RESEARCH PAPER

Hypovitaminosis D and Hypocalcemic Seizures in Infancy

P MEHROTRA, RK MARWAHA*, S ANEJA, ANJU SETH, B M SINGLA, GANIE ASHRAF*, B SHARMA*, APARNA SASTRY* AND N TANDON#

*From the Department of Pediatrics, Kalawati Saran Hospital, New Delhi; *Department of Endocrinology and Thyroid Research Centre, Institute of Nuclear Medicine and Allied Sciences, Delhi; and #Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, New Delhi, India.*

Correspondence to: Dr Raman K Marwaha, Department of Endocrinology and Thyroid Research Centre,INMAS, Delhi 110 054, India. marawaha_raman@hotmail.com

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Background: Hypocalcemia accounts for a majority of seizures in infants reporting to the emergency ward of our hospital.

Objective: To evaluate the role of Vitamin D deficiency in the etiology of hypocalcemic seizures in infancy.

Design and Setting: Cross sectional hospital based study, from April 2006-March 2007.

Subjects: 60 infants with hypocalcemic seizures and their mothers (study group) and 60 healthy breastfed infants with their lactating mothers (control group).

Measurements: Vitamin D [25(OH) D] and intact parathormone levels.

Results: High prevalence of hypovitaminosis D [25(OH)D levels <10 ng/mL] was observed in study mothers (85%), control mothers (50%), study infants (90%), and control infants (41.7%). Mean serum 25(OH) D values in study mothers and their infants (6.54 ± 5.32 ng/mL and 4.92 ± 4.62 ng/mL) were significantly lower than those of mother-infant pairs (9.06 ± 4.78 ng/mL and 9.03 ± 4.63 ng/mL) in the control group (P<0.001). A strong positive correlation of 25(OH) D levels between mothers and their infants was seen in both the study and control populations (P<0.001). Of the 54 study infants who had 25(OH)D levels <10ng/mL, 48 (89%) were born to mothers who also had 25(OH) D levels <10ng/mL.

Conclusions: Vitamin D deficiency is a major cause of hypocalcemic seizures in infants. Infants born to vitamin D deficient mothers are at a significantly higher risk to develop hypocalcemic seizures.

Key words: *Hypocalcemia, Hypovitaminosis D, Infant, Seizures, Vitamin D*.

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Hypocalcemia due to vitamin D deficiency constitutes a major cause of infantile seizures in developing countries. Infants are a vulnerable population for development of vitamin D deficiency because of their high rate of skeletal growth(1-3). During early infancy, vitamin D stores depend on intrauterine accretion and breastmilk, in addition to sunlight exposure. Breastfed infants born to and nursed by vitamin D deficient mothers have been shown to have low serum 25(OH) D levels(4,5). Maternal vitamin D deficiency may therefore represent an important risk factor for hypovitaminosis D in early infancy, thereby resulting in hypocalcemia and rickets in this age group(6,7).

Reports have indicated that there is a high prevalence of hypovitaminosis D in India, particularly amongst pregnant and lactating women(8-10). We conducted this study to evaluate the role of vitamin D deficiency in the etiology of symptomatic hypocalcemia during infancy.

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METHODS

A total of 60 consecutive infants, 15 days to 6 months of age, presenting with hypocalcemic seizures (Study infants) to the pediatric emergency ward of a tertiary level children's hospital, on two days a week, were recruited for the study along with their mothers

(Study mothers), between April 2006 and March 2007. Hypocalcemia was considered to be the cause of seizures if total serum calcium level was <8mg/dL or ionized calcium level was <4mg/dL, with normal levels of serum albumin. A similar number of age and socioeconomically matched breastfed infants (Control infants) and their mothers (Control mothers), attending an immunization clinic were taken as controls. The study was approved by the Ethics Committee of the Hospital. Written informed consent was obtained from the mothers enrolled in the study.

Only infants from full term singleton deliveries, without congenital malformations were chosen as subjects for the study. Babies were considered full term based on a review of history obtained from the mother, corroborated by hospital records when available. Exclusion criteria included birthweight ≤2 kg and history of intake of drugs or supplements known to affect bone mineral metabolism.

Mothers known to have hepatic, renal or bone disorders, malabsorption or intake of any drugs/ supplements known to affect the calcium -vitamin D - PTH axis were also excluded from the study. The selected mother-infant pairs underwent concurrent clinical, radiological (only for study infants), biochemical and hormonal assessment on the first visit as described below. Dietary assessment of total calories, protein, carbohydrate, fat, calcium, fiber and phytate was done in study and control mothers by a 24 h recall method as per published guidelines(11). Sun exposure was quantified by calculating the UV score for the mothers. This was done by assessing the mean body surface area (m^2) (using rule of 9) exposed to the sun and the mean duration (min/day) of sunlight exposure in a day between 9AM to 4PM. UV score ($min.m^2$/day) was calculated by multiplying the above two parameters(12).

Blood was drawn by venepuncture for total and ionic calcium (Ca), inorganic phosphate (P), alkaline phosphatase (ALP), magnesium (Mg), vitamin D (25(OH)D) and intact parathormone (iPTH) from both mothers and infants. Routine investigations to exclude other causes of seizures in infants were also performed. Serum was separated in a cold centrifuge and supernatant removed in three aliquots. While serum Ca, P, ALP were estimated on the same day, the remaining aliquots were stored at –80º C until 25(OH) D and iPTH were estimated. Radiographs of the wrist joint were done to look for radiological evidence of rickets in hypocalcaemic infants.

Hormone assays were performed at the Institute of Nuclear Medicine and Allied Sciences (INMAS). The serum concentration of 25(OH) D (reference range 9.0-37.6 ng/mL) was measured by RIA (Diasorin, Stillwater, MN). Analytical sensitivity of the kit was 1.5ng/mL. We classified hypovitaminosis D based on the measurement of serum 25 (OH)D concentration(13), as follows: mild hypovitaminosis D: 10-20 ng/mL; moderate hypovitaminosis D: 5-10 ng/mL; and severe hypovitaminosis D: <5 ng/mL. However, in view of many earlier studies taking 10ng/mL as the cut-off for vitamin D deficiency, we have also discussed our results based on this value. Serum iPTH (reference range 13-54 ng/L) was measured using IRMA (Diasorin, Stillwater, MN). Analytical sensitivity of the kit was 0.7 ng/L.

Serum ionized calcium was estimated by ion-exchange method (Electrolyte Analyzer, Roche, Mannheim, Germany). The reference range for ionized calcium was 4-5 mg/dL (infants 10 days-2 yrs) and 4.7-5.2 mg/dL (adult women). Total serum calcium was measured by colorimetric method and inorganic phosphate and alkaline phosphatase were measured by photometric method (Randox Lab Ltd, UK). The reference range for total calcium was 8.4-10.8 mg/dL (infants 10 days-2 yrs) and 8.8-10.2 mg/dL (adult women). Serum albumin was measured using bromo-cresol green (BCG) dye method. The normal range of inorganic phosphate was 3.0 to 7.0mg/dL (infants) and 2.7 to 4.5 mg/dL (adults). The upper limit of normal for ALP in infants was 1076 IU/L while that in non-pregnant women was 240 IU/L. Serum magnesium was measured by colorimetric method (Bio-chemistry Analyzer, Roche, Mannheim, Germany) and the normal range was 1.5-2.7 mg/dL.

Statistical analysis was carried out using SPSS 11.0. Independent *t* test was used to compare difference of means between the study and control groups. Spearman correlation analysis was perfor-

med to examine the relation of variables between infants and their respective mothers. Logistic and linear regression analysis was used wherever necessary.

RESULTS

The mean age of study and control infants was 3.0 ± 0.16 and 3.0 ± 0.14 months, respectively, with male to female ratio of 1.14:1 and 0.66: 1. Among study infants, 41 (68.3%) were exclusively breastfed, 9 (15%) were supplemented with animal milk and 10 (16.7%) were exclusively on animal milk. The control infants were predominantly breastfed with some of them receiving water or 1-2 animal milk feeds along with breast feeds. Associated infections were demonstrable in 29 (48.3%) study infants [lower respiratory tract infection in 15 (25%), acute gastroenteritis in 11 (18.3%) and sepsis in 3 (5%)]. Subtle signs of vitamin D deficiency in the form of wide anterior fontanel (>2.5 × 2.5 cm) and craniotabes were present in 8 (13%), while radiological evidence (metaphyseal fraying and osteopenia) was observed in 18 (30%) of study infants. There was no clinical evidence of rickets in any of the control infants.

Dietary evaluation revealed gross differences in the nutritional status and sun exposure of study and control mothers (***Table* I**).The mean/median serum values of total and ionic Ca, inorganic phosphate, ALP, 25(OH) D and iPTH in mother – infant pairs are shown in ***Table* II.** The distribution of serum 25(OH)D levels in mother-infant pairs is shown in ***Fig.*1.** Elevated serum ALP was observed in a significantly higher proportion of study infants (72%) as compared to study mothers (6.1%) (P <0.001). A significant inverse correlation between 25 (OH) D and PTH was noted in both study (r = –0.22, P<0.04) and control (r = –0.72, P<0.0001) mothers. An inverse correlation between 25 (OH) D and ALP was seen only in study mothers (r = –0.042, P = 0.0008). No significant correlation was noted between 25 (OH) D and calcium in either group.

TABLE I ANTHROPOMETRY AND DIETARY CHARACTERISTICS OF STUDY AND CONTROL MOTHERS

Parameter (mean±SD)	Controls (n=60)	Study (n=60)	P value
Age, (y) (mean ± SD)	25.0 ± 2.0	24.7 ± 2.0	0.08
BMI (Kg/m^2) (mean ± SD)	20.9 ± 1.6	19.6 ± 1.4	0.001
Calorie intake (Kcal/day)	1545 ± 197	1384 ± 273	0.0003
Crude fiber (g)	11.5 ± 3.2	12.6 ± 2.4	0.047
Calcium intake (mg)	671 ± 171	454. ± 62	0.0001
Phosphate intake(mg)	815 ± 162	827 ± 89	0.60
Sun exposure (UV score) min m^2/ day	7.60 ± 4.17	2.67 ± 1.93	0.001
Multiparity (>2) %	11.6	61.7	0.001

Among study infants with 25 (OH) D <10ng/mL, 75% had raised PTH in contrast to only 3.1% infants with 25(OH) D>10ng/mL. The mean PTH level in infants with serum 25(OH)D level <10 ng/mL was significantly higher than those with levels >10ng/mL (94.2 ± 78.3 pg/mL *vs* 28.9 ± 11.6 pg/mL, P=0.001). No significant correlation was found between serum 25(OH) D and PTH, calcium, or ALP in study infants. In control infants, a significant inverse correlation was observed between 25(OH)D and PTH (r = –0.65, P <0.0001).

TABLE II BIOCHEMICAL PARAMETERS IN INFANT-MOTHER PAIRS

Parameter	Mothers			Infants		
	Control	Study	P value	Control	Study	P value
Serum calcium (mg/dL)	9.83±0.67	9.57±0.48	0.014	9.79±0.78	7.11±0.46	0.0001
Serum ionized calcium (mg/dL)	4.70±0.27	4.42±0.34	0.0001	4.54±0.28	3.30±0.26	0.0001
Serum ionized phosphorus (mg/dL)	3.84±0.73	4.16±0.89	0.087	4.37±0.83	3.33±1.35	0.005
Alkaline phosphatase (IU/L)	310.48±102.77	654.83±41.41	0.0001	557.23±167.41	1738.45±499.24	0.0001
25 OH vitamin D (ng/mL)	9.06±4.78	6.54±5.32	0.007	9.03 ± 4.63	4.92±4.62	0.0001
Serum parathormone (pg/mL)	64.36±56.16	60.55±37.57	0.56	69.10 ± 72.43	132.72±91.65	0.0001

All values are mean ± SD; Ca, Calcium; P, Phosphorus.

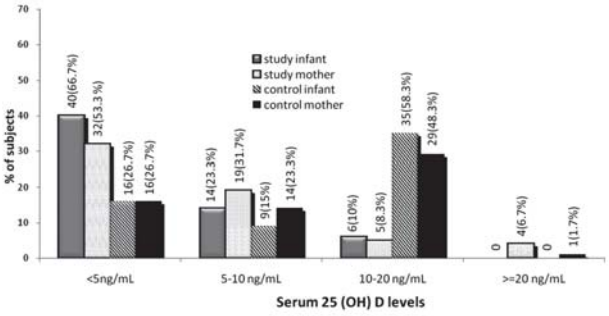

FIG. 1 *Serum 25 (OH) D levels in control and study subjects.*

Hypomagnesemia was noted in 4 infants with seizures and none of the controls. Seizures in these infants responded only when magnesium therapy was instituted.

A strong positive correlation was noted between serum 25(OH)D levels of mother-infant pairs in both study (r = 0.64; P<0.0001) and control populations (r = 0.39; P<0.002). However, no such correlation was seen for serum calcium. Of the 54 study infants who had 25(OH)D levels <10ng/mL, 48 (89%) were born to mothers who also had 25(OH) D <10ng/mL. Using logistic regression, infants born to mothers with 25 (OH) D <10 ng/mL had a 40 times increased risk of hypovitaminosis D when compared to those born to mothers with 25(OH) D levels ≥10 ng/mL.

DISCUSSION

Vitamin D deficiency continues to be a public health problem prevalent in many Asian countries, including India, despite abundant sunlight (8,9,14). Results of the present study demonstrate that majority of mother-infant pairs from our population were vitamin D deficient with mean levels of 25(OH)D being <10ng/mL. Using Lips criteria(13), all study and control infants were having vitamin D deficiency. However, even if a 25(OH)D level of <10ng/mL was taken as the cut off value, as reported in some earlier studies(4,6), 90% of the study and 41.7% of control infants were still affected. The seasonal impact on 25(OH)D levels in children was not evaluated because, during early infancy, most infants are kept indoors and covered due to prevailing sociocultural beliefs.

Balasubramanian, *et al.*(3), have reported that all 13 exclusively breast-fed infants with hypo-calcemic seizures had low serum 25(OH)D (mean 3.8 ± 2.08 ng/mL) and elevated PTH (106 ± 25.4 pg/mL). In a similar study conducted in Pakistan, in 65 infants presenting with hypocalcemic seizures, all the 15 mother-infant pairs in whom 25 (OH)D was estimated had levels <10ng/mL(3). Evaluating medical records of infants with a diagnosis of vitamin D deficiency and/or nutritional rickets, Hatun, *et al.*(15) reported that 79% had presented with seizures.

A large number of reports are available in the literature highlighting the high prevalence of 25(OH) D deficiency in women of childbearing age(16-21), during pregnancy(10,22-24), and lactation(15,25, 26), resulting in adverse effects in women, fetus, infants and children(22). Two earlier studies from India have also highlighted the high prevalence of hypovitaminosis D in women during pregnancy(8,10). Our study demonstrates a continuing high prevalence of vitamin D deficiency in lactating mothers, with 85% of the study mothers and 50% of control mothers having serum 25(OH)D levels <10ng/mL. Using similar cut offs for 25(OH)D, Dawodu, *et al.*(25) showed 61% of lactating mothers in Saudi Arabia to be vitamin D deficient.

The strong correlation between maternal and infant serum 25(OH)D levels noted in the present study support the fact that infant serum 25 (OH)D levels are dependent not only on the maternal serum 25(OH)D levels at birth, but also on the breastmilk vitamin D content and on sunlight exposure of the mother and the child, which are probably very similar as they share a common environment(27-29). A positive relationship between maternal and fetal (cord blood) circulating 25(OH)D levels has been reported in recent studies(10,30,31). A similar relationship in vitamin D status has also been reported between mothers and infants suffering from rickets(2,32,33). The only study where no correlation between maternal-infant 25(OH)D levels was reported was from Turkey(15).

We conclude that hypovitaminosis D in lactating mothers is strongly correlated with hypovitaminosis D in neonates and infants. Given this correlation, infants born to vitamin D deficient mothers are prone to develop hypocalcemic seizures. There is a need to

WHAT IS ALREADY KNOWN?

- Hypocalcemia is a well known cause of seizures, especially in infants.

WHAT THIS STUDY ADDS?

- Vitamin D deficiency is an important predisposing factor for symptomatic hypocalcemia in young infants and maternal vitamin D deficiency is a risk factor for its deficiency during infancy.

assess the vitamin D status of all pregnant and lactating women and to consider routine vitamin D supplementation to breastfed infants, and pregnant and lactating women.

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