RESEARCH PAPER

Bronchopulmonary Dysplasia in Preterm Neonates in a Level III Neonatal Unit in India

SAVITA BHUNWAL[1], KANYA MUKHOPADHYAY[1], SHALMOLI BHATTACHARYA[2], PRANAB DEY[3] AND LAKHBIR KAUR DHALIWAL[4]

From Departments of [1]Pediatrics, [2]Biophysics, [3]Cytology, and [4]Obstetrics and Gynecology, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

Correspondence to: Dr Kanya Mukhopadhyay, Professor, Neonatology, Department of Pediatrics, PGIMER, Chandigarh 160 012, India. kanyapgi@gmail.com

Received: January 02, 2017; Initial review: April 11,2017; Accepted: November 24, 2017.

Objective: To find out the incidence and associations of bronchopulmonary dysplasia (BPD) in preterm neonates.

Design: Descriptive cohort.

Methods: All consecutively born neonates <33 weeks gestation requiring oxygen or respiratory support during first 3 days of life were enrolled from a level III neonatal unit in Chandigarh, India. Those with malformations were excluded. Placenta was examined for histological chorioamnionitis in preterm rupture of membranes and/or preterm spontaneous onset of labour. Serum Malondialdehyde (MDA) and Superoxide dismutase (SOD) and Catalase levels were estimated on day 3 of life. All recruited neonates were followed up till discharge or death.

Results: Out of 250 neonates enrolled, 170 (68%) survived till day 28 and BPD developed in 19 (11.2%) infants. The mean gestation and birth weight were significantly lower in infants who developed BPD. Chorioamnionitis (clinical 5.3% *vs* 1.9%, *P*=0.375; and histological 37.5% *vs* 16.7%, *P*<0.001), patent ductus arteriosus (PDA) (52.6% *vs* 8.9%, *P*<0.001), median (IQR) sepsis episodes [2 (2,3) *vs* 1 (1,2), *P*<0.001], invasive ventilation (84.2% *vs* 11.3%, *P*<0.001), and duration of ventilation [56 (4) d *vs* 4 (5) d, *P*=0.001] were significantly higher in infants with BPD. Serum MDA, SOD and Catalase levels were comparable between the two groups.

Conclusion: Chorioamnionitis, PDA and sepsis were significantly associated with BPD.

Keywords: *Anti-oxidant enzymes, Chorioamnionitis, Chronic lung disease, Oxidants.*

Published online: December 14, 2017. PII:S097475591600101

Broncho-pulmonary dysplasia (BPD) is one of the most important chronic complications in very preterm neonates [1], especially those born before 28-30 weeks of gestation and weighing ≤1000 grams [2]. Ehrenkranz, *et al*. [3] validated the consensus definition of BPD in a cohort of preterm (<32 weeks) extremely low birth weight (ELBW) infants alive at 36 weeks of post menstrual age (PMA) and reported an incidence of 44%, as against the older criteria [3]. BPD decreases with increasing gestational age and birth weight with the highest incidence at lower extremes of birth weight and gestation age [3,4].

In India, with the increasing availability of surfactant and intensive care, survival at lower gestational age is steadily increasing; however, data regarding the incidence and risk factors of BPD are scarce [5,6]. This study was planned to generate recent data on incidence and risk factors of BPD in infants <33 weeks of gestation.

METHODS

This study was carried out at a level III neonatal unit in Chandigarh, India between July 2012 and June 2013 after clearance by Institutional research ethics committee. All consecutively born infants <33 weeks gestational age, who received any form of respiratory support (oxygen by hood/nasal cannula or continuous positive airway pressure (CPAP) or non-invasive mechanical ventilation (NIMV) or mechanical ventilation (MV) within first 3 days of life were prospectively enrolled. Infants with congenital malformations (including congenital heart disease other than PDA) were excluded. Written informed consent was obtained from parents. Gestational age was based on maternal last menstrual period and postnatally by New Ballard Score [7]. Neonates were followed up till discharge or death during hospital stay.

BPD was defined based on the criterion of receiving oxygen therapy of >21% for 28 days in those who

received initial respiratory support. Severity of BPD was assessed at 36 weeks PCA. Mild BPD was defined as a need for supplemental oxygen (O2) for ≥28 days but not at 36 weeks postmenstrual age (PMA) or discharge, moderate BPD as need for ≤30% O2 at 36 weeks PMA and severe BPD as need for ≥30% O2 (CPAP, HFNC, and/or positive pressure) at 36 weeks PMA [3]. No baby was discharged on home oxygen.

In cases of preterm rupture of membrane (PTROM) and/or preterm spontaneous onset of labor at <33 weeks of gestation, where infants developed respiratory distress soon after birth, placenta was examined by a histopathologist to look for any evidence of Chorio-amnionitis. Clinical chorioamnionitis was diagnosed in the setting of maternal fever (≥100.4ºF/38° C) and at least two of the following: maternal leukocytosis (>15,000 cells/ mm^3), maternal tachycardia (>100 bpm), fetal tachycardia (>160 bpm), uterine tenderness, stained or foul smelling amniotic fluid. Cases of maternal upper respiratory infection and urinary tract infection were excluded [11]. Histological chorioa-mnionitis was diagnosed in presence of amniotropic infiltration by both maternal and fetal neutrophils in the chorioamniotic membranes and the umbilical cord [12]. Pneumonia was diagnosed if there was respiratory distress, in the presence of a positive blood culture or if any two of the following were present - existing or predisposing factors like maternal fever, foul smelling liquor, prolonged rupture of membranes or gastric polymorphs more than 5 per high power field, Clinical picture of septicemia (poor feeding, lethargy, poor reflexes, hypo- or hyper-thermia, abdominal distension etc.), *X*-ray suggestive of pneumonia, and positive septic screen. Sepsis was defined as either blood culture positive sepsis, or suspect sepsis if septic screen was positive in presence of clinical features but blood culture negative. All antenatal details including clinical chorioa-mnionitis, demographic characteristics and morbidities including details of ventilation were recorded.

Serum Malondialdehyde (MDA) and antioxidant enzymes (Superoxide dismutase (SOD) and Catalase) were estimated on day 3 of life in all infants. MDA level was measured as described by Ohkawa (1979), *et al.* [8], SOD was measured by method reported by Kono, *et al.* [9], and Catalase was estimated by method described Luck, *et al.* [10].

Each year in our institution, nearly 600-700 infants are born at <33 weeks gestation, of which approximately 50% receive respiratory support, with average survival of about 63% [6]. Hence it was expected 250-300 infants could be enrolled in this study with about 160-190 infants surviving.

Statistical analysis was performed using SPSS version 18.0. Comparisons of demographic characteristics and co-morbidities were made using Student's *t* test or chi square test, as appropriate in two groups (BPD *vs* No BPD).

RESULTS

A total of 670 infants were born at <33 weeks of gestation, of which 350 infants required respiratory support within the first 72 hours of life (***Fig.* 1**). Demographic details of the study population is described in ***Table* I**.

Of the 250 infants enrolled, 170 (68%) survived till day 28 of life and 19 (11.2%) developed BPD. Severity of BPD could be assessed in 16 infants as one baby died and two infants were off oxygen at 36 weeks PCA. Amongst all infants <33 weeks gestation born during the study period (*n*=670) who survived till 28 days, BPD incidence was 3.9%.

Infants with BPD were lesser in gestation, lighter at birth, more likely to be growth retarded, and had a higher occurrence of chorioamnionitis and PTROM (***Table* II**). When stratified by gestation, BPD was more frequent in infants <28 weeks (7 out of 16) than those between 28-30 weeks (10 out of 70). Similarly, infants weighing <1 kg had a higher incidence of BPD than those between 1-1.5 kg (8 out of 34 *vs* 9 out of 91).

Infants with BPD had a significantly higher prevalence of PDA, pneumonia, sepsis, need for blood transfusions, and need for invasive ventilation (***Table* III**).

There was no difference in the levels of MDA and antioxidant enzymes (Catalase and SOD) in those with and without BPD (***Fig.* 2**).

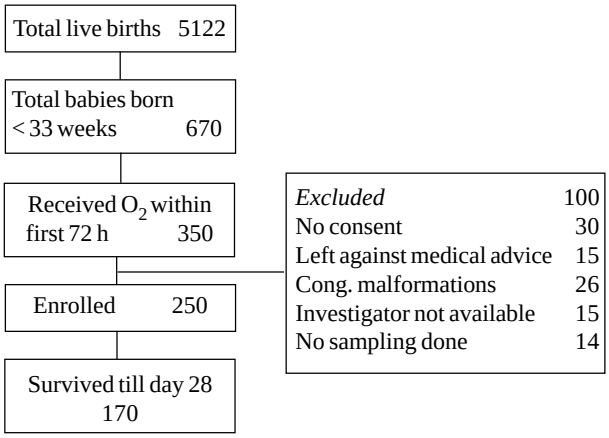

FIG. 1 *Flow of patients.*

TABLE I DEMOGRAPHIC AND MORBIDITY CHARACTERISTICS OF THE STUDY PARTICIPANTS (*N*=250)

Characteristic	*Value*
#Gestational age (wk)	29 (2.1)
Gestational age categories	
<28 wk	41 (16.6)
28 to 30 wk	111 (44.4)
31 to 32 wk	98 (39.4)
#Birthweight (g)	1203 (335)
Birthweight categories	
<1 kg	74 (29.6)
1-1.5 kg	124 (49.6)
>1.5 kg	52 (20.8)
SGA	82 (32.8)
Male gender	147 (58.8)
Mechanical Ventilation	33 (13.2)
Only CPAP	110 (44)
Suspect sepsis	203 (81.2)
Culture positive sepsis	31 (12.4)
PDA (ECHO-proven)	29/224 (12.9)
ROP (*n*=106)	61 (57.5)

Values expressed as n (%) or #Mean(SD).

DISCUSSION

In the present study 11.2% preterm neonates (<33 wk gestation) with respiratory distress developed BPD with a higher incidence in infants <1 kg and <28 weeks gestation. PTROM, histological chorioamnionitis, pneumonia, sepsis, mechanical ventilation, PDA were present in higher proportion in BPD infants along with longer duration of ventilation.

The limitations of our study are small sample size due to time constraints, and lower rates of survival at extremes of gestation due to infrastructure limitations.

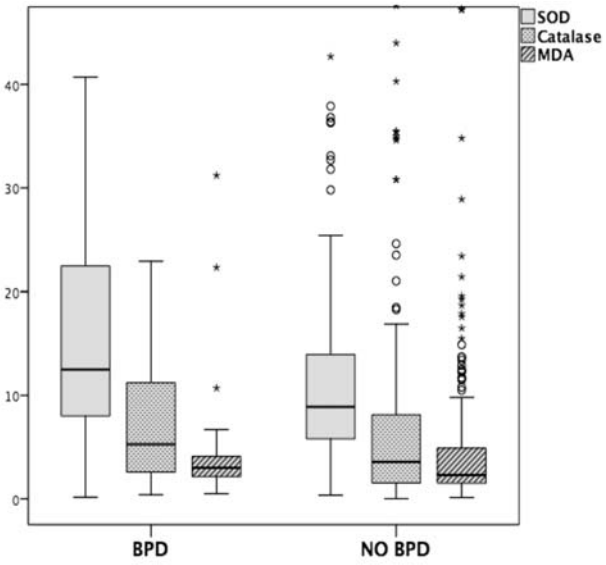

FIG. 2 *Box and Whisker plot showing Superoxide dismutase (SOD), Catalase and Malondialdehyde (MDA) levels in infants with or without bronchopulmonary dysplasia (BPD) on day 3 of life.*

There has been an increase in incidence of BPD (from 5.8% to 11.5%) in the past decade in our institution [5,6]. Narang, *et al*. [5] reported an incidence of BPD of 28.7%, 10.7% and 1.7% in infants less than 28 weeks, 29-30 weeks and 31- 32 weeks, respectively, and 50%, 8.1% and 2.3% in infants with birthweight <1000 g, 1000-1249 g and 1250-1499 g, respectively [5]. Ehrenkranz, *et al*. [3] reported BPD incidence of 52% in infants with birthweight 501 to 750 g, 34% in infants weighing 750-1000 g, and 15% in infants weighing between 1001-1200 g while it was 7% in infants weighing 1201-1500 grams. We observed a much lower incidence of BPD at corresponding birthweights probably due to higher gestation as well as lower rate of survival at lower gestation. Higher proportion of BPD in SGA infants as compared to AGA infants can probably be explained by significantly lower gestational age of

TABLE II DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF INFANTS WITH AND WITHOUT BRONCHOPULMONARY DYSPLASIA

Variable	*BPD, n=19*	*No BPD, n=151*	*P value*	*RR/RD (95% CI)*
#Birth weight (g)	1090 (312)	1305 (304)	0.004	- 215 (-34 , -378)
#Gestation (wk)	9 (47.4%)	35 (23.2%)	0.023	2.58 (1.12, 5.93)
Antenatal steroid	11 (57.9%)	80 (52.98%)	0.686	1.19 (0.51, 2.82)
Clinical chorioamnionitis	1 (5.3%)	3 (1.99%)	0.375	2.31 (0.40, 13.3)
Histological chorioamnionitis	6/16 (37.5%)	10/60 (16.7%)	<0.001	5.06 (1.63, 15.76)
PTROM	12 (63.2%)	51(33.8%)	<0.001	2.91 (1.21, 7.01)

Values are expressed as n(%) or #mean(SD); BPD: bronchopulmonary dysplasia; SGA: Small for gestational age, PTROM: Preterm prolonged rupture of membrane.

TABLE III COMPARISON OF CO MORBIDITIES IN INFANTS WITH OR WITHOUT BRONCHOPULMONARY DYSPLASIA

Variables	*BPD (n=19)*	*No BPD (n=151)*	*P value*
HMD	11 (57.9)	75 (49.7)	0.499
Surfactant	14 (73.6)	59 (39.1)	0.004
PDA	10 (52.6)	11 (7.3)	<0.001
Pneumonia	4 (21)	10 (6.6)	0.031
#Sepsis episodes	2 (2-3)	1 (1-2)	<0.001
Sepsis (All)	19 (100)	116 (76.8)	<0.001
Culture-positive sepsis	11 (57.9)	36 (23.8)	0.002
Blood transfusion	16 (84)	22 (14.5)	<0.001
CPAP within 48 h (*n*=144)	18 (94.7)	126 (83.4)	0.197
Invasive ventilation at 48 h	8 (42.1)	9 (6)	<0.001
Mechanical ventilation	16 (84.2)	17 (11.3)	<0.001
#Duration of ventilation (d)	48 (37-60)	2 (1-6)	<0.001
ROP (All stages)	19/19 (100)	42/87 (48.3)	<0.001
#Hospital stay (d)	56 (50-76)	22 (14-35)	<0.001

All values in n(%), except #Median (IQR); BPD – Bronchopulmonary dysplasia; HMD – Hyaline membrane disease; PDA – Patent Ductus Arteriosus; CPAP – Continuous Positive Airway Pressure; ROP – Retinopathy of Prematurity.

SGA infants in our cohort which was also observed in our previous study [6]. The overall gestational age of our BPD infants is higher (mean 28.3 weeks) than those reported in Western literature [3]. BPD developing at higher gestation in our set-up may be due to higher incidence of sepsis and related factors rather than prematurity alone as compared to Western data. Reports of BPD from developing countries are infrequent. Ho and Chang [13] from Malaysia reported an incidence of 3.3% BPD in their VLBW cohort of 2003 infants (mean gestation 29.5 wk), of whom 72% infants were ventilated. A recent report of Malaysian national neonatal registry reported a similar incidence of BPD (8-11.7%) in their VLBW cohort [14], as in the present study.

Gagliardi, *et al*. [15] reported 15.9% incidence of BPD in a cohort of 23-32 weeks and <1500 grams infants and observed mechanical ventilation, greater severity of illness as measured by Clinical Risk Index for Infants (CRIB) score, and PDA to be significantly associated risk factors for BPD. Use of antenatal steroids had no independent effect on BPD. These findings are similar to the findings of the present study. There are conflicting reports of use of continuous positive airway pressure (CPAP) and reduction of BPD. A Cochrane systematic review showed no difference in BPD defined as oxygen dependency in preterm neonates [16]. Boo, *et al.* [14] noted that CPAP significantly reduced BPD among survivors in a cohort of VLBW infants in Malaysia. Narang, *et al.* [5] also reported higher incidence of BPD in ventilated infants and who also received higher oxygen concentration. PTROM and histological chorioamnionitis was detected more amongst BPD infants in the present study. A recent large cohort study did not find any association of BPD and chorioamnionitis [17]. Sepsis and pneumonia were significantly higher in infants with CLD, as also reported in a previous study [5].

Though free radicals play a role in BPD pathogenesis, the present study did not find elevated levels of free radicals or deficiency of anti-oxidant enzymes in the BPD infants. These are similar to the observations of Ryan, *et al.* [18], where only pulmonary concentrations of free radical product malondialdehyde was noted to be elevated but this elevation was weakly correlated with the development of BPD. The probable explanation may be that these oxidant biomarkers are elevated in other conditions like sepsis or pneumonia and the oxidative injury is only one amongst the multiple factors that plays a role in development of BPD.

With improving neonatal survival in our country, we may experience more and more children with BPD. Recognition of country-specific risk factors like sepsis, pneumonia, PDA, chorioamnionitis may help us to reduce the incidence of BPD. Adequate infrastructure will be required for optimum long-term management of these infants.

WHAT IS ALREADY KNOWN?

- Extreme prematurity, PDA, prolonged ventilation, and invasive ventilation are common risk factors for BPD.

WHAT THIS STUDY ADDS?

- BPD occurs in higher gestational age and birthweight infants in India; chorioamnionitis, sepsis and pneumonia are commonly associated.

Contributors: SB: prepared the protocol, enrolled patients, collected and analyzed the data and drafted the manuscript; KM: conceptualized and designed the study, supervised data collection and analysis, and critically revised the manuscript; SB: conducted biochemical analysis and reviewed the manuscript. PD: Conducted the histopathological examination of placenta. LKD: Helped in data collection and reviewed the manuscript.
Funding: None. *Competing interests:* None stated.

REFERENCES

1. Fanaroff AA, Wright LL, Stevenson DK, Shankaran S, Donovan EF, Ehrenkranz RA, *et al*. Very low birth weight outcomes of the National Institute of Child Health and Human Development Neonatal Research Network, May 1991 through December 1992. Am J Obstet Gynecol. 1995;173:1423-31.
2. Lemons JA, Bauer CR, Oh W, Korones SB, Papile LA, Stoll BJ, *et al.* Very low birth weight outcomes of the National Institute of Child Health and Human Development Neonatal Research Network, January 1995 through December 1996. NICHD Neonatal Research Network. Pediatrics. 2001;107:E1.
3. Ehrenkranz RA, Walsh MC, Vohr BR, Jobe AH, Wright LL, Fanaroff AA, *et al*. National Institutes of Child Health and Human Development Neonatal Research Network. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. Pediatrics. 2005;116:1353-60.
4. Kinsella J, Greenough A, Abman SA. Bronchopulmonary dysplasia. Lancet. 2006;367:1421-31.
5. Narang A, Kumar P, Kumar R. Chronic lung disease in neonates: emerging problem in India. Indian Pediatr. 2002;39:158-62.
6. Mukhopadhyay K, Louis D, Murki S, Mahajan R, Dogra MR, Kumar P. Survival and morbidity among two cohorts of extremely low birth weight neonates from a tertiary hospital in northern India. Indian Pediatr. 2013;50: 1047-50.
7. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score expanded to include extremely premature infants. J Pediatr. 1991;119:417-23.
8. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Ann Biochem. 1979;95:351-8.
9. Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys. 1978;186:189-95.
10. Luck H. Catalase, method of enzymatic analysis. In: Bermeyer HO, editor. London; New York: Academic Press. 1971. p. 855-93.
11. Newton ER. Chorioamnionitis and intraamniotic infection. Clin Obstet Gynecol. 1993;36:795-808.
12. Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol. 2003;6:435-48.
13. Ho JJ, Chang AS. Changes in the process of care and outcome over a 10 year period in a neonatal nursery in a developing country. J Trop Pediatr. 2007;53:232-7.
14. Boo NY, Cheah IG, Neoh SH, Chee SC. Malaysian National Neonatal Registry. Impact and challenges of early continuous positive airway pressure therapy for very low birth weight neonates in a developing country. Neonatology. 2016;110:116-24.
15. Gagliardi L, Bellù R, Rusconi F, Merazzi D, Mosca F. Antenatal steroids and risk of bronchopulmonary dysplasia: a lack of effect or a case of over-adjustment. Paediatr Perinat Epidemiol. 2007;21:347-53.
16. Ho JJ, Subramaniam P, Davis PG. Continuous distending pressure for respiratory distress in preterm infants. Cochrane Database Syst Rev. 2015;4:CD002271.
17. Ballard AR, Mallett LH, Pruszynski JE, JB Cantey JB. Chorioamnionitis and subsequent bronchopulmonary dysplasia in very-low-birth weight infants: a 25-year cohort. J Perinatol. 2016;36:1045-8.
18. Ryan R, Ahmed Q, Lakshminrusimha S. Inflammatory mediators in the immunology of bronchopulmonary dysplasia. Clinic Rev Allergy Immunol. 2008;34: 174-90.