Risk Factors of Metabolic Bone Disease in Bronchopulmonary Dysplasia Premature Infants With Gestational Age Less Than 32 Weeks

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Research article

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Abstract

Background

Metabolic bone disease (MBD) is a complication of multifactorial aetiology in preterm infants. Several risk factors have been identified in general. Bronchopulmonary Dysplasia (BPD) infants present an increased incidence of MBD, but it is unknown which factors contribute to this. The aim of this study was to determine the risk factors for developing MBD in BPD infants.

Methods

A retrospective review of the medical records of BPD infants admitted to the Neonatal Intensive Care Unit (NICU) at Zhangzhou Hospital between Jun 2016 and May 2020. BPD infants with MBD were identified, two contemporaneous without MBD matched by gestational age and gender were randomly selected as control infants for each case of MBD. The association between putative risk factors and MBD was estimated with ORs and 95% CIs. A *P*-value threshold ≤ 0.2 was used in univariate analysis for inclusion into a multivariate (adjusted) model with a *P*-value of < 0.05 as statistically significant.

Results

A total of 156 BPD infants were enrolled with 52 cases of MBD and 104 controls. Fetal growth restriction (OR 5.60, 95% CI, 1.77–17.72), extremely low birth weight (OR 3.70, 95% CI, 1.35–10.10), feeding volume ≥ 80 mL/kg/day at the end of the 4th week after birth (OR 12.21, 95% CI, 3.89–38.33), cholestasis (OR 4.29, 95% CI, 1.65–11.15), and late onset sepsis (OR 3.79, 95% CI, 1.12–12.77) were found to be statistically significant risk factors for MBD in BPD infants.

Conclusion

In gestational age homogeneous BPD infants, fetal growth restriction, extremely low birth weight, feeding volume ≥ 80 mL/kg/day at the end of the 4th week after birth and late onset sepsis are significant risk factors for MBD. These findings provide potential predictive factors for MBD in BPD infants but still warrant prospective validation.

Introduction

Metabolic Bone Disease (MBD) of prematurity is a multifactorial disorder characterized by low bone mass and demineralization of bone tissue posing a consequent increase in bone fragility and long-term reduced linear growth and childhood or even adult height, which is commonly observed in very low birth weight (VLBW, $<1,500$ g), especially in extremely low birth weight (ELBW, $<1,000$ g) infants with a reported incidence of about 16%–40% [1]. BPD infants have a higher incidence (60% vs 34%, compared with infants without BPD) of MBD in infants $\geq 1,250$ g [2]. Among infants born $\geq 1,500$ g suffered from severe BPD, about one third developed severe MBD [3].

Several factors associated with increased risk of MBD had been identified. Costa R et al found that the majority of MBD infants were male and presented lower gestational age, lower birth weight, and prolonged duration of parenteral nutrition [4]. Chen W et al found that birth at <30 weeks of gestation, vitamin D supplementation at >14 days of age, and achievement of total enteral nutrition (TEN) beyond 28 days of age were independent risk factors for MBD in infants $\geq$ 34 weeks gestational age [5]. Montaner Ramón A et al pointed out only restricted fetal growth was independently associated with the development of severe MBD (OR 9.65, 95% CI 3.48–26.76) in infants $\geq$ 32 weeks gestational age and/or weight $\geq$ 1,500 g [6], and Ukarapong S et al indicated that only cholestasis remained significantly risk factors (OR 9.6, 95% CI 2.1–45.3) in infants <30 weeks gestational age and/or weight <1,000 g [7]. Although BPD infants presented an increased risk for developing MBD, previous studies did not evaluate the risk factors that would be associated with MBD in BPD infants.

The purpose of this study was to assess the risk factors for MBD in BPD infants, which might contribute to identify high-risk individuals and propose further recommendations for management of the comorbidities.

Materials And Methods

Study population and setting

We performed a retrospective review of the medical records of BPD infants admitted to the Neonatal Intensive Care Unit (NICU) at Zhangzhou Hospital between Jun 2016 and May 2020. The study was approved by the Institutional Review Board of the Zhangzhou Hospital, with waiver of informed consent. Cases with MBD were identified as the case group. Inclusion criteria consisted of the following: (1) gestational age $\geq$ 32 weeks, (2) survival up to 36 weeks post-menstrual age (PMA), (3) diagnosis of BPD according to National Institutes of Health (NIH) that defined as requirement of oxygen support (>21%) for at least 28 days and a subsequent assessment at 36 weeks PMA or discharge, whichever comes first. At the time of assessment, infants with no oxygen requirement were classified as having mild BPD, infants requiring <30% oxygen were classified as having moderate BPD and infants with a need for positive pressure ventilation/continuous positive pressure (PPV/CPAP) and/or oxygen requirement $\geq$ 30% were classified as having severe BPD [8], and (4) diagnosis of MBD that defined as peak serum ALP higher than 900 U/L and serum phosphate level lower than 1.8 mmol/L, which yielded a sensitivity of 100% at a specificity of 70%, with or without radiographic changes of long bones [9]. Exclusion criteria included the following: (1) gestational age $\geq$ 32 weeks, (2) presence of significant congenital anomalies, (3) death before 36 weeks PMA. For each MBD case, two contemporaneous with BPD but without MBD matched at equivalent gestational age and gender (male: female=1:1) were randomly selected.

Clinical data on demographics and putative risk factors for MBD were collected, including birth weight, gender, use of antenatal steroids, histologic chorioamnionitis (HCA), fetal growth restriction (FGR), maternal hypertensive disorders without FGR, prolonged rupture of membranes (PROM), type of feeding, initiation of oral vitamin D supplementation, feeding volume at the end of the 4th week after birth,

postnatal dexamethasone application, patent ductus arteriosus (PDA), cholestasis, use of mechanical ventilation, late onset sepsis (LOS), thyroid function tests and platelet (PLT) count.

FGR was defined as antenatal diagnosis by any 2 of the following: estimated fetal weight below the 10th percentile according to the care provider reference curve, abnormal fetal Doppler findings, growth arrest, or maternal hypertensive disorders. PROM was defined as rupture of the membranes occurring earlier than 24 h before delivery [10]. HCA was defined as the infiltration of neutrophils in any of the following structures: placental disc, the chorioamniotic membranes, and the umbilical cord [11]. Cholestasis was defined as [direct bilirubin](#) >2 mg/dL [12]. Maternal hypertensive disorder was defined as systolic blood pressure ≥ 140 mmHg and (or) diastolic blood pressure ≥ 90 mmHg [13]. PDA was diagnosed with echocardiography confirmation. Thyroid-stimulating hormone (TSH) = 10 mU/L was used as the cutoff level for hypothyroidism [14] and $PLT < 100 \times 10^5/L$ was considered to be thrombocytopenia [15]. LOS was diagnosed with clinical suspicion and evaluated by microbial culture or two non-specific blood indicators occurring after 72 h of birth [16, 17].

In our NICU, we started parenteral nutrition in the first hours after birth and introduced enteral feeding with breast milk as soon as the infant reached a tolerated respiratory rate without hemodynamic instability. The amount increased gradually with a rate of 20–30 mL/kg/day without signs of feeding intolerance. Oral vitamin D (800–1000 IU) supplementation was started when the amount of breast milk feed reaches 50 mL/kg/day, and liquid human milk fortification was added when the amount of breast milk feed reaches 80 mL/kg/day. Premature formula was also used if the amount of breast milk is insufficient.

Statistical Analyses

Data were analyzed using SPSS version 26. Dichotomous or categorical variables were compared between cases and controls with the use of χ^2 analysis or the Fisher exact test or z-test. Continuous variables were compared between groups via the Mann-Whitney *U* test. A risk model was derived via logistic regression, with 95% CIs calculated for ORs using the forward LR method. A *P*-value of ≤ 0.2 was used in univariate analysis for inclusion of putative risk factors into the multivariate (adjusted) model. A *P*-value of < 0.05 was considered statistically significant [16].

Results

During the study period, a total of 156 infants with BPD were enrolled with 52 cases of MBD and 104 controls. Demographic and clinical characteristics among cases with MBD and controls was shown in Table 1. There is no significant difference between the two groups in the distribution of BPD severity (mild BPD 59.6% vs. 70.2%; moderate BPD 23.1% vs 21.2%; severe BPD 17.3% vs 8.7% in MBD cases and controls respectively; *P*=0.236).

Univariate logistic regression analyses ([Table 2](#)) revealed that, among the putative risk factors evaluated, FGR (OR 6.16, 95% CI, 2.34–16.20) extremely low birth weight (ELBW, birth weight < 1000 g) (OR 2.28, 95% CI, 1.04–5.00), feeding volume < 80 mL/kg/day at the end of the 4th week after birth (OR 13.00, 95% CI,

4.82–34.84), cholestasis (OR 3.50, 95% CI, 1.66–7.41), and LOS (OR 3.60, 95% CI, 1.37–9.47) were all statistically significantly associated with development of MBD in BPD. After adjustment in multiple logistic regression, each of these remained as statistically significant, independent risk factors for MBD (FGR [OR 5.60, 95% CI, 1.77–17.72], ELBW [OR 3.70, 95% CI, 1.35–10.10]), feeding volume $\geq$ 80 mL/kg/day at the end of the 4th week after birth [OR 12.21, 95% CI, 3.89–38.33], cholestasis [OR 4.29, 95% CI, 1.65–11.15], LOS [OR 3.69, 95% CI, 1.12–12.77]).

Discussion

The growing advances in the intensive care of preterm infants have led to a decrease in mortality, but cause more frequent morbidity such as MBD and BPD, etc. Previous studies have proved that MBD is inversely associated with gestational age [4, 5], hence we matched the gestational age equal to the cases when selecting the controls to allow evaluating other putative factors in homogeneous groups. This study provides novel data on risk factors for MBD in BPD infants. FGR, ELBW (birth weight $\leq$ 1000 g), feeding volume $\geq$ 80 mL/kg/day at the end of the 4th week after birth, cholestasis and LOS each served as statistically significant, independent risk factors, on the basis of their associated ORs from multiple logistic regression.

The association between FGR and MBD in BPD infants was confirmed in our study which also in accordance with other data showing that FGR was a risk factor for BPD published [8]. This early life factor seemed to ease MBD development in a programmed process and might be used as an early predictive indicator for screening of MBD. FGR commonly coexists with preeclampsia at gestation, which results in placental abnormalities that deteriorates placental transfer of calcium, magnesium and phosphorus [19], and thus contribute to MBD. Furthermore, our study showed that maternal hypertensive disorder without FGR was not associated with MBD, indicating the disrupted placental as key pathogenesis. In addition to FGR, ELBW was also an independent risk factor for MBD in BPD infants of equal gestational age in our study samples, revealing an inverse association between birth weight and MBD, which was found even in ELBWs exclusively by Viswanathan S et al [20].

Feeding problems are almost inevitable in the very preterm infants. Generally, it is not difficult to introduce enteral feeding but hard to reach total enteral feeding owing to uncomfortable abdominal distention or signs of necrotizing enterocolitis (NEC), which may disrupt the feeding schedule. Prolonged PN were demonstrated to associated with MBD by several researches elsewhere [21]. Similarly our study revealed that an enteral feeding volume $\geq$ 80 mL/kg/d at the end of the 4th week after birth remarkably increased the risk for MBD in BPD infants. This may be explained by the following reasons. First, the small breast-fed volume did not allow the introduce of breast milk fortifier in our NICU. Breast milk provides inadequate protein and mineral content which cannot guarantee enough calcium and phosphate intake for preterm infants. Second, the sufficient feeding volume still require PN supplement which pose the possibility of aluminum contamination and the risk of mineral precipitation in the solution due to the small volumes [22]. It has been demonstrated that newborns fed exclusively with breast milk showed lower levels of phosphate [23] and fortified breast milk feeding significantly increase bone mineral

density (BMD) values via linear regression analysis [24]. Therefore, an earlier begin of fortification of the breast milk begins might be taken into consideration in breast-fed preterm infants with high risk for BPD, as appropriate. Some studies showed that vitamin D supplementation at >14 days of age was also associated with MBD, however, our study did not consistent with it. A possible explanation is that it is the blood levels of 25(OH)D3 that is directly associated with MBD [6]. A usual intake dose of 800–1000 IU/d for preterm infants with BPD did not achieve the protective level, which is probable due to fat-soluble vitamin deficiency caused by the coexisting cholestasis [25]. Simultaneously, cholestasis was also a risk factor for MBD in BPD infants confirmed by our study and other researches [21]. In other words, these infants may be at a greater demand for vitamin D supplementation.

LOS remains an important problem and are associated with complications of preterm infants including BPD [27]. Jensen EA et al found that blood culture confirmed sepsis were associated with increased odds of severe MBD in infants with severe BPD [28]. Our study reenforced the association between LOS and MBD in BPD infants. It has been acknowledged that important interactions occur between immune and skeletal systems [29]. Lipopolysaccharide exposures could result in bone loss [30], which might be due to the activation of B and T cells that potentially regulate bone resorption [29]. Approaches, such as a strict hygiene protocol and the minimization of invasive procedures in NICUs should be employed in prevention of LOS in order to reduce MBD.

The strengths of this study are as follows, first, we enrolled only infants with BPD and matched gestational age to ensure the comparison in homogeneous groups, second, the monocentric study guaranteed that the enrollments were managed strictly under the same perinatal practices including diagnosis and treatments. Still, there are some limitations. MBD is a multifactorial disease. The small sample size may mask some risk factors that could not be displayed. However, we determine some risk factors that could be interpreted. The results in our study revealed that MBD in BPD infants shared some same risk factors with preterm infants in general.

Conclusion

In gestational age homogeneous BPD infants, fetal growth restriction, ELBW, feeding volume ≥ 80 mL/kg/day at the end of the 4th week after birth and LOS are significant risk factors for MBD. Given the observational nature of this study, further longitudinal/prospective studies are required to validate these findings.

Abbreviations

MBD Metabolic bone disease

BPD Bronchopulmonary dysplasia

HCA Histologic chorioamnionitis

FGR Fetal growth restriction

ELBW Extremely low birth weight

VLBW Very low birth weight

PROM Prolonged rupture of membranes

PDA Patent ductus arteriosus

LOS Late onset sepsis

PMA Post-menstrual age

NICU Neonatal intensive care unit

OR Odds ratio

aOR Adjusted odds ratio

CI Confidence interval

Declarations

Acknowledgements

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Authors' contributions

Xu LP had primary responsibility for the study design and protocol development. Chen WW performed the final data analyses and contributed to the writing of the manuscript. Zhang ZH was involved in the data collection. Dai SZ was involved in the table drawing. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

The study was approved by the Institutional Review Board of the Zhangzhou Hospital. The study is based on register data, and individual informed consent from each participant is not required by waiver from the ethical committee and national guidelines.

Consent for publication

Not applicable.

Competing interests

None declared.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1 Demographic and clinical characteristics among cases with MBD and controls				
	Cases		Controls	
	n	%	n	%
Antenatal factors	52		104	
Steroids	37	71.2	79	76.0
HCA	29	55.8	50	48.1
FGR	16	30.8	7	6.7
Maternal hypertensive disorders without FGR	5	9.6	11	10.6
PROM	11	21.2	27	26.0
Postnatal factors				
ELBW	16	30.8	17	16.3
Breast milk-fed	39	75.0	71	68.3
Initiation of oral vitamin D supplementation ≥ 2 weeks after birth	20	38.5	26	25.0
Feeding volume ≥ 80 mL/kg/day at the end of the 4th week after birth	23	44.2	6	5.8
Dexamethasone	37	71.2	79	76.0
PDA	25	48.1	47	45.2
Cholestasis	22	42.3	18	17.3
LOS	12	23.1	8	7.7
Mechanical ventilation	28	53.8	57	54.8
Hypothyroidism	15	28.8	24	23.1
Thrombocytopenia	9	17.3	10	9.6

MBD, Metabolic bone disease; HCA, histologic chorioamnionitis; FGR, fetal growth restriction; ELBW, extremely low birth weight; PROM, prolonged rupture of membranes; PDA, patent ductus arteriosus; LOS, late onset sepsis.

Table 2 Unadjusted OR and aOR for putative risk factors for development of MBD in BPD infants from univariate and multiple logistic regression

Putative risk factors	Unadjusted				Adjusted			
	95%		95%		95%		95%	
	OR	LCL	UCL	<i>P</i>	OR	LCL	UCL	<i>P</i>
Antenatal factors								
Steroids	0.78	0.37	1.65	0.517				
HCA	1.36	0.70	2.66	0.366				
FGR	6.16	2.34	16.20	□ 0.001	5.60	1.77	17.72	0.003
Maternal hypertensive disorders without FGR	0.90	0.30	2.74	0.852				
PROM	0.77	0.35	1.70	0.765				
Postnatal factors								
ELBW	2.49	1.14	5.41	0.022	3.70	1.35	10.10	0.011
Breast milk-fed	0.72	0.34	1.52	0.386				
Initiation of oral vitamin D supplementation ≥2 weeks after birth	1.98	0.96	4.05	0.063				
Feeding volume ≥80 mL/kg/day at the end of the 4 th week after birth	12.95	4.82	34.84	□ 0.001	12.21	3.89	38.34	□ 0.001
Dexamethasone	1.73	0.72	4.12	0.220				
PDA	1.12	0.58	2.19	0.733				
Cholestasis	3.50	1.66	7.41	0.001	4.29	1.65	11.15	0.003
LOS	3.60	1.37	9.47	0.009	3.79	1.12	12.77	0.032
Mechanical ventilation	0.96	0.49	1.88	0.909				
Hypothyroidism	1.35	0.64	2.87	0.434				
Thrombocytopenia	1.97	0.75	5.2	0.172				

MBD, Metabolic bone disease; HCA, histologic chorioamnionitis; FGR, fetal growth restriction; ELBW, extremely low birth weight; PROM, prolonged rupture of membranes; PDA, patent ductus arteriosus; LOS, late onset sepsis.