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Respiratory status in preterm babies on basis of surfactant maturation

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Abstract
This prospective hospital based study was conducted on 100 preterm neonates delivered in Department of Obstetrics and Gynaecology and admitted to Neonatology section of Department of Pediatrics, Govt. Medical College/Rajindra Hospital Patiala. The surfactant maturation was estimated by Shake Bubble Stability Test on gastric aspirate. The respiratory status of babies was determined on the basis of clinical definition of Respiratory Distress in a newborn after observing for 6 hours after birth. Surfactant maturation, as reflected by shake bubble test, had a significant correlation with gestational age ($p<0.001$). 31% of the study neonates had RD and the incidence of respiratory distress varied significantly with shake bubble grades ($p=0.0001$) i.e. the immature the surfactant status of a newborn, the more likely it is to suffer from respiratory distress. With gestational age ($r= 0.650$, $p=0.0001$) i.e. lesser the gestational age of a newborn, immature would be its surfactant. The incidence and severity of respiratory distress had a significant inverse correlation with surfactant maturation, reflected by increasing shake bubble grade, ($r= - 0.546$, $p<0.001$), i.e. immature the surfactant status of a newborn, more would be the incidence and severity of R.D. in the newborn. The respiratory status of a neonate varied significantly with shake bubble grades ($p=0.046$ for birth weight < 1000g, $p<0.001$ for 1000-1499g, $p<0.001$ for 1500-1999g, $p=0.004$ for 2000-2499g).Incidence and severity of respiratory distress varied significantly with birth asphyxia ($p<0.001$). Key Words: Preterm, Surfactant, Respiratory distress.

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Introduction
Respiratory distress is a common emergency responsible for 30 to 40 percent of neonatal admissions [1]. It is defined clinically as a state with respiratory rate of the newborn more than 60/min, and/or, intercostal retractions, and/or, presence of expiratory grunt. Causes of neonatal respiratory distress include causes affecting respiration at alveolar level (HMD, Pneumonia, MAS, Pneumothorax, Pulmonary hemorrhage, PPHN, TTN) or structural anomalies of the respiratory tract (Choanal atresia, Tracheo-esophageal fistula, Congenital diaphragmatic hernia, Congenital lobar emphysema) and extrapulmonary causes (bony defects of the chest wall, congenital heart disease and metabolic acidosis) [2]. Respiratory Distress Syndrome, also known as, Hyaline Membrane Disease, is a breathing disorder of premature babies. RDS is, infact, the commonest cause of neonatal mortality in preterm babies [3]. The basic abnormality in HMD is lack of surfactant due to immaturity of lungs. Respiratory Distress Syndrome (RDS) affects about 1 percent of newborn infants and is the leading cause of death in babies who are born prematurely. The incidence of the disease is inversely related to gestational age and birth weight. It occurs in 60 to 80 % of infants less than 28 wks of gestational age, 15 to 30% of infants between 32 to 36 weeks, 5% of infants beyond 37 weeks [4].

The chemical entity responsible for this ailment is surfactant. The term "SURFACTANT" is a blend of "surface active agent", usually amphiphillic organic compounds. Pulmonary surfactant is a mixture of Dipalmitoyl phosphatidyl choline (lecithin), phosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl inositol, surface proteins (SP-A, SP-B, SP-C, SP-D), neutral lipids and cholesterol.[4] Further studies on RDS revealed that the deficiency of surfactant was a consequence of either insufficient production by immature lungs or a generic mutation in one of the surfactant proteins, SP-B. The rarer genetic form of disease is not associated with premature birth and occurs in full term babies [5].

Surfactant is produced by Type II alveolar cells of fetal lungs in the form of lamellar bodies. These lamellar bodies are secreted by exocytosis into the surface water layer lining the alveolar airspace, where the surfactant forms a meshwork of tubular myelin. It is only by 35 weeks of gestation that mature levels of surfactant are present in the lungs.

Fetal lung maturity, on the basis of surfactant levels, can be assessed by several tests.

Shake bubble Test (Clements) is a simple bedside test, the interpretation of which is interfered by blood or meconium. The interpretation of Shake Bubble test has been categorized from 0 to 4+. The risk of Hyaline Membrane disease is minimal if the result is 2+ or more [6].

Amniotic fluid Lecithin/Sphingomyelin (L/S) ratio is performed by thin layer chromatography. The risk of RDS is very low if the L/S ratio is >2. Fluorescent Polarization test (TDx-FLM) measures surfactant to albumin ratio. A value of >55 mg of surfactant/gm of albumin correlates with pulmonary maturity. Contamination with blood or meconium interferes with the results [7].

Lamellar Body Count of >50000 lamellar bodies/microliter predicts fetal lung maturity (FLM) [7].

Amniotic Fluid Optical Density Test indirectly measures the lamellar body count and thus predicts fetal lung maturity [7].

Amniotic Fluid Phosphatidyl Glycerol has the advantage that it is not affected by contamination of amniotic fluid by blood or meconium. Its sensitivity is low and can give false negative results when other tests are indicating mature fetal lungs [7].

Shake Bubble Test, the test used in this project, is a commonly performed bedside maneuver that assesses the surfactant level by means of the ability of bubble production when equal amounts of absolute alcohol and gastric aspirate, taken in a test tube, are shaken and allowed to stand undisturbed.

The pathogenesis of HMD revolves around the basic functionalities of Hypoxia and Hypercapnia. Deficient synthesis or release of surfactant together with small respiratory units and a compliant chest wall produces atelectasis and results in perfused but not ventilated alveoli, causing hypoxia. Decreased lung compliance, small tidal volumes, increased physiological dead space, increased work of breathing, insufficient alveolar ventilation eventually results in hypercapnia. The combination of hypoxia, hypercapnia and acidosis causes pulmonary artery vasoconstriction with increased right to left shunt through the foramen ovale and ductus arteriosus and within the lungs itself. Pulmonary blood flow is reduced and ischemic injury to the cells producing surfactant and to the vascular bed results in effusion of proteinaceous material into the alveolar spaces, forming the well-known Hyaline Membrane which further interferes with gaseous exchange and clinically manifests as Respiratory Distress.

Hyaline Membrane Disease is clinically characterized by a respiratory rate >60/min, dyspnea (intercostal and subcostal indrawing, sternal retractions) with a predominantly diaphragmatic breathing pattern and a characteristic expiratory grunt or moan, all presenting within 4-6 hours of delivery [8].

Respiratory Distress Syndrome never occurs beyond 6 hours of life [8].

However, there are contradictory results regarding various factors influencing surfactant maturity and respiratory status in preterm neonates. An attempt has been made to decipher the same in the present study.

Subjects and Methods

The study “Respiratory Status in Preterm Babies on the Basis of Surfactant Maturation” was conducted on 100 preterm neonates delivered in Department of Obstetrics and Gynaecology, Govt. Medical College, Patiala and admitted to Neonatology section of Department of Pediatrics, Govt. Medical College, Patiala.

Inclusion criteria

All preterm babies, whether AGA, LGA or SGA, were included in the study.

Preterm were the babies delivered before 37 completed weeks of gestational age [9].

A newborn was said to have respiratory distress when the respiratory rate of the newborn was more than 60/min, and/or, there were intercostal retractions, and/or, expiratory grunt was present.

Respiratory Distress Syndrome was characterized clinically by a respiratory rate >60/min, dyspnea (intercostal, subcostal indrawing, sternal retractions) with a predominantly diaphragmatic breathing pattern and a characteristic expiratory grunt or moan, all presenting within 4-6 hours of delivery.[8]

AGA were the babies with birth weight between 10^{th} to 90^{th}percentile of weight for that gestational age.

LGA were the babies with birth weight beyond 90^{th} percentile of weight for that gestational age.

SGA were the babies with birth weight less than 10^{th} percentile of weight for that gestational age. [10]

Exclusion criteria

- Term Babies
- Post term Babies

Term were the babies born between 37 to 41 completed weeks of gestation.

POSTTERM were the babies born with 42 weeks of gestation or more. [9]

Methods

The respiratory status was determined in premature babies of the study group on the basis of clinical definition of Respiratory Distress in a newborn. The surfactant maturation in this study was estimated by Shake Bubble Stability Test. Every newborn baby was observed for a period of six hours for the possible development of respiratory distress syndrome. [8]

Respiratory distress (R.D.) in a newborn

Clinically, a newborn was said to have respiratory distress if the respiratory rate of the newborn was more than 60/min, and/or, there were subcostal retractions, and/or, an expiratory grunt was present.

Shake bubble stability test [11]

Initially described by Clements (1972), it was further modified by Bhagwanani, Fahmy and Turnbull.

Shake test was performed on gastric aspirate to predict RDS (Respiratory Distress Syndrome) as follows:

A) Gastric aspirate was obtained before the baby was one hour of age by passing a suction catheter with a mucus trap

into the stomach. Specimens less than 1ml were considered inadequate.
B) 0.5ml of absolute alcohol was added to 0.5 ml of gastric aspirate into a 4 ml test tube. The capped test tube was shaken vigorously for 15 seconds and allowed to stand undisturbed for 15 minutes.

Results and interpretation

1. Immature = no bubbles : high risk of RDS (60 percent)
2. 1+ = very small bubbles in meniscus extending one third or less distance around the test tube (a magnifying glass was usually required to determine that these were bubbles, as opposed to particles): intermediate risk of RDS (20 percent).
3. 2+ = single rim of bubbles extending one third to all the way around the test tube.
4. 3+ = A rim of bubbles all the way around the test tube, with a double row in some areas: low risk of RDS (less than 1 percent).
5. 4+ = A double row or more of bubbles all the way around the test tube: fully mature.

In this study, the respiratory status in preterm babies was analyzed on the basis of surfactant maturation as depicted by shake bubble stability test. The data so obtained was analyzed statistically

Results

The study population consisted of 100 preterm neonates out of which 49 (49%) were female and 51 (51%) were male. The gestational age group with maximum neonates (30%) was ≥ 35 wks while minimum (6%) belonged to ≤ 28 weeks group (Table 1). Statistically, the difference in the distribution of neonates amongst different gestational age groups was significant (p<0.0001). However, there was no significant difference in the distribution of male and female neonates within different gestational age groups (p=0.279) (Table 1). Birth asphyxia was observed in 16 (16%) neonates, 4 (4%) had mild birth asphyxia, 5 (5%) had moderate birth asphyxia and 7 (7%) had severe birth asphyxia. The maximum fraction of neonates with birth asphyxia (30%) was in gestational age group 29-30 wks (Table 2). Statistically, the incidence and degree of birth asphyxia did not vary significantly with gestational age (p=0.363) (Table 2). In the present study, there were 6 (6%) neonates with shake bubble grade zero, 29 (29%) with 1+, 45 (45%) with 2+, 19 (19%) with 3+ and 1 (1%) with 4+ shake bubble grade. Maximum fraction of the neonates with gestational age ≤ 28 weeks (66.7%) had shake bubble grade 0, with 29-30 weeks (60%) had 1+ grade, with 31-32 wks (62.1%) and 33-34 wks (48%) had 2+ grade while those with gestational age ≥ 35 wks had equal fraction (40%) of 2+ and 3+(Table 3). Surfactant maturation, as reflected by shake bubble test, had a significant correlation with gestational age (r= 0.650) i.e. lesser the gestational age of a newborn, immature would be its surfactant (Table 3). In the study, 69 (69%) of the study neonates were free from R.D. Amongst the 31 (31%) neonates with R.D., 1 (1%) had R.D. in the form of tachypnea (1/3), 10 (10%) had tachypnea and retractions (2/3), 20 (20%) had tachypnea, retractions and expiratory grunt (3/3). It was observed that with increasing surfactant maturation, there was a decrease in the incidence and severity of R.D. (Table 4). The incidence and severity of respiratory distress had a significant inverse correlation with surfactant maturation, reflected by increasing shake bubble grade (r= - 0.546), i.e. immature the surfactant status of a neonate, more would be the incidence and severity of R.D. in the neonate (Table 4).

It was observed that within each gestational age group, there was decrease in the incidence of R.D. with increasing shake bubble grade. Also, with increasing gestational age, there was decrease in the incidence of R.D. and increase in shake bubble grade. But within individual shake bubble grades, incidence of RDS did not vary with gestational age (Table5). Statistically, there was a decrease in the incidence of respiratory distress with increasing gestational age, but in all neonates with a particular shake bubble grade, incidence of respiratory distress did not vary significantly with gestational age (Table 5). All the newborns with birth weight <1000g had shake bubble grade 0 and majority of these had R.D. It was seen that surfactant maturation increased with increasing birth weight. Also, the incidence of R.D. decreased with increasing birth weight (Table 6). Although, there was a decrease in the incidence of respiratory distress with increasing birth weight, in all infants with a particular shake bubble grade, incidence of respiratory distress did not vary significantly with birth weight except in 2+ shake bubble grade, where with increasing birth weight, there was a decrease in the incidence of R.D (Table 6). All the newborns with birth asphyxia had R.D. Majority of them had shake bubble grade 0 and 1+. Within shake bubble grade 1+ and 2+, incidence and severity of respiratory distress appeared to be more in newborns with birth asphyxia than those without birth asphyxia. Incidence and severity of R.D. decreased with increasing S.B. grade, regardless of the presence or absence of birth asphyxia (Table 7). Statistically, it was revealed that incidence and severity of respiratory distress of a neonate varied significantly with birth asphyxia. However when the evaluation was done for individual shake bubble grades, this association was statistically significant for 1+ and 2+ (Table 7).

There were 31 neonates in the study group who developed distress and maximum 29 (93.6%) developed distress at birth. Only 1 (3.2%) neonate developed distress between 2nd and 4th hour of life while 1 (3.2%) developed distress between 4th and 6th hour of life. Clearly, maximum newborns developed distress at birth (Table 8). Statistical evaluation confirmed that majority of the neonates had R.D. at birth (p<0.001).

Table 1: Distribution of neonates as per gestational age

Gestational age (wks)	Total (n) (% of total)	Female (n) (% within gestation)	Male (n) (% within gestation)
≤28	6 (6%)	5 (83.3%)	1 (16.6%)
29-30	10 (10%)	4 (40%)	6 (60%)
31-32	29 (29%)	11 (37.9%)	18 (62.1%)
33-34	25 (25%)	14 (56%)	11 (44%)
≥ 35	30 (30%)	15 (50%)	15 (50%)
Total	100 (100%)	49 (49%)	51 (51%)

Statistical Analysis

Comparison Group	Pearson Chi Square Value	df	p-value
Distribution of neonates as per gestational age	25	4	<0.0001
Distribution of male and female neonates as per gestational age	5.078	4	0.279

Table 2: Distribution of neonates as per grade of birth asphyxia

Gestational Age (wks)	Total (% within gestation)	No (% within gestation)	Mild (% within gestation)	Moderate (% within gestation)	Severe (% within gestation)
≤28	6 (100%)	5 (83.3%)	0 (0%)	0 (0%)	1 (16.7%)
29-30	10 (100%)	7 (70%)	1 (10%)	1 (10%)	1 (10%)
31-32	29 (100%)	22 (75.9%)	2 (6.9%)	4 (13.8%)	1 (3.4%)
33-34	25 (100%)	23 (92%)	0 (0%)	0 (0%)	2 (8%)
≥35	30 (100%)	27 (90%)	1 (3.3%)	0 (0%)	2 (6.7%)
Total	100(100%)	84 (84%)	4 (4%)	5 (5%)	7 (7%)

Statistical Analysis

Pearson Chi square value	df	p value
13.090	12	0.363

Table 3: Surfactant maturation in neonates as per gestational age

Gestational Age (wks)	0 (% within gestation)	1+ (% within gestation)	2+ (% within gestation)	3+ (% within gestation)	4+ (within gestation)	Total (% within gestation)
≤28	4 (66.7%)	1 (16.7%)	1 (16.7%)	0 (0%)	0 (0%)	6 (100%)
29-30	2 (20%)	6 (60%)	2 (20%)	0 (0%)	0 (0%)	10 (100%)
31-32	0 (0%)	11 (37.9%)	18 (62.1%)	0 (0%)	0 (0%)	29 (100%)
33-34	0 (0%)	6 (24%)	12 (48%)	7 (28%)	0 (0%)	25 (100%)
≥35	0 (0%)	5 (16.7%)	12 (40%)	12 (40%)	1 (3.3%)	30 (100%)
Total	6 (6%)	29 (29%)	45 (45%)	19 (19%)	1 (1%)	100 (100%)

Statistical analysis

Pearson chi square value	df	p-value
74.8	16	0.0001

Table 4: Respiratory status (R.D.) in neonates as per surfactant maturation

Shake bubble Grade	No. of neonates (n)	R.D. (-) (% within grade)	R.D. (+) (% within grade)	Severity of R.D.		
				1/3 (% within grade)	2/3 (% within grade)	3/3 (% within grade)
0	6	1 (16.6%)	5 (83.3%)	0 (0%)	0 (0%)	5 (83.3%)
1+	29	14 (48.2%)	15 (51.7%)	0 (0%)	0 (0%)	15 (51.7%)
2+	45	35 (77.8%)	10 (22.2%)	0 (0%)	10 (22.2%)	0 (0%)
3+	19	18 (94.7%)	1 (5.3%)	1 (5.3%)	0 (0%)	0 (0%)
4+	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	100	69 (69%)	31 (31%)	1 (1%)	10 (10%)	20 (20%)

Statistical analysis

Pearson chi square value	df	p value
62.764	12	<0.001

Table 5: Respiratory Status (R.D.) in Relation to Surfactant Maturation as Per Gestational Age

Shake Bubble grade	No. of Cases (n)	≤28 wks (% within Gestation and S.B. Grade)		29-30 wks (% within Gestation and S.B. Grade)		31-32 wks (% within Gestation and S.B. Grade)		33-34 wks (% within Gestation and S.B. Grade)	≥35 wks (% within Gestation and S.B. Grade)		
		R.D. (+)	R.D. (-)	R.D. (+)	R.D. (-)	R.D. (+)	R.D. (-)	R.D. (+)	R.D. (-)	R.D. (+)	R.D. (-)
0	6	4 (100%)	0 (0%)	1 (50%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
1+	29	1 (100%)	0 (0%)	3 (50%)	3 (50%)	5 (45.5%)	6 (54.5%)	3 (50%)	3 (50%)	3 (60%)	2 (40%)
2+	45	1 (100%)	0 (0%)	1 (50%)	1 (50%)	4 (22.2%)	14 (77.8%)	2 (16.7%)	10 (83.3%)	2 (16.7%)	10 (83.3%)
3+	19	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (14.3%)	6 (85.7%)	0 (0%)	12 (100%)
4+	1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Total	100	6 (100%)	0 (0%)	5 (50%)	5 (50%)	9 (31.0%)	20 (69.0%)	6 (24%)	19 (76%)	5 (16.7%)	25 (83.3%)

Statistical analysis

Comparison Group	Pearson chi square value	Df	p- value
Incidence of R.D. with gestational age	18.497	4	0.001

Comparison of incidence of R.D. with gestational age in individual shake bubble grades	Shake Bubble Grade	p-value
	0	0.333
	1+	0.868
	2+	0.306
	3+	0.368
	4+	-

Table 6: Respiratory Status (R.D.) in Relation to Surfactant Maturation as Per Birth Weight

Shake bubble grade	No. of cases (n)	<1000g (% within Birth Weight and S.B. Grade)	1000-1499g (% within Birth Weight and S.B. Grade)	1500-1999g (% within Birth Weight and S.B. Grade)	2000-2499g (% within Birth Weight and S.B. Grade)		≥2500g (% within Birth Weight and S.B. Grade)				
		R.D. (+)	R.D. (-)	R.D. (+)	R.D. (-)	R.D. (+)	R.D. (-)	R.D. (+)	R.D. (-)	R.D. (+)	R.D. (-)
0	6	3 (100%)	0 (0%)	2 (66.7%)	1 (33.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
1+	29	0 (0%)	1 (100%)	5 (33.3%)	10 (66.7%)	9 (75%)	3 (25%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)
2+	45	0 (0%)	0 (0%)	7 (77.8%)	2 (22.2%)	2 7.2%)	26 (92.8%)	1 (14.3%)	6 (85.7%)	0 (0%)	1 (100%)
3+	19	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	7 100%)	1 (11.1%)	8 (88.9%)	0 (0%)	3 (100%)
4+	1	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Total	100	3 (75%)	1 (25%)	14 (51.6%)	13 (48.4%)	11 (23.4%)	36 (76.6%)	3 (17.6%)	14 (82.3%)	0 (0%)	5 (100%)

Statistical Analysis

Comparison Group	Pearson chi square value	df	p-value
Incidence of R.D. with birth weight	14.040	4	0.007

Comparison of incidence of R.D. with birth weight in individual shake bubble grades	Shake Bubble Grade	p- value
	0	1.000
	1+	0.084
	2+	0.001
	3+	0.556
	4+	-

Table 7: Respiratory status (R.D.) in relation to surfactant maturation as per birth asphyxia

Shake bubble grade		No. of neonates (n)	R.D. (-) (% within B.A. status)	R.D. (+) (% within B.A. status)	Severity of R.D.		
					1/3 (% within B.A. status)	2/3 (% within B.A. status)	3/3 (% within B.A. status)
0	B.A.+	2	0 (0%)	2 (100%)	0 (0%)	0 (0%)	2 (100%)
	B.A. -	4	1 (25%)	3 (75%)	0 (0%)	0 (0%)	3 (75%)
1+	B.A. +	11	0 (0%)	11 (100%)	0 (0%)	0 (0%)	11 (100%)
	B.A. -	18	14 (77.8%)	4 (22.2%)	0 (0%)	0 (0%)	4 (22.2%)
2+	B.A.+	3	0 (0%)	3 (100%)	0 (0%)	3 (100%)	0 (0%)
	B.A. -	42	35 (83.3%)	7 (16.7%)	0 (0%)	7 (16.7%)	0 (0%)
3+	B.A. +	0	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	B.A. -	19	18 (94.7%)	1 (5.3%)	1 (5.3%)	0 (0%)	0 (0%)
4+	B.A. +	0	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	B.A. -	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Statistical analysis

Comparison Group	Pearson chi square value	df	p-value
Comparison of Respiratory status in neonates with and without birth asphyxia	50.521	3	<0.001

Shake Bubble Grade	Pearson chi square value	Df	p- value
0	0.600	**1**	1.000
1+	16.541	**1**	0.001
2+	11.250	**1**	0.008
3+	-	-	-
4+	-	-	-

Table 8: Age at onset of respiratory distress syndrome

Age at onset of R.D. (hrs)	No. of neonates (n)
0	29 (93.6%)
<2	0 (0%)
≥2 - <4	1 (3.2%)
≥4 - <6	1 (3.2%)
Total	31 (100%)

Statistical analysis

Comparison group	p – value
Distribution of neonates on the basis of age at onset of R.D.	<0.001

Discussion

The present study was done on 100 preterm newborns whose surfactant maturation was estimated by Shake Bubble Stability Test and respiratory status was determined. Every newborn was observed for a period of six hours for the possible development of respiratory distress. The aim of the present study was to depict the respiratory status in preterm babies in relation to surfactant maturation.

Efforts were made to collect more evidence to appraise ourselves about the various factors affecting surfactant maturation and respiratory status of preterm babies. It is well known that there are numerous perinatal factors that affect the respiratory outcome of a preterm newborn. The present study evaluated some of these factors.

Surfactant Maturation and Gestational Age

The present study revealed that surfactant maturation varied significantly with gestational age i.e. lesser the gestational age of a newborn, immature would be its surfactant (r=0.650, p=0.0001) (Table 3).

The present study was consistent with that of Edwards and Baillie [12] (1973), who assessed surfactant maturity by performing bubble test on uncontaminated amniotic liquor specimens. The results were interpreted as 0 to 4+ and analysis revealed increasing surfactant maturity with increasing gestational age.

Barreiro *et al* [13] (1981) also found a statistically significant correlation between Clement's test (shake test) and gestational age, as in the present study.

Betz *et al* [14] (1992) estimated the amount of surfactant-producing alveolar type II cells in human fetal lung using polyclonal antibodies against apoprotein B and C of human pulmonary surfactant. An age-dependent increase in the number of surfactant producing type II alveolar cells was found between the 22nd and 29th week of gestation, implying an increase in the surfactant maturation with increasing gestational age, as in the present study.

Respiratory status of newborn and Surfactant maturation

The incidence and severity of respiratory distress varied significantly with surfactant maturation i.e. immature the surfactant status of a newborn, more would be the incidence and severity of R.D (r= - 0.546, p<0.001) (Table 4).

Edwards and Baillie [12] (1973) estimated surfactant maturation by bubble test and results were interpreted as negative and positive. The positive results were graded from 1+ to 4+. None of the neonates with positive tests developed HMD (27.7% developed HMD in the present study), while 50% of the neonates with negative tests developed HMD (83.3% in the present study) (Table 4), thus implying increased incidence of RDS with decreased surfactant maturation, as depicted in the present study. The differences in the incidence of HMD in the study conducted by Edwards and Baillie [12] (1973) and the present study could be because the specimens in the former were obtained from patients with gestational age ranging from 28 – 42 weeks while the present study was conducted exclusively on neonates with gestational age < 37 weeks.

Gunston and Davey[15] (1978) conducted bubble test on amniotic fluid samples and found that no newborn developed RDS when the bubble score was 2+ or more, 10 of 66 patients with a bubble score of 0 or 1 + developed HMD. The incidence of RDS in the present study was 10 of 45 in newborns with SBG 2+ or more, 20 of 35 in newborns with SBG 0 or 1+ (Table 4). Thus both the present study and that of Gunston and Davey [15] (1978) showed similar trend of increasing incidence of RDS with decreasing surfactant maturation.

Parekh *et al* [16] (1983) in their study found that 85% of preterm neonates with negative shake test developed RDS (p<0.001), comparable with the figure of 83.3% in the present study (Table 4).

Vermeulen *et al* [17] (1979) performed gastric aspirate foam test in the prediction of HMD in LBW infants. In 59 of 61(96.7%) infants with no respiratory distress, the test was positive (The figure in the present study was 68 of 69 i.e. 98.6%) (Table4). All infants who developed hyaline membrane disease (HMD) had negative or intermediate foam test results.

Dewhurst *et al* [18] (1973) used "shake" or bubble test as one of the means of surfactant estimation. There was no case of RDS when the shake test was positive in a dilution of 1:4 or more, thereby signifying decreased risk of RDS with increasing surfactant maturation, as observed in the present study.

Mansour *et al* [19] (2009) analyzed gastric aspirates of 81 neonates with gestation less than 34 weeks for Gastric Aspirate Shake Test (GAST). Results were interpreted as negative, intermediate or positive. All infants who developed HMD had negative or intermediate test results, thereby emphasizing the relation between surfactant deficiency and RDS, as depicted in the present study.

Chida *et al* [20] (1993), Verder *et al* [21] (2003) and Fiori *et al* [22] (2004) used stable microbubble test for surfactant estimation and emphasized the increased risk of RDS with immature levels of surfactant, as observed in the present study.

McElrath *et al* [23] (2004) (used amniotic fluid surfactant-to-albumin ratio for assessing surfactant maturity) and Daniel *et al* [24] (2010) (used LBC and SMT for assessing surfactant maturity) revealed that immature the surfactant status, more would be the risk of RDS, as observed in the present study.

Respiratory status of newborn and Gestational age

There was a decrease in the incidence of respiratory distress with increasing gestational age (p =0.001), i.e. incidence was 100% in neonates with ≤28 wks, 50% in 29-30 wks, 31% in 31-32 wks, 24% in 33-34 wks and 16.7% in ≥ 35 wks. But incidence of RDS within a particular shake bubble grade did not vary significantly with gestational age (p=0.333 for SBG 0, p=0.868 for SBG 1+, p=0.306 for SBG 2+ and 0.368 for SBG 3+) (Table 5). The present study revealed that there was a significant inverse correlation between incidence and severity of RD and gestational age (r= - 0.392).

Respiratory status was assessed in the following studies as per gestational age in relation to surfactant maturation, but maturity of surfactant was assessed by methods other than Shake Bubble Test.

McElrath *et al* [23] (2004) assessed neonatal respiratory distress syndrome as a function of gestational age and surfactant to albumin ratio. Neonatal RDS was diagnosed by the criteria of the presence of 2 or more of the following: evidence of respiratory compromise shortly after delivery and a persistent oxygen requirement for more than 24 hours, administration of exogenous pulmonary surfactant, and/or radiographic evidence of hyaline membrane disease. They reported both gestational age and surfactant to albumin ratio to be independent predictors of RDS and found that with increasing gestation, surfactant maturation increases and lesser is the chance of RDS, as depicted in the present study. Similar results were reported by St. Clair *et al* [25] (2008) who attempted to assess the probability of neonatal respiratory distress syndrome as a function of gestational age and surfactant maturity by using lecithin/sphingomyelin ratio.

Ashton *et al* [26] (1992) estimated phosphatidylcholine (surfactant) composition of endotracheal aspirates in intubated neonates with RDS as a measure of surfactant maturity and found that neonates with a DPPC: POPC ratio less than 3.0 developed RDS irrespective of gestational age, thereby signifying that in neonates with a particular surfactant maturity level, incidence of RDS did not vary with gestational age, as observed in the present study.

Parvin *et al* [27] (2005) predicted RDS using gestational age and fetal lung maturity by fluorescent polarization based TDx FLM II. They found that chances of RDS decrease 31% for each increasing week of GA and decrease 67% for each 10 mg/g increase in the TDx FLM II ratio, thereby implying decreased chances of RDS with increasing gestational age and increasing surfactant maturity, as observed in the present study. Within a gestational age, increasing surfactant (TDx FLM II ratio) decreased the chances of RDS, in agreement with the observations in the present study. Karcher *et al* [28] (2005) also found similar results when they attempted to derive GA specific predicted risk of RDS using lamellar body count and surfactant to albumin ratio in amniotic fluid.

The results of the present study were consistent with the results of the following studies, although the risk of RDS as per gestational age was estimated in these studies without assessing surfactant maturity.

Harms *et al* [29] (1997) retrospectively analyzed 1109 premature newborns with birth weight < 1500g and RDS was assumed if the infants needed mechanical ventilation with oxygen supplementation and the typical radiological signs were present on chest x-ray. They found that at ≤ 28 weeks gestational age 90% of infants suffered from RDS (55% severe RDS grade III or IV). The incidence was 75% (grade III or IV: 32%) for infants born at 29 and 30 weeks, 48% (grade III or IV: 15%) at 31 and 32 weeks and 33% (grade III or IV: 6%) for neonates born at 33 weeks of gestation.

Reed *et al* [30] (1978) and Bryan *et al* [31] (1990) ($p<0.0001$) found increased risk of RDS with decreasing gestational age. Wach *et al* [32] (1994) found a neonate with gestation less than 30 weeks to be at a higher risk of RDS. Fidanovski *et al* [33] (2005) tried to decipher the relation between perinatal characteristics, disease severity and outcome in premature infants with clinical and radiological signs of RDS requiring mechanical ventilation. Shorter gestational age was found to be associated with increased severity and mortality. Hack *et al* [34] (1991) found increased severity of RDS and requirement for ventilation as well as increased mortality among lower gestational age newborns. Madar *et al* [35] (1999) found that incidence of RDS requiring ventilator support decreased with increasing gestational age

Brus *et al* [36] (1999) conducted a study on 26 preterm neonates who were diagnosed as cases of RDS on the basis of clinical and radiological criteria. They found that the severity of RDS was independent of gestational age of the neonate. The dissimilar results might be due to the fact that the inclusion criteria was different (only neonates with gestational age 27-33 wks having no evidence of infection upto 3 days after study completion were included) and surfactant maturation was not assessed, as done in the present study.

Respiratory status of newborn and birth weight

The present study revealed that the incidence of respiratory distress decreased with increasing birth weight ($p=0.007$), but in all neonates with a particular shake bubble grade, incidence of respiratory distress did not vary significantly with birth weight ($p=1.000$ for SBG 0, $p=0.084$ for SBG 1+, $p=0.556$ for SBG 3+) except in 2+ shake bubble grade ($p=0.001$) (Table 6). The present study revealed that there was a significant inverse correlation between incidence and severity of RD and birth weight ($r= - 0.419$).

The results of the present study were consistent with the results of the following studies, although risk of RDS as per birth weight was estimated in these studies without assessing surfactant maturity. Studies assessing incidence and severity of RDS as per birth weight in relation to surfactant maturation could not be traced in the literature.

As depicted in the present study, Hack *et al* [34] (1991) found increased severity of RDS and requirement for ventilation among lower birth weight newborns. Morbidity increased with decreasing birth weight. Fidanovski *et al* [33] (2005) tried to decipher the relation between perinatal characteristics and disease severity in premature newborns with clinical and radiological signs of RDS requiring mechanical ventilation. Lower birth weight was found to be associated with increased severity, as observed in the present study. Reed *et al*[30] (1978) found low birth weight to be a major risk factor for RDS in their study depicting epidemiology of RDS, as observed in the present study.

In contrast to the present study, Brus *et al* [36] (1999), however, found that the severity of RDS was independent of birth weight of the neonate. The dissimilar results might be due to the fact that the inclusion criteria was different (only neonates with gestational age 27-33 wks having no evidence of infection upto 3 days after study completion were included) and surfactant maturation was not assessed, as done in the present study.

Respiratory status of newborn and Birth asphyxia

The present study found that there was a significant correlation between respiratory status of a neonate and birth asphyxia ($r=0.665$, $p<0.001$). However when the evaluation was done for individual shake bubble grades, incidence of RDS was more in neonates with birth asphyxia than those without birth asphyxia, in shake bubble grades 1+ and 2+ ($p=1.000$ for SBG 0, $p=0.001$ for SBG 1+, $p=0.008$ for SBG 2+) (Table 7).

The risk of RDS was estimated in the following studies as per birth asphyxia without assessing surfactant maturity. Studies assessing incidence and severity of RDS as per birth asphyxia in relation to surfactant maturation could not be traced in the literature.

Harms *et al*[29] (1997) conducted a study in which RDS was assumed to be present in a newborn if it needed mechanical ventilation with oxygen supplementation and the typical radiological signs were present on chest x-ray. They found that APGAR score at 5 min < 7 increased the risk of RDS significantly ($p < 0.05$), thereby signifying association of RDS and birth asphyxia, as depicted in the study.

Brus *et al* [36] (1999), who assessed preterm newborns and prospectively classified them as newborns with mild, moderate and severe RDS according to the clinical and radiologic criteria. They found that lower APGAR scores both at one and five minutes were significant •31% of the study neonates had RD and the incidence of respiratory distress varied significantly with shake bubble grades (p=0.0001) i.e. the immature the surfactant status of a newborn, the more likely it is to suffer from respiratory distress.

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