.3%.

Shake bubble test showed a false positivity in 20 cases and false negativity in 3 cases giving a sensitivity of 91.7%, specificity of 73.7%, positive predictive value of 50% and negative predictive value of 96.6%. (Table 2). Lamellar body count seems to be comparable to shake bubble test with respect to sensitivity and negative predictive value, whereas found superior to shake bubble test with respect to specificity and positive predictive value.

Thus, lamellar body count is proved to be superior to shake bubble test.

The lamellar body count predicted accurately the accelerated lung maturity in PIH and PROM cases and delayed lung maturity in a diabetic mother.

The effect of blood and meconium contamination on lamellar body count remains to be resolved. Ashwood et al 1990, reported a decrease in lamellar body count by 22% with 1% whole blood contamination, while Dubin in 1989 showed no significant effect on addition of whole blood. Further work is needed to clarify and quantify the effect of blood contamination of lamellar body count, since it will be useful in cases of blood stained liquor. Dubin 1989 observed

non-interference in lamellar body count 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.

CONCLUSION: The cut-off level of amniotic fluid lamellar body count of 30,000/microlitre is considered to be optimum to predict foetal lung maturity and respiratory distress syndrome.

Lamellar body count is comparable to shake bubble test in simplicity and in cost effectiveness and found superior to the latter as it is rapid and more reliable (Table 4).

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