Effects of seasonal variation and maternal clothing style on vitamin D levels of mothers and their infants

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In Turkey, vitamin D deficiency has been seen in children as well as in their mothers. We assessed the vitamin D status of 83 infants and their mothers, categorized into four groups according to season and mothers' clothing style. We measured serum levels of 25-hydroxy vitamin D, calcium, inorganic phosphorus and alkaline phosphatase in each mother-infant pair. In the summer, the rate of vitamin D deficiency was higher in mothers who wore clothing that covered nearly all of the body (55%) than in mothers whose clothing covered less of the body (13.6%) (p=0.016). In the winter, nearly half of both groups of mothers had vitamin D deficiency or insufficiency, and there were no significant differences based on their clothing style (p>0.05). In all groups of infants, vitamin D deficiency and insufficiency rates were found to be very low. It seems that 400 IU/daily vitamin D intake is adequate for infants in Turkey; for mothers, we propose vitamin D supplementation during the pregnancy and lactation period.

Key words: clothing, infant, mother, seasonal variation, vitamin D deficiency.

Because of the long half-life of 25-OHD, serum 25-OHD concentration is the best indicator of vitamin D status, which reflects the body's vitamin D stores[1,2]. Several factors have significant effects on serum vitamin D levels, including season, sunlight exposure, age and diet. Because of rapid growth in the skeletal system, infants aged 4 months to 2 years are at a high risk for vitamin D deficiency[3]. The majority of cases of rickets have been found to occur in the situation of prolonged breastfeeding without vitamin D supplementation, due to the inadequate vitamin D content of breast milk and inadequate exposure to sunlight[3]. Serum 25-OHD levels are dependent on vitamin D intake, maternal vitamin D stores and cutaneous synthesis[4]. Most foods have vitamin D content that is not adequate for normal plasma levels, and vitamin D fortification of food is not routine in many countries, so cutaneous production is the most important source[5,6]. Eighty to 100% of the body's vitamin D requirement is provided by the skin upon exposure to sunlight[7,8]. To maintain a sufficient vitamin D level in the body, at least 20% of the body's surface should be exposed to sunlight[9]. Vitamin D deficiency is a preventable condition, and the cost of prevention is inexpensive in most the countries. Although Turkey receives a considerable amount of sunshine, vitamin D deficiency remains a major public health problem among children. Although no vitamin D fortification of milk was taking place, routine vitamin D supplementation began being given to all infants from the first days of life, regardless of nutrition type, in 2005[10,11].

Even in the presence of adequate sunshine, a "covered" style of clothing and inadequate vitamin D intake can lead to vitamin D deficiency and insufficiency among breastfeeding mothers and their infants. In this study we aimed to evaluate vitamin D status in healthy infants and their mothers according to season and clothing style.

Material and Methods

The study was conducted in 2010-2011 during

two seasonal periods: summer (May-September) and winter (November-February). Eighty-three infants aged 3-4 months-old and their mothers were recruited for the study. All of the infants were born at term (38-42 weeks' gestation) and had birth weights above 2500 g. All of the infants were children who were breastfed exclusively; those receiving formula were excluded from the study. Infants and mothers with disorders likely to affect vitamin D status, e.g., liver, kidney or bone disorders, gastrointestinal tract diseases, and chronic disease or medication intake, were excluded. Four groups were designated, according to the mothers' clothing style and the season.

Group 1: Uncovered mothers and their infants in summer

Group 2: Covered mothers and their infants in summer

Group 3: Uncovered mothers and their infants in winter

Group 4: Covered mothers and their infants in winter

The study was approved by Ethics Committee of the Ankara Child Diseases Hematology Oncology Training and Research Hospital, Ankara, Turkey.

Each mother completed a questionnaire that covered the following information: season at admission, infant feeding history and intake of vitamin D supplementation, vitamin D supplementation during pregnancy, and sunlight exposure according to the clothing style of the mother and infant when outdoors.

Data collected from the mothers included biosocial characteristics, i.e., age, exposure to sunshine outdoors and dress style were recorded. Total sunshine exposure was calculated by multiplying the average number of minutes per exposure by the number of exposures per week.

The style of clothing worn while outdoors was assessed for how much skin it exposed to the sunshine. A "covered" style of dress was defined as one that involved covering the hair and wearing garments that completely covered the arms and legs.

The average duration of sunlight exposure per day was derived from the minutes spent outdoors during the week preceding completion of the questionnaire.

Biochemical tests were performed on each mother-infant pair, with serum concentrations of 25-hydroxy vitamin D (25-OHD), calcium (Ca) (8.6-10.8 mg/dl), inorganic phosphorus (P) (3.8-6.5 mg/dl) and alkaline phosphatase (ALP) (females:355-1037 U/L, males:297-1178 U/L) being measured. Serum 25-OHD was measured using liquid chromatography and tandem mass spectrometry (LC-MS/MS). Serum Ca, P and ALP levels were measured using the spectrophotometric method

In this study, vitamin D deficiency, insufficiency and sufficiency were defined as serum 25-OHD levels of less than 15 ng/ml, 15-20 ng/ml, and over 20 ng/ml, respectively[9].

Statistical Analysis

Statistical analysis was conducted using SPSS, version 11.0. Continuous variables were presented as means ± SD (range). Comparisons of the data were done between sets of two groups (covered and uncovered mothers, mothers in winter and summer, infants whose mothers were covered and uncovered, etc). For nominal data, the Mann-Whitney U test was used for comparison of the groups. The chi-square test was used to assess associations between categorical variables. Differences with $p<0.05$ were considered significant.

Results

All of the infants were born at term with a birth weight above 2500 g. The general characteristics of the study group are summarized in Table I. There were no statistical differences between the groups ($p>0.05$).

During the pregnancy period, all of the mothers had been recommended iron supplements by their obstetricians; none had used vitamin supplements containing vitamin D. All of the infants were receiving 3 drops (400 IU) of vitamin D3 per day as recommended. The mean 25-OHD levels of mothers and infants were 20.5±9 ng/ml and 39±12.8 ng/ml, respectively.

When the groups of mothers were compared for serum concentration levels of Ca, P and ALP, no significant differences were found between the summer-winter and covered-uncovered groups ($p>0.05$) (Table II). In the summer, mothers whose clothing style was uncovered *(group 1)* had mean serum 25-OHD levels higher than

Table I. Descriptive Characteristics of Infants and Mothers*

	Group 1	Group 2	Group 3	Group 4	p
Gender (n/%) (Male/Female)	16/6 (72.7/27.3)	8/12 (40/60)	9/11 (45/55)	10/11 (47.6/52.4)	0.139
Birth weight (g)	3216.36±347.15 (2680-3800)	3221.5±515.07 (2500-4700)	3195.5±445.07 (2500-4100)	3261.9±488.34 (2500-4000)	0.972
Mother's age (years)	26.31±3.68 (22-36)	26.5±5.14 (20-36)	27.6±4.12 (21-35)	26.52±4.51 (20-36)	0.781
Weight (g)	6377.27±1072.12 (3900-8500)	6440±934.93 (4700-8800)	6050±608.27 (5000-7900)	5980.95±925.53 (4700-8000)	0.270
Height (cm)	61.22±3.63 (53-68)	62.17±2.8 (57-68)	60.85±3.61 (54-68)	60.9±3.61 (54-68)	0.593
Head circumference (cm)	40.31±1.46 (37-43)	40.27±1.35 (38-43)	39.5±1.40 (36-42)	39.69±1.40 (37-42)	0.159

**All values are given as mean±standard deviation*
Group 1: Uncovered mothers and their infants in summer
Group 2: Covered mothers and their infants in summer
Group 3: Uncovered mothers and their infants in winter
Group 4: Covered mothers and their infants in winter

those of mothers wearing a covered style of clothing *(group 2)*; the difference was found to be statistically significant ($p<0.001$). In the winter, differences in mean serum 25-OHD levels between the two groups of mothers were not found to be statistically significant ($p=0.391$) (Fig. 1). In all groups of infants, regardless of seasonal variation or mothers' clothing style, serum 25-OHD levels and serum concentrations of Ca, P and ALP were found to be similar, i.e., demonstrating no statistically significant differences ($p>0.05$) (Table II).

When mothers and infants were categorized on the basis of their vitamin D levels into groups displaying vitamin D deficiency, insufficiency or sufficiency, 30.1% of mothers (n=25) and 2.4% of infants (n=2) fell into the vitamin D-deficient category (Table III). Serum levels of Ca, P and ALP were within the normal range in all three groups of mothers, with no statistically significant differences between them ($p=0.986$, 0.287, 0.498, respectively).

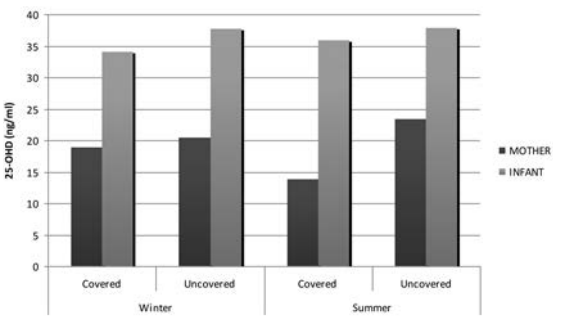

Fig. 1. Vitamin D levels of mothers and infants according to season and clothing style

In the summer, the rate of vitamin D deficiency was higher in mothers whose clothing type were covered (55%) than in mothers whose clothing type was uncovered (13.6%); the difference was found statistically significant ($p=0.016$). The rate of vitamin D sufficiency in these two groups was found to be 72.7% and 35.0%, respectively; here too the difference was found statistically significant ($p=0.014$). In the winter, nearly half of both groups of mothers were vitamin D deficient or insufficient; there were no significant differences between mothers with different clothing styles in the winter ($p>0.05$). Vitamin D levels of half of the mothers were found to be <20 ng/ml. In all groups of infants, regardless of seasonal variation or mothers' clothing style, vitamin D deficiency and insufficiency rates were found to be very low (Table III).

Discussion

We studied 3- and 4-month-old infants and their mothers; in the mothers, the overall prevalence of vitamin D deficiency (Vitamin D level<15 ng/ml) was found to be 30.1% (n=25); 64.0% in mothers with a covered style of dress and 36.0% in mothers with an uncovered style. This could be explained by the fact that food products are not fortified with

Table II. Serum Concentration Levels of Ca, P, ALP and 25-OHD in Mother-Infant Groups*

	Group 1		Group 2		Group 3		Group 4	
	Mother	Infant	Mother	Infant	Mother	Infant	Mother	Infant
Ca (mg/dl)	9.55 (8.8-10.1)	10.8 (10.37-11)	9.45 (8.95-9.97)	10.8 (10.37-11.2)	9.1 (8.8-10)	10.55 (10.22-10.87)	9.4 (8.5-10.1)	10.6 (10.3-10.85)
P (mg/dl)	3.55 (3.4-4.5)	6.1 (5.6-6.6)	4 (3.52-4.6)	6.4 (6-6.77)	3.4 (3.12-3.67)	6 (5.8-6.57)	3.5 (3.3-4.2)	6.1 (5.65-6.7)
ALP (U/L)	254 (168.75-369.75)	697 (644.25-882.25)	254.5 (207.75-306.25)	660 (546-893)	201 (174.25-371.25)	655.5 (362-718.5)	289 (199-399)	362 (285.5-520)
25-OHD (ng/ml)	23.4** (19.62-28.57)	37.9 (25.57-50.7)	13.8** (10.2-21.9)	35.95 (30.82-46.57)	20.35 (12.87-29.8)	37.7 (32.22-45.92)	18.9 (13.85-26)	34.1 (28.35-50)

**All values are given as medians (25-75 quartiles)*
***: p=0.001*

vitamin D and vitamin D supplementation is not routine the during pregnancy and lactation period, so that the main source of vitamin D is cutaneous synthesis. However, for religious and cultural reasons, some women choose to wear concealing clothing and have limited outdoor activity. In addition, mothers in Turkey have traditionally kept their infants completely bundled up when outdoors, especially in the winter, with only their faces showing. This way of dressing thus results in vitamin D deficiency in both infants and mothers because of the absence of cutaneous synthesis. In Turkey, vitamin D insufficiency has been reported in up to 46-80% of pregnant women and others of reproductive age[5,12]. Similar results have been reported in up to 18-84% of pregnant women elsewhere[7]. Therefore, vitamin D deficiency and insufficiency can be seen among breastfeeding mothers and their infants. Even in the presence of adequate sunshine, a covered style of dress and inadequate vitamin D intake can lead to low vitamin D levels in both breastfeeding mothers and infants; articles reporting similar results have been published by researchers studying various countries of the Middle East or other countries with Muslim communities[6,9,12,13]. It has also been shown that mean dietary vitamin D intake among Turkish pregnant and nursing women was below the recommended dietary allowance[12]. In our study, we asked only about vitamin D oral supplementation, not dietary intake, and this could be a limitation of our study. Although 30.1% of mothers had a vitamin D deficiency, none of them had symptoms suggesting hypovitaminosis D. Only two of these mothers' infants (8%) had vitamin D insufficiency; vitamin D levels were found sufficient in the others. We attributed this result to the practice of giving regular vitamin D supplementation to infants. If exogenous vitamin D is not provided, infants become vitamin D deficient after several weeks[14].

In one study, high-dose maternal (60,000 IU/monthly) and infant (400 IU/daily) vitamin D supplementation resulted in a 64% reduction in the prevalance of vitamin D deficiency among exclusively breastfed infants[6]. Another study showed that if mothers were provided with daily 400 IU vitamin D supplementation during the lactation period, the vitamin D content of milk ranged from <25 up to 78 IU/L[15].

It is known that there exists a close relationship between the vitamin D levels of mothers and infants[9]. Furthermore, the most important risk factor for low 25-OHD levels in infancy has been found to be a maternal 25-OHD level lower than 10 ng/ml[12]. 25 OHD is one of the major metabolites to cross the placenta. Many mothers have low 25-OHD levels during pregnancy, and this is associated with their infants having low 25-OHD levels at birth[9]. Infants who were diagnosed with vitamin D deficiency before the age of 3 months and whose mothers had low 25-OHD levels all had a history of limited sunshine exposure. The authors attributed this result to the fact for the first 6 weeks of life infants were kept indoors for reasons of tradition[4].

It is known that vitamin D stores in infancy are based on fetal stores and maternal vitamin

Table III. Vitamin D Status of Mothers and Infants According to Season and Clothing Style

Season	Clothing style		Vitamin D levels (ng/ml) <15 (Deficiency) n (%)	15-19.9 (Insufficiency) n (%)	>20 (Sufficiency) n (%)
Summer	Uncovered	Mother	3 (13.6)*	3 (13.6)	16 (72.7)**
		Infant	1(4.5)	1 (4.5)	20 (90.9)
	Covered	Mother	11 (55.0)*	2 (10.0)	7 (35.0)**
		Infant	-	-	20 (100.0)
Winter	Uncovered	Mother	6 (30.0)	4 (20.0)	10 (50.0)
		Infant	1 (5.0)	1 (5.0)	18 (90.0)
	Covered	Mother	5 (23.8)	7 (33.3)	9 (42.9)
		Infant	-	1 (4.8)	20 (95.2)

*p=0.016, **p=0.014

D status[15,16]. A recommendation that has been proposed for instances where there is no vitamin D supplementation is to expose the face, arms and hands for a short period at midday several times a week in order to maintain normal vitamin D levels [16,17]. Despite ample sunlight in places including Arab and South Asian countries, infantile vitamin D deficiency is common; this situation has been attributed to their mothers' low 25-OHD levels. This is in contrast to the situation in developed countries, where mean maternal serum 25-OHD levels have been found to correlate with normal levels of 25-OHD in infants. In agreement with previous studies[18,19], we found the mean level of 25-OHD to fall in the insufficiency range (20.52±9.03 ng/ml) in mothers, regardless of season or clothing style. Although mothers who dressed in an uncovered style had mean serum 25-OHD levels significantly higher than those of the "covered" mothers in the summer, mean serum 25-OHD levels of both groups of mothers were low in the winter. Since 2011, 1200 IU/daily vitamin D supplementation has been recommended in Turkey for all pregnant or breastfeeding mothers from the second trimester up to the end of the sixth month of lactation[20]. Although the vitamin D supplementation campaign began around the time of our study, none of our mothers reported an adequate vitamin D intake.

In previous studies, seasonal variation in 25-OHD levels has been clearly demonstrated. During the winter, children of all ages have lower vitamin D levels than in summer[9]. To our knowledge, our study is the first that has been conducted with mother-infant pairs addressing the influence of clothing style and season in Turkey. In our study, we found that in all groups of infants, regardless of seasonal variation and mothers' clothing style, vitamin D levels were generally adequate. That is, vitamin D deficiency and insufficiency rates were found to be low, which could be related to regular vitamin D supplementation for infants in Turkey. In past years, the recommended daily amount of vitamin D for all children and infants was 200 IU [21], but the American Academy of Pediatrics (AAP) currently recommends a daily amount of 400 IU from the first days of life[9]. Some authors have thought that for Turkey, this amount may be not sufficient to prevent vitamin D deficiency, while others have indicated that clinical rickets and severe vitamin D deficiency have diminished dramatically in Turkey following the vitamin D supplementation campaign[12]. According to our results, it seems that 400 IU daily vitamin D intake is adequate for Turkey. Mutlu et al.[22] concluded that although the maternal vitamin D deficiency rate is high, daily 400 IU vitamin D supplementation has been shown to be sufficient for infants. However, Onal et al.[23] suggested that even though daily 400 IU vitamin D intake appears to be sufficient during the first year, given the fact that vitamin D deficiency continued even after the supplementation campaign, vitamin

D supplementation should be at least 600 IU daily in Turkey.

In infants, limited exposure to sunlight due to being completely wrapped up means that skin synthesis of vitamin D is prevented, leading to vitamin D deficiency[24]. The fact that significant seasonal differences in vitamin D levels were not found even though infants had more sunlight exposure in summer could be related to regular vitamin D supplementation. Although no differences were found between the vitamin D levels of infants, in the uncovered mothers' group vitamin D levels <15 ng/ml were found in one infant in the summer and one in the winter. This could be related to problems in administering the supplement, such as missing a dose or vomiting after a dose, or to individual differences of vitamin D absorption.

In mothers, rates of vitamin D deficiency and insufficiency were 33.3% and 11.9%, respectively, in the summer; rates of deficiency and insufficiency were both the same, 26.8%, in the winter. Thus, in the summer only 54.7% of mothers had sufficient levels of vitamin D; this rate dropped yet further in the winter, to 46.3%. These data indicate that despite adequate sunshine, vitamin D deficiency and insufficiency can be seen in breastfeeding mothers and/or their infants. In view of this, advising women to utilize sunshine and oral vitamin D replacement, especially during the pregnancy and lactation period, should be considered, even though, according to our results, it seems that 400 IU daily vitamin D intake is adequate for infants in Turkey. As have other authors, we propose that vitamin D supplementation may be necessary during the pregnancy and lactation period. At the same time, an educational campaign regarding sufficient exposure to sunlight could be initiated, such as to encourage pregnant and lactating mothers to go outdoors themselves and/or ensure that their infants are taken outdoors for 10-15 minutes of sun exposure (at times other than the middle of the day) on a regular basis. In addition, adequate vitamin D intake and fortification of food with vitamin D should be promoted, and vitamin D prophylaxis for infants continued.

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