Original Research

Factors Affecting Metabolic Bone Disease of Prematurity: Is Hypothyroxinemia Included?

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Abstract

Objectives: The association between transient hypothyroxinemia of prematurity (THOP) and metabolic bone disease of prematurity (MBD) is not clearly known. We aimed to evaluate the effects of THOP and other risk factors on MBD in very low birth weight infants.

Methods: This study included infants born at <30 weeks gestational age and <1500 g birth weight who were hospitalized between July 2016 and December 2019. The following information was obtained from medical records: Demographic characteristics; clinical follow-up data; morbidities; initial thyroid function tests; and calcium (Ca), phosphorus (P), and alkaline phosphatase (ALP) levels at postnatal 4–6 weeks. Newborns with an ALP level >500 IU/L were diagnosed with MBD. Patients without MBD were defined as Group 1 and patients with MBD were defined as Group 2.

Results: Our study enrolled 145 infants who met the inclusion criteria. The incidences of MBD and THOP were 16.5% and 56.5%, respectively. Gestational age and birth weight were significantly lower in Group 2 than in Group 1. It was observed that these infants received total parenteral nutrition for a longer period of time and had a longer transition period to full enteral feeding. In addition, duration of non-invasive mechanical ventilation, duration of oxygen treatment, frequencies of moderate-severe bronchopulmonary dysplasia, and postnatal steroid use were found to be significantly higher in babies in Group 2 compared to babies in Group 1. There was no significant difference between the groups in terms of THOP. However, multivariate logistic regression analysis revealed no risk factors for the development of MBD. The presence of MBD and Ca, P, and ALP levels did not differ significantly between patients with and without THOP.

Conclusion: Our study reveals that MBD is a multifactorial disease and THOP is not a risk factor for the development of MBD.

Keywords: Metabolic bone disease of prematurity, prematurity, transient hypothyroxinemia of prematurity, very low birth weight infant

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Metabolic bone disease (MBD) of prematurity remains a major morbidity for premature infants with very low birth weight (VLBW).[1] MBD is characterized by decreased bone mineralization and may cause bone fractures in patients with severe disease.[2] As there is no generally accepted definition, the incidence of MBD varies considerably across studies. The incidences are estimated to be 20–30% in VLBW infants and 50–60% in extremely low birth weight (ELBW) infants. Furthermore, this condition is negatively associated with both birth weight and gestational

age.[1-3] Most mineral content is stored in bone tissue during the third trimester. Thus, preterm newborns are often born with insufficient mineralization.[4] In addition to the increased need for adequate mineralization in the postnatal period, factors contributing to MBD include infections, prolonged immobility due to respiratory support, feeding with unenriched breast milk, delayed enteral feeding, some drugs (e.g., steroids, methylxanthine, and loop diuretics), and prolonged total parenteral nutrition (TPN).[5-7]

Low thyroxine (T4)/triiodothyronine (T3) with normal/low thyroid-stimulating hormone (TSH) concentrations are common laboratory findings of transient hypothyroxinemia of prematurity (THOP) that improves at postnatal weeks 6–8.[8] Insufficient production and secretion of thyrotropin-releasing hormone, an inadequate response of the thyroid gland to TSH, poor iodine organification, and insufficient conversion of T4 into T3 are potential reasons for the low serum concentrations of thyroid hormones detected in preterm newborns with gestational age <30 weeks.[8,9] With decreasing gestational age and the onset of morbidities (e.g., sepsis, shock, or asphyxia), the incidence of THOP may reach 35–50%.[8,10]

It is well defined that congenital and juvenile acquired hypothyroidism delays bone development and leads to growth retardation. In severe cases, a postnatal growth arrest may occur with a complex skeletal dysplasia including absence of ossification centers. Thyroid hormone replacement induces rapid "catch up" growth and accelerated skeletal maturation in children with hypothyroidism. In contrast, juvenile thyrotoxicosis accelerates skeletal development and growth, and may result in advanced bone age. [11] At the cellular level, studies demonstrated that thyroid hormone receptors were expressed in bone tissue, and that the thyroid hormone-specific transporter was present in osteoblasts and osteoclasts. These clinical and molecular data suggest that thyroid hormones contribute to the progression of endochondral ossification and linear growth; thus, normal levels of thyroid hormones are indispensable for adequate bone development and maintenance in childhood.[12] However, the effects of transient hypothyroxinemia on MBD of prematurity still remain unclear.[13,14]

The aim of this study was to evaluate the risk factors for MBD and whether there is an association between THOP and MBD in VLBW newborns with a gestational age <30 weeks.

Methods

This retrospective cross-sectional study considered preterm babies hospitalized in our neonatal intensive care unit between July 2016 and December 2019. The study was approved by the Institutional Ethics Committee (No: 2021/50-01).

Inclusion Criteria

Preterm infants with gestational age <30 weeks and birth weight <1500 g were included in the study.

Exclusion Criteria

Death or referral to another center in the first 4 weeks of life, presence of a major congenital anomaly, diagnosis with primary or secondary hypothyroidism, and data being unavailable for analysis were determined as exclusion criteria.

Data Collection

The following information was obtained from the patients' medical records: Infants' demographic and maternal characteristics (e.g., gestational age, birth weight, mode of delivery, sex, antenatal steroid treatment, 5 min Apgar score, multiple pregnancy, small for gestational age, maternal age, preeclampsia, gestational diabetes, preterm premature rupture of membranes, chorioamnionitis, and maternal thyroid disease), clinical data and morbidities (e.g., duration of invasive and non-invasive mechanical ventilation, duration of oxygen treatment, time of achieving full enteral feeding, duration of TPN, drug use [surfactant, inotropic agents, loop diuretics, and steroids], late-onset sepsis, Grade ≥3 intraventricular hemorrhage, hemodynamically significant patent ductus arteriosus, Stage ≥2 necrotizing enterocolitis, cholestasis, THOP, and moderate-to-severe bronchopulmonary dysplasia [BPD]), length of hospital stay, and mortality data. Free T4 (fT4) and TSH levels were measured during the first 2 weeks of life. Calcium (Ca), phosphorus (P), and alkaline phosphatase (ALP) levels were recorded during postnatal weeks 4–6.

Evaluation of MBD

Samples were analyzed on an Abbott Architect ci4100 (Abbott Park, IL, USA). Serum Ca concentrations were measured by a complexometric method using Arsenazo-III (Architect Ca reagent). Serum P concentrations were determined by an end-point method using a direct phosphomolybdate reaction (Abbott Architect). ALP activities were measured kinetically through catalyzed hydrolysis of p-nitrophenylphosphate to p-nitrophenol and phosphoric acid (Abbott Architect).

MBD was defined as a serum ALP concentration >500 IU/L at postnatal 4–6 weeks. Patients with ALP >500 IU/L + P ≥4.5 mg/dl were diagnosed with mild MBD; patients with ALP >500 IU/L + P < 4.5 mg/dl were diagnosed with severe MBD.[15] Patients were divided into two groups: Without MBD (Group 1) and with MBD (Group 2). Demographic, clinical, and laboratory data were compared between the two groups. To evaluate the effects of THOP on bone metabolism, patients were regrouped according to the pres-

ence of THOP; Ca, P, and ALP levels were then compared between groups.

Measurement and Evaluation of Thyroid Function

The chemiluminescent microparticle immune study method (Abbott Laboratories, Abbott Park, IL, USA) was used to measure fT4 and TSH concentrations. The coefficient of variation and the analytical sensitivity for TSH were 1.9–5.2% and 0.0025 μIU/mL, respectively. The coefficient of variation for fT4 was 3.6–7.8%, and the analytical sensitivity was 0.4 ng/dL.

THOP was defined for a gestational age of 23–27 weeks as a fT4 level <0.91 ng/ml on postnatal day 7 and <0.95 ng/ml on postnatal day 14. For a gestational age of 28–30 weeks, it was defined as fT4 concentration <1.16 ng/ml on postnatal day 7 and <1.21 ng/ml on postnatal day 14.[16]

Management of Parenteral and Enteral Nutrition

During the study period, TPN was initiated in all patients on the 1st day of life with 6 mg/kg/min glucose perfusion containing 2 g/kg/day amino acid and 1 g/kg/day lipid solution. If no problem was detected in the first 3 postnatal days, the amino acid and lipid doses were increased to 4 g/kg and 3 g/kg, respectively. For the first 3 days, 35 mg/kg Ca (calcium gluconate) was added to TPN. After the 3rd day, high Ca (60 mg/kg/day) and high phosphate content (40 mg/kg/day) were administered until TPN withdrawal. Vitamins were added to TPN from the second postnatal day; trace elements were added after the 1st week of life.

In all clinically stable patients, minimal enteral nutrition was initiated as soon as possible if breast milk was available; it was initiated with preterm formula on the 4th postnatal day if breast milk was unavailable. The volume of enteral feeding was gradually increased (20–30 cc/kg/day) as tolerated. Lipid supplementation was discontinued when the volume of enteral nutrition exceeded 50% of the total volume; TPN was withdrawn when enteral nutrition reached 100 ml/kg/day. When breast milk reached 100 ml/kg/day, 1.1 g of breast milk fortifier was added to each 25 ml of breast milk. Each patient was administered 400 IU Vitamin D daily after TPN withdrawal.

Definitions

Diagnosis and classification of BPD were made according to the National Institutes of Health consensus.[17] Modified Bell's criteria were used to diagnose Stage ≥2 necrotizing enterocolitis.[18] The Papile classification[19] was used for the diagnosis of Grade ≥3 intraventricular hemorrhage. A positive blood culture after the 3rd day of life was considered late-onset sepsis.[20] PDA diagnosis and treatment guidelines of the Turkish Neonatology Society were used for hemodynamically significant PDA diagnostic criteria.[21]

Statistical Analysis

The data were analyzed using IBM SPSS software (ver. 23.0; IBM Corp., Armonk, NY, USA). The normalities of data distributions were evaluated using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The categorical variables among groups were compared using the Chi-squared test. The Mann–Whitney U-test was used for pairwise comparisons of non-normally distributed data between groups. Binary logistic regression analysis was used to examine risk factors affecting the existence of MBD. Analysis results are presented as median (Q1-Q3) for quantitative data and as frequency for categorical data. The significance level was set at p<0.05.

Results

During the study period, 187 newborns admitted to our unit met the criteria for birth weight and gestational age. Forty-two infants were excluded, including six with major congenital anomalies, one with skeletal dysplasia, 20 who died within the first 4 weeks of life, three who were referred to another center, six who had a diagnosis of primary or secondary hypothyroidism, and six who had missing data. Finally, the study was conducted with 145 cases meeting the inclusion criteria. The incidences of MBD were 16.5% (24/145) in all study groups, 24% (19/79) in patients with birth weight <1000 g, and 7.6% (5/66) in patients with a birth weight of 1000–1500 g. Of these infants, 12 had mild MBD and 12 had severe MBD. Demographic and maternal characteristics of the groups are shown in Table 1. Gesta-

Table 1. Demographic characteristics of the groups

	Group 1 (n=121)	Group 2 (n=24)	p
Gestational age (weeks)	27 (26–28)	26.00 (24–27)	0.009
Birth weigh (grams)	1000 (770–1185)	795 (662.5–965)	0.001
Male, n (%)	64 (52.9)	12 (50)	0.972
Cesarean delivery, n (%)	95 (78.5)	18 (75)	0.913
5 min Apgar score	7 (6–7.5)	6 (5–7)	0.138
Multiple pregnancy, n (%)	27 (22.3)	7 (29.2)	0.645
SGA, n (%)	9 (7.4)	1 (4.2)	1.000
Antenatal steroid, n (%)	42 (34.7)	7 (29.2)	0.773
Maternal age (years)	28 (25–35)	29.5 (23–34)	0.62
pPROM >18 h, n (%)	23 (19)	6 (25)	0.577
Chorioamnionitis, n (%)	4 (3.3)	2 (8.3)	0.259
Preeclampsia, n (%)	21 (17.4)	1 (4.2)	0.126
Gestational diabetes, n (%)	3 (2.5)	0 (0)	1.000
Maternal thyroid disease, n (%)	3 (2.5)	0 (0)	1.000

SGA: Small for gestational age; pPROM: Preterm premature rupture of membranes.

tional age and birth weight were significantly lower in Group 2 than in Group 1. No significant differences were found between the two groups in terms of maternal characteristics or other demographic data (p>0.05).

The clinical features and morbidities of the two groups are shown in Table 2. Postnatal steroid use, presence of moderate-to-severe BPD, time of achieving full enteral feeding, duration of TPN, non-invasive mechanical ventilation, and oxygen treatment duration were significantly greater in Group 2 than in Group 1. There were no significant differences between groups in terms of inotropic or diuretic drug use (p>0.05). As shown in Table 2, no significant difference was obtained between infants with and without MBD according to the presence of THOP (p>0.05). The incidence of THOP was 56.5% (82/145). When the patients were grouped according to the presence of THOP, no differences between the groups were detected in terms of MBD and Ca, P, and ALP levels (p>0.05).

Univariate analysis showed that the risk of MBD decreased 0.74-fold with increasing gestational age (p=0.012). The risk of MBD increased 1.05-fold with increasing duration of TPN infusion (p=0.03), 0.3-fold with postnatal steroid use (p=0.023), and 4.91-fold with BPD (p=0.001). However, the risk factors that might have an impact on the development of MBD (e.g., gestational age, THOP, duration of TPN infusion, postnatal steroid use, and moderate-to-severe BPD) were not statistically significant in the multivariate logistic regression analysis (Table 3).

Discussion

In this study, the incidences of MBD were 24% in preterm with birth weight <1000 g, 7.6% in preterm with a birth weight of 1000–1500 g, and 16.5% in the whole cohort. The previous reports have suggested that MBD might be observed in approximately 20–30% of VLBW infants and in ap-

Table 2. Clinical characteristics and neonatal morbidities of the groups

	Group 1 (n=121)	Group 2 (n=24)	P
Surfactant, n (%)	98 (81)	22 (91.7)	0.253
HsPDA, n (%)	37 (30.6)	10 (41.7)	0.411
Grade ≥ 3 IVH, n (%)	16 (13.2)	5 (20.8)	0.346
Late-onset sepsis, n (%)	13 (10.7)	2 (8.3)	1.000
Stage ≥ 2 NEC, n (%)	6 (5)	3 (12.5)	0.170
Moderate-severe BPD, n (%)	35 (28.9)	16 (66.7)	0.001
Cholestasis, n (%)	4 (3.3)	2 (8.3)	0.259
THOP, n (%)	65 (53.7)	17 (70.8)	0.187
Postnatal steroid, n (%)	64 (52.9)	19 (79.2)	0.031
Duration of TPN infusion (days)	14 (10 - 18)	18.5 (12.5–29.5)	0.003
Achievement time of full enteral feeding (days)	17 (14–22)	22.5 (16–32.75)	0.003
Duration of invasive MV (days)	4 (1–15)	7.5 (1–19.25)	0.322
Duration of non-invasive MV (days)	12 (4–22)	22 (10–41.75)	0.004
Duration of oxygen treatment (days)	11 (3–34)	31 (25.5–38.75)	0.001
Length of stay (days)	68 (54–97.5)	90 (69–116)	0.003
Death, n (%)	4 (3.3)	1 (4.2)	1

HsPDA: Hemodynamically significant PDA; IVH: Intraventricular hemorrhage; NEC: Necrotizing enterocolitis; BPD: Bronchopulmonary dysplasia; THOP: Transient hypothyroxinemia of prematurity; TPN: Total parenteral nutrition; MV: Mechanical ventilation.

Table 3. Risk factors for metabolic bone disease revealed by binary logistic regression analysis

	Univariate		Multivariate	
	OR	P	OR	P
Gestational age	0.74 (0.58–0.94)	0.012	0.86 (0.65–1.12)	0.256
THOP	2.09 (0.81–5.41)	0.128	1.14 (0.38–3.36)	0.819
Duration of TPN infusion	1.05 (1.00–1.09)	0.030	1.02 (0.97–1.06)	0.452
Postnatal steroid	0.30 (0.10–0.84)	0.023	1.31 (0.34–4.98)	0.697
BPD	4.91 (1.93–12.52)	0.001	2.97 (0.88–9.99)	0.079

THOP: Transient hypothyroxinemia of prematurity; TPN: Total parenteral nutrition; BPD: Bronchopulmonary dysplasia.

proximately 50–60% of ELBW.[1,3] However, in recent years, the incidence has decreased to 10–20% in ELBW infants with early aggressive TPN support, as well as the routine use of breast milk fortifiers and premature formulas.[2,22] The frequencies of MBD in our study were consistent with the current published literature.

MBD of prematurity is usually caused by a deficiency of Ca and/or P because of reduced intake and/or absorption. [1,2] Known risk factors for MBD include insufficient mineral accumulation in fetal bones due to preterm birth; lower supplemental doses of Ca and P in the postnatal period (compared with the intrauterine period); long-term use of TPN, methylxanthine, diuretic, and steroid treatments; conditions such as sepsis, cholestasis, BPD, and necrotizing enterocolitis; immobilization; and feeding with unfortified breast milk.[1,4-7,23] In our study, infants with MBD had a lower gestational age and a lower birth weight, compared with newborns who did not have MBD. The time of achieving full enteral feeding and the duration of TPN were longer in infants with MBD. We found that the duration of non-invasive mechanical ventilation and oxygen therapy was longer in infants with MBD. However, we found no between-group difference in terms of invasive mechanical ventilation duration. We consider that this might be related to our approach, which aimed to end invasive mechanical ventilation support as soon as possible, then maintain respiratory support with non-invasive methods.

The use of diuretics and steroids in infants with BPD has been considered as a risk factor for MBD due to increased renal calcium excretion by diuretics and the suppressive effects of steroids on bone formation.[24] In our study, moderate-to-severe BPD and postnatal steroid use were both more frequent in infants with MBD. Although univariate analysis showed that the risk of MBD increased in relation to various risk factors, none were statistically significant in the multivariate logistic regression analysis. We presume that the low number of patients with MBD and the multifactorial etiology of MBD might have contributed to this finding.

The development of MBD peaks at 4–8 weeks of life; thus, preterm infants should be screened at postnatal 4–6 weeks. For this purpose, the following screening parameters may be used: Serum Ca, P, and ALP values; parathyroid hormone (PTH) level; tubular phosphate reabsorption; and radiological imaging.[1,7] There is no consensus regarding the diagnosis of MBD; thus, reported incidences differ among hospitals. Backström et al.[25] reported that the sensitivity and specificity of an ALP level >900 U/L and a P level <1.8 mmol/L were 100% and 70%, respectively. Mitchell et al.[2] recommended screening for MBD if two ALP values record-

ed at least 1 week apart were > 800 IU/L and/or a single value exceeded 1000 IU/L. According to recent studies, lower ALP levels are likely associated with MBD. In their study of 336 VLBW infants, Figueras-Aloy et al.[15] measured bone mineral density before discharge from the hospital using dual-energy X-ray absorptiometry; they then determined the optimal threshold values of ALP and P to diagnose and determine the severity of MBD. The findings of their study suggested that an ALP level >500 IU/L and a P level ≥4.5 mg/dl should be classified as mild MBD, while an ALP level >500 IU/L and a P level <4.5 mg/dl should be classified as severe MBD. In a subsequent study, the optimal cutoff value for ALP, with a sensitivity of 100% and a specificity of 80.77%, was 500 IU/L.[3] Following these recommendations, we used a serum ALP level of >500 IU/L in the diagnosis of MBD; we classified ALP >500 IU/L and p<4.5 mg/dl as severe MBD.

THOP is generally a self-limiting condition in which thyroid function tests return to normal ranges around the 6th–8th weeks of life.[8] Although different incidence values for hypothyroxinemia have been reported in the literature, the incidence can reach 35–50% with decreasing gestational age and in the presence of morbidities.[10,26] A study from Turkey by Demirel et al.[13] evaluated 124 infants born at ≤34 weeks' gestational age; it showed that the incidence of THOP was 15.3%. However, the incidence in our cohort was 56.5%. We suspect that this discrepancy is because we included infants born at <30 weeks' gestational age and with a birth weight <1500 g. Furthermore, the reference values that we used for the diagnosis of THOP were greater than the values used by Demirel et al.[13]

Thyroid hormones contribute to regulating skeletal development, achieving peak bone mass, and maintaining adult bones. Abnormal thyroid hormone levels not only impair bone maturation and linear growth in childhood but also alter bone remodeling and increase fracture risk in adults. [12] Although the effects of thyroid function on bone metabolism in adults and older children are well defined, studies evaluations of the impacts of thyroid hormones on bone development in infants (especially preterm) are limited. The study by Demirel et al.,[13] which was the first to evaluate the relationship between THOP and MBD, showed no significant association between THOP and MBD. In another study from Turkey, which included 543 VLBW infants and evaluated the relationship between congenital hypothyroidism and MBD, thyroid hormones had no effect on MBD. [14] Although in the present study, we used different definitions and reference intervals for both MBD and THOP, our results were similar to those of Demirel et al.[13] When we grouped our patients according to the presence of THOP, we found no differences between groups in terms of MBD

and serum Ca, P, and ALP levels. MBD is a major morbidity of bone metabolism in VLBW infants. While it is demonstrated that thyroid hormone disorders affect negatively bone development and structure, our study revealed that transient hypothyroxinemia is not directly associated with the development of MBD in these infants.

The main limitation of our study was that it comprised a retrospective analysis of medical records. Therefore, the homogeneity between groups could not be achieved. In addition, the diagnosis of MBD was reached using only biochemical markers without accompanying radiological imaging and dual-energy X-ray absorptiometry data. As we did not use in the diagnosis of MBD, PTH levels were missing in most of the patients and were not included in the study data. Furthermore, we did not obtain information regarding MBD that might have developed after discharge. However, the main strength of our study is that it assessed the association between MBD and THOP, which was evaluated in a limited manner in previous studies, using a cohort with lower gestational age.

Conclusion

Our study showed that infants with MBD had a lower gestational age, a lower birth weight, a longer duration of TPN, and a later time of achieving full enteral feeding, compared with those who did not develop MBD. Risk factors such as moderate-to-severe BPD, postnatal steroid use, and longer respiratory support were more common in infants with MBD. Although hypothyroidism may affect negatively bone metabolism in children, we did not find any statistically significant relationship between THOP and MBD in VLBW infants.

Disclosures

Ethics Committee Approval: The study was approved by the Institutional Ethics Committee (No: 2021/50-01).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – M.D., B.O., M.S.; Design – M.D., B.O., M.S.; Supervision – M.D, B.O.; Materials – M.D., M.S.; Data collection &/or processing – M.D., M.S.; Analysis and/or interpretation – M.D., B.O., M.S.; Literature search – M.D., B.O., M.S.; Writing – M.D., B.O., M.S.; Critical review – M.D., B.O.

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