Serum Sclerostin Levels in Newborns Born to Mothers With Vitamin D Deficiency

Gonca Sandal, MD, Ozgur Pirgon, MD,† Bumin Dundar, MD,‡ Hasan Cetin, MD,* and Halil Ibrahim Bayram, MD§*

Aim: Sclerostin inhibits osteoblast functions, differentiations, and survival rates. The aim of this study was to investigate the association between circulating sclerostin (an emerging biomarker and important regulator of bone formation) and neonatal parameters in mothers with vitamin D deficiency.

Method: Forty-five mothers and their newborns were recruited in the study. The mothers were divided into 2 groups as vitamin D–deficient group 25 (OH)D (25-hydroxyvitamin D3 < 20 ng/mL) and vitamin D–sufficient group 25(OH)D (>20 ng/mL). Their newborns had measurements of weight, height, calcium, phosphate, alkaline phosphatase, sclerostin, and 25(OH)D at birth.

Results: The mothers with vitamin D deficiency had significantly lower vitamin D levels than the mothers with vitamin D sufficiency (8.7 [3.4] ng/mL vs 26.7 [4.0] ng/mL, $P < 0.001$). There were no significant differences between women with vitamin D deficiency and women with vitamin D sufficiency for sclerostin concentrations (205.4 [64.8] pg/mL vs 291.6 [122.9] pg/mL). However, 25(OH)D (10.1 [8.1] ng/mL vs 33.4 [11.6] ng/mL, $P < 0.001$) and sclerostin concentrations (182.9 [15.3] pg/mL vs 288.8 [32.3] pg/mL, $P = 0.01$) were lower in newborns born by mothers with vitamin D deficiency compared and with newborns of mothers with vitamin D sufficiency. Circulating sclerostin measurements were not associated with 25(OH)D levels of both mothers and their newborns.

Conclusions: We found significantly lower sclerostin levels in newborns born by women with vitamin D deficiency compared with newborns of nondeficient mothers.

Key Words: sclerostin, vitamin D, newborn, mother

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Maternal vitamin D status during pregnancy may program skeletal development and body composition in the offspring by influencing the interaction between osteoblasts and adipocytes.[1] The regulation of maternal placental-fetal mineral homeostasis and skeletal development has remained largely unknown.[2] Previous studies have shown that maternal vitamin D status, defined by serum 25(OH)D (25-hydroxyvitamin D) concentration, tightly associates with cord blood vitamin D concentration.[3,4] Low maternal 25(OH)D level is associated with shorter duration of gestation and, consequently, reduced growth of long bones in newborns.[1] In addition, children of mothers with low vitamin D status during late pregnancy had reduced whole body bone mineral content at the age of 9 years.[5]

Sclerostin is a glycoprotein that inhibits osteoblast differentiation and bone formation.[6] Although the underlying mechanisms are unclear, it was hypothesized that sclerostin has an inhibitory effect on bone formation by directly blocking the Wnt signaling pathway.[7] In healthy adults, significantly higher concentrations of sclerostin in men than in women and a positive correlation between sclerostin and age, body mass index (BMI), and bone mineral density were observed.[8] In an another study, no correlation between serum sclerostin and markers of bone turnover was found in young adults; however, in groups of the elderly subjects, they found negative correlations between some of the bone parameters.[9] Recently, intensive studies have been conducted to assess serum sclerostin concentrations in adults, but there are few articles on sclerostin levels in newborns and children. The primary aim of the study was to determine whether there was an association between maternal 25(OH)D levels and serum neonatal sclerostin levels.

MATERIAL AND METHODS

Subjects

Forty-five pregnant women and their newborns born by normal spontaneous vaginal delivery at the Hospital of Süleyman Demirel University, Medical Faculty between December 2013 and February 2014 were enrolled in the present study. Women with singleton term pregnancies were recruited consecutively from the delivery suite. We excluded infants whose mothers had any clinical conditions such as parathyroid, bone, renal, diabetes mellitus, and gastrointestinal disorders. The mothers were divided into 2 groups according to their vitamin D status (vitamin D–deficient group, 23; vitamin D–sufficient group, 22). We defined vitamin D deficiency as a serum concentration of less than 20 ng/mL, and the mothers were divided into 2 groups as vitamin D–deficient group 25(OH)D (25-hydroxyvitamin D3 < 20 ng/mL) and vitamin D–sufficient group 25(OH)D (>20 ng/mL).[10,11]

None of the infants of the mothers had congenital malformations, chromosomal abnormalities, or intrauterine infections. Apgar scores in the fifth minute were greater than 8 and physical examinations were normal. Those with chronic conditions, systemic infections, and nutrition defects were excluded from the study. Anthropometric measurements (height and weight) were recorded and blood samples were taken. Those without evidence of systemic disease, requiring no therapeutic intervention and growing normally, were enrolled. Birth weight and length were obtained from each neonate immediately after birth. Weight measurements were made with naked babies by an electronic weighing machine. Height measurements were accomplished with a height measuring board (head portion stable, feet portion mobile).

Süleyman Demirel University, Medical Faculty Ethic Committee approval (November 2013 and 95 session number) has been received. All women participating in the study provided a

From the *Department of Pediatrics, Division of Neonatology, Faculty of Medicine, Süleyman Demirel University; †Department of Pediatrics, Division of Pediatric Endocrinology, Faculty of Medicine, Süleyman Demirel University, Isparta; ‡Department of Pediatrics, Division of Pediatric Endocrinology, Faculty of Medicine, Katip Celebi University, Izmir; §Department of Biochemistry, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey.
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Reprints: Ozgur Pirgon, MD, Department of Pediatrics, Division of Pediatric Endocrinology, Faculty of Medicine, Süleyman Demirel University, 32260, Cunur, Isparta, Turkey. E-mail: ozgurpirgon@gmail.com.
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TABLE 1. Comparison of Mothers and Their Newborns According to Maternal 25(OH)D Concentrations

	Mothers		
	Vitamin D Deficient	Vitamin D Sufficient	
	<20 ng/mL	>20 ng/mL	*P*
Mothers			
n	23	22	—
Age, y	30.2 (6.0)	27.6 (5.46)	0.155
Weight, kg	80.6 (12.2)	75.1 (11.7)	0.158
BMI, kg/m^2	31.9 (12.2)	28.4 (4.2)	0.250
Prepregnancy weight, kg	62.9 (17.5)	63.3 (13.5)	0.929
Height, cm	163.0 (4.3)	162.0 (6.5)	0.571
Gestational age, wk	39.2 (0.9)	39.4 (0.8)	0.465
Calcium, mg/dL	8.5 (0.6)	8.7 (0.5)	0.243
Phosphate, mg/dL	3.8 (1.1)	4.0 (1.1)	0.706
ALP, U/L	157.2 (51.6)	187.7 (98.1)	0.214
25(OH)D, ng/mL	8.7 (3.4)	26.7 (4.0)	<0.001
Maternal sclerostin, pg/mL	205.4 (64.8)	291.6 (122.9)	0.870
Newborns			
Weight, g	3409.5 (398.4)	3269.4 (407.7)	0.280
Height, cm	49.5 (2.4)	50.4 (3.6)	0.330
Head circumference, cm	34.5 (1.2)	32.6 (6.0)	0.150
Calcium, mg/dL	8.9 (0.6)	9.2 (0.8)	0.186
Phosphate, mg/dL	5.6 (0.8)	5.9 (0.9)	0.472
ALP, U/L	157.1 (51.6)	179.2 (86.3)	0.869
25(OH)D, ng/mL	10.1 (8.1)	33.4 (11.6)	<0.001
Neonatal sclerostin, pg/mL	182.9 (15.3)	288.8 (32.3)	0.012

Data are presented as mean (SD), unless otherwise stated.

written informed consent in accordance with the Declaration of Helsinki.

Blood Samples

Blood was collected from mothers and their newborns in the first 24 hours after birth. The blood samples were immediately centrifuged after clotting and the supernatant serum was kept frozen at −80°C until the time of the assay.

Serum 25(OH)D concentrations, as the best estimates of overall vitamin D status, were measured using an automated chemiluminescence immunoassay Cobas 6000 E601 analyzer (Roche Diagnostic, Mannheim, Germany). To avoid seasonal variations, all 25(OH)D samples were collected during the winter months (between December 2013 and February 2014) in both groups. Determination of serum sclerostin levels was performed by enzyme immunoassay (2nd Generation High Sensitive Human Soluble Sclerostin Elisa reagent; Aviscera Bioscience Quidel Corporation, Santa Clara, CA). All assays were performed according to the manufacturer's instructions. The intra-assay and interassay coefficient of variation were 10% for all assays. Serum levels of calcium, phosphate, and alkaline phosphatase (ALP) were measured on the same day with an auto analyzer.

Statistical Analysis

Data were expressed as mean (SD). Differences in the means of variables were tested using both parametric and nonparametric tests depending on the distribution of the variables. Correlation analyses were conducted using Spearman or Pearson correlation coefficients depending once again on the distribution of the variables. A P value of <0.05 was considered significant. SPSS Version 17 (SPSS, Chicago, IL) was used for analysis.

RESULTS

The characteristics of the 45 mothers and their newborns were summarized in Table 1. Both of the groups (mothers with vitamin D deficiency and vitamin D sufficiency) showed no significant differences in terms of age, weight, prepregnancy weight, height, BMI, calcium, phosphate, ALP, and serum sclerostin levels. The mothers with vitamin D deficiency had significantly lower vitamin D levels than mothers with vitamin D sufficiency (8.7 [3.4] ng/mL vs 26.7 [4.0] ng/mL, $P < 0.001$). There were no significant differences between women with vitamin D deficiency and women vitamin D sufficiency for sclerostin concentrations (205.4 [64.8] pg/mL vs 291.6 [122.9] pg/mL).

When compared their newborns of the 2 groups, there were no significant differences for gestational age, sex, weight, height, head circumference, serum calcium, phosphate, and ALP levels. However, the infants of mothers with vitamin D deficiency had lower 25(OH)D (10.1 [8.1] ng/mL vs 33.4 [11.6] ng/mL, $P < 0.001$) and serum sclerostin levels (182.9 [15.3] pg/mL vs 288.8 [32.3] pg/mL, $P = 0.01$) than the infants of mothers with vitamin D sufficiency (Fig. 1). There were no significant associations between neonatal sclerostin levels and neonatal/maternal parameters (Table 2, Figs. 1 and 2).

DISCUSSION

Maternal vitamin D insufficiency has been associated with several adverse health outcomes such as infections, cesarean section, and fetal growth restriction. In the present study, we reported maternal vitamin D status and its effect on maternal and neonatal sclerostin levels in a cohort of women with vitamin D deficiency. Plasma levels of 25(OH)D in the neonate correspond to approximately 60% to 70% of maternal levels, although some studies have reported even lower values.[12,13] Consistent with previous reports, we found low 25(OH)D levels in infants born to mothers with vitamin D deficiency. More studies on birth weight, infants of mothers with 25(OH)D concentrations of less than 37.5 nmol/L (15 ng/mL) during pregnancy, had reported lower birth weight.[14] However,

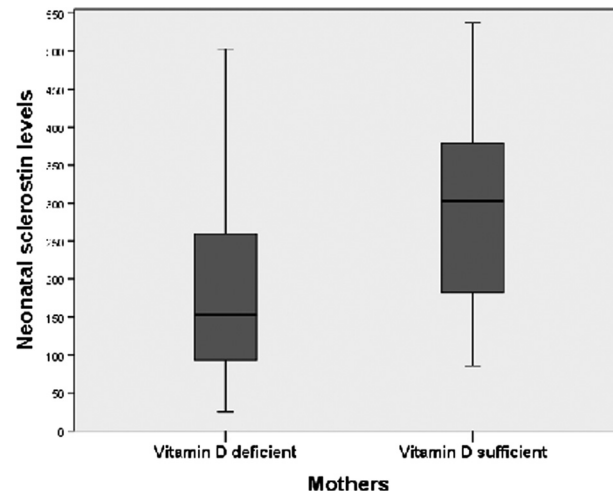

FIGURE 1. Comparison of neonatal sclerostin in mothers with vitamin D deficiency/sufficiency.

they found no differences in birth length and head circumference. We also investigated existing evidence on the effect of maternal 25(OH)D levels on birth variables (birth weight, length, and head circumference). Similar to the other studies, we did not find significant differences on neonatal antropometric parameters at birth.

Our data demonstrated that neonatal sclerostin levels were decreased as well as 25(OH)D in these infants born to mothers with vitamin D deficiency. Sclerostin is solely produced by osteocytes, the mechanoreceptors in the skeleton. Although sclerostin has been studied for many years, the exact mechanism of its activity is unknown.[15] Sclerostin is inhibitor of the Wnt signaling pathway and thus could be involved in the pathogenesis of age-related bone fragility. As an endogenous inhibitor of the Wnt/β-catenin pathway, the sclerostin should be related to decreased bone masses, although several studies indicate opposite results.[16,17] Van Bezooijen et al.[18] demonstrated that although sclerostin is seen as an inhibitor of bone formation, it does not inhibit the activity of ALP, one of the markers of bone formation. Research on sclerostin in patients with osteoporosis is of particular interest, but study results are inconclusive. Both higher as well as reduced sclerostin concentrations were observed in these patients. Dovjak et al.[19] found that lower sclerostin levels in patients with hip fractures in elderly subjects with osteoporosis. Ambroszkiewicz et al.[20] found significantly lower mean sclerostin levels in the group of children with cow's milk allergy compared with healthy children.

There were no data in the literature to date regarding the relationship between vitamin D and serum sclerostin levels in newborns. In this study, we found that the infants of the mothers with vitamin D deficiency had lower serum sclerostin levels than the controls. Godang et al.[21] found a significantly higher level of sclerostin in the umbilical cord plasma compared with the maternal circulation and a significant effect of umbilical cord sclerostin on neonatal total body bone mineral content. We did not analyze the umbilical cord samples because the cord blood

TABLE 2. Correlations Neonatal Sclerostin Levels and Maternal/Neonatal Parameters in the Group of Mothers With Vitamin D Deficiency

	r	*P*
Maternal		
Age	0.066	0.777
Maternal weight	−0.167	0.468
BMI	−0.199	0.387
Prepregnancy weight	0.081	0.727
Height	−0.213	0.355
Gestational age	−0.026	0.909
Calcium	0.077	0.742
Phosphate	−0.225	0.326
ALP	−0.167	0.469
25(OH)D	0.170	0.461
Maternal sclerostin	0.258	0.224
Newborns		
Birth weight	−0.05	0.807
Height	0.147	0.526
Head circumference	−0.06	0.781
Calcium	0.049	0.834
Phosphate	0.329	0.146
ALP	0.154	0.506
25(OH)D	0.270	0.237

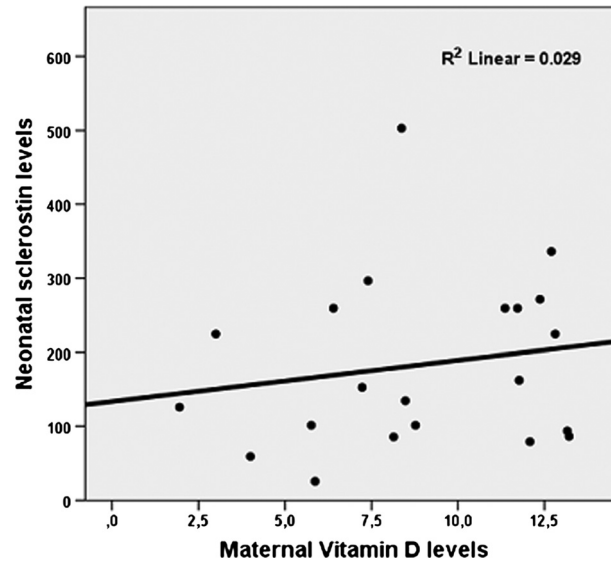

FIGURE 2. The relationship between serum neonatal sclerostin and maternal vitamin D levels (R^2 = 0.029) in group of mothers with vitamin D deficiency.

25(OH)D concentrations in populations have been similar to maternal late-pregnancy serum 25(OH)D concentrations.[10,22] Serum sclerostin levels exhibit a significant positive correlation with bone mineral density. However, Ryan et al.[23] showed that sclerostin not only alters bone mineralization but also influences mineral metabolism by altering concentrations of hormones that regulate mineral accretion. Although there was no statistically significant correlation between serum sclerostin and 25(OH)D levels, lower sclerostin levels in vitamin D–deficient groups suggest that the 25(OH)D/sclerostin relationship may be one of the factors linking fetal bone health. Ryan et al.[23] suggested that in the absence of sclerostin, 24-hydroxylase activity is decreased, resulting in the formation of decreased amounts of 24,25(OH)2D.

In conclusion, our results suggested that maternal vitamin D status affects neonatal 25(OH)D and serum sclerostin levels during the intrauterine period and may influence fetal bone health. Based on the findings of this study, there was no statistically significant relationship between neonatal and maternal sclerostin levels and, also, sclerostin was not strongly associated with maternal/neonatal 25(OH)D levels possibly due to the small numbers of the groups. Thus, it remains unclear whether sclerostin is a clinically useful predictor of neonatal bone health, at least in this setting. In addition, vitamin D supplementation in pregnancy has the potential to be a simple intervention with significant benefits on fetal bone health.

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The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. All women provided written informed consent. In addition, for investigations involving newborns, informed consent has been obtained from the parents of participants involved.

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