Vitamin D Status in A Sample of Preschool Children Aged From 1 to 6 Years Visiting the Pediatrics Clinic at Jordan University Hospital

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Abstract

Objectives: Vitamin D status was investigated in 200 children (100 males and 100 females) visiting the Pediatrics Clinic at Jordan University Hospital.

Methods: Subjects were categorized into two age groups: 1-3 years (toddlers) and 3-6 years (preschool children). Data were collected on children's lifestyle factors, dietary intake, height, weight and BMI. Serum 25-hydroxyvitamin D_3 (25 (OH) D_3) concentration was determined.

Results: 16.5% of the children had vitamin D deficiency (serum 25 (OH) $D_3 \leq 15$ ng/mL), while 15.5% had vitamin D insufficiency (serum 25 (OH) D_3 from 15 to 20 ng/mL). Serum 25 (OH) D_3 levels for the toddlers (26.25 ± 1.16) were significantly higher ($P<0.05$) than those of the preschool children (21.49 ± 1.16). Serum 25 (OH) D_3 was significantly higher ($P<0.05$) for exclusively formula-fed children (27.42 ± 0.86) or children with mixed feeding (26.69 ± 0.92) than for those who were exclusively breast-fed (17.02 ± 0.90). Also, serum 25 (OH) D_3 levels were positively correlated with the duration of formula feeding ($r = 0.4849$, $P<0.0001$), duration of outdoor physical activity ($r = 0.3940$, $P<0.0001$), monthly frequency of outdoor physical activity ($r = 0.61087$, $P<0.0001$), and vitamin D ($r = 0.4678$, $p<0.0001$) and calcium ($r =0.4951$, $p<0.0001$) intakes. However, serum 25 (OH) D_3 levels were negatively correlated with duration of breast feeding ($r = -0.3554$, $P<0.0001$) and BMI ($r = -0.4235$; $P<0.0001$).

Conclusions: It is concluded that hypovitaminosis D is common in preschool children and that lifestyle conditions influence vitamin D status.

Keywords: Hypovitaminosis D, Jordan, 25-OH D_3.

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Introduction

Vitamin D or cholecalciferol is the "the sunshine vitamin" and is produced photochemically in the skin from 7-dehydrocholesterol (7-DHC). It may be considered a prohormone, rather than a vitamin, as it is converted in the body from cholecalciferol to the hormone 1, 25-dihydroxyvitamin D_3 (calcitriol) . [1, 2] Vitamin D plays an important role in the maintenance of normal blood levels of calcium and phosphate, which are in turn needed for the normal mineralization of bone, muscle contraction, nerve conduction and general cellular function in all

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cells of the body.[3-5] Its sufficiency, especially during the childhood and adolescent years, is critically important not only for bone health, but also for the prevention of many serious chronic diseases, including cancer, cardiovascular and autoimmune diseases. It has been suggested that vitamin D deficiency during infancy and childhood may increase the risk of these chronic diseases for the rest of one's life. [6]

Vitamin D deficiency has been known to cause skeletal deformities as early as the mid-17th century in London, England.[7] It is well known that, currently, vitamin D deficiency is prevalent in children and adults living in the United States, Europe, Middle East, India, Australia, New Zealand and Asia. [8]

Deficiency of vitamin D can occur when the usual intake is lower than recommended levels over time, or due to limited exposure to sunlight, impairment in the metabolic activation of the vitamin D or inadequate absorption of vitamin D from the digestive tract. [9] In addition, several factors potentially affect vitamin D status. These include genetic factors, adiposity and factors affecting the cutaneous synthesis of vitamin D such as skin pigmentation, age, season, latitude, time spent outdoors, clothing and use of sunscreens. [10, 11] The most common symptoms of vitamin D deficiency are rickets in young children and osteomalacia in adults. Rickets is characterized by continued formation of osteoid matrix and cartilage that are improperly mineralized resulting in soft, pliable bones.[12]

Serum concentration of 25-hydroxyvitamin D (25 (OH) D) may be considered the best and most practical indicator of the vitamin D status. It reflects both vitamin D produced cutaneously and that obtained from food and supplements and has a fairly long circulating half-life of 15 days. [13]

Hypovitaminosis D has been reported in children in sunny countries, including many Middle Eastern countries.[14, 15] Accordingly, it is expected that a high number of vitamin D deficient children can be found in Jordan. To the best of our knowledge there have been no previous studies performed on preschool children in Jordan to evaluate their vitamin D status.

Therefore, this study is aimed at the investigation of vitamin D status among a sample of toddlers and preschool children with the goal of finding out the relationship between their vitamin D status and certain sociodemographic factors, lifestyle factors and dietary intake of the vitamin.

Materials and Methods

Selection of the Sample

This study was conducted at Jordan University Hospital during the months of May and June of 2009. A sample of 200 preschool children was recruited from the Pediatrics Clinic at Jordan University Hospital. Children were assigned from both sexes and chosen from two major age group categories: 1-3 years (toddler) and 3-6 years (preschool children). A physical examination and full medical history for each of the subjects were conducted by pediatricians. Exclusion criteria included hepatic or renal disease, metabolic bone disease, fat malabsorption, type I diabetes and medications influencing bone metabolism such as antacids, anticonvulsants and antirejection medications. Children were also excluded if they had received supplements containing vitamin D before testing. The study was approved by the Research Ethics Committee at Jordan University Hospital and a written informed consent was obtained from all parents or legal guardians.

Questionnaire

The parents or legal guardians of each participant answered, through a personal interview, a detailed basic information questionnaire that was prepared in Arabic. This questionnaire included personal information such as the subject's name, sex and date of birth and information on the child's lifestyle factors such as duration of outdoor physical activity, monthly frequency of outdoor physical activity in the past month and intensity of indoor activity and TV-watching. The reliability of the questionnaire was confirmed by Cronbach's Alpha test. [16]

In addition, parents provided nutritional data for the children including type of milk fed during infancy (breast or formula), duration of breast feeding and/or formula feeding and dietary calcium and vitamin D intake using a food frequency table. Parents or caretakers were requested to record the frequency of intake of 31 selected food items that are considered sources of vitamin D and/or calcium. Photographs and standard portion sizes (measuring cups and spoons) were used to support the estimation of portion sizes. Vitamin D and calcium intakes were calculated based on food composition tables and available literature. [1, 9, 17- 19]

Anthropometric Measurements

Height and weight were measured while subjects wore light clothing and were without shoes. The recumbent length of toddlers less than 2 years of age was measured on a length board with a fixed head board and moveable foot board. Stature or standing height of preschool children was measured using a height board. Body weight and height were measured to the nearest 0.1 kilogram (kg) and 0.1 centimeter (cm), respectively, using a digital scale (BA Wang DT 150) and a height board (Seca 200, Germany). BMI was calculated as weight (in kg)/height (in m). The BMI cutoffs provided by the Center for Disease Control and Prevention (CDC) were used to classify children as underweight, normal, with risk of becoming overweight or overweight. [20]

Biochemical Assessment

Non-fasting venous blood samples were obtained from all subjects. Blood samples were taken by a registered nurse, using blue butterfly needle gage 23 (Enteplin, Egypt) to obtain 5 milliliters of blood by vein puncture. The blood samples were placed in plain tubes (Zoimed, Germany) and left to coagulate at room temperature, then centrifugation was done at 3000 rpm for 15 minutes to get blood serum. Serum specimens were then stored frozen at -18ºC until analyzed**.**

Serum 25 (OH) D_3 was measured by the electrochemiluminescence immunoassay method (ECLIA) (Roche, USA), in the Medical Laboratories of Khalidi Medical Center (Amman, Jordan). Inter-assay coefficients of variation for 25 (OH) D_3 were 9.0% to 15.0%. The limit of detection was 4 ng/mL or 10 nmol/L.

Criteria of Vitamin D Status

The children were divided into three diagnostic categories according to their serum 25 (OH) D_3 concentration. In increasing order of severity, the serum 25 (OH) D_3 levels were as follows: vitamin D insufficiency, from 15 to 20 ng/mL; vitamin D deficiency, ≤ 15 ng/mL; and severe vitamin D deficiency, ≤ 5 ng/mL. The definition of severe vitamin D deficiency, vitamin D deficiency and insufficiency were based on data from previous studies. [18, 21, 22]

Statistical Analysis

Statistical analysis was performed using the Statistical Analysis System (SAS) software package, version 9 at the University of Jordan. The results of each variable studied were subjected to analysis of variance (ANOVA) followed by least significant difference (LSD). The association between serum vitamin D and other parameters were assessed using the Pearson correlation coefficient. Results were expressed as mean ± standard error of means (SEM) and frequency distribution. Differences at $P < 0.05$ were considered significant.

Results

Description of the Study Sample

A total of 200 children were studied in May through June 2009, with equal numbers from both genders**.** As shown in Table 1, the means for the ages of the toddler groups (boys and girls) were 23.7 and 23.5 months respectively. Whereas the means for the ages of the preschool children groups (boys and girls) were 55.37 and 57.12 months respectively. In both genders and age groups, there were no significant differences (P>0.05) in BMI, duration of breast feeding, duration of formula feeding, duration of outdoor physical activity, calcium intake and vitamin D intake. The mean value of serum 25 (OH) D_3 was

significantly higher (P<0.05) in toddler groups than those of preschool children groups (Table 1).

Age and Gender Groups

The percentage distribution of the study groups according to their vitamin D status is presented in Table 2. It can be noted that 16.5% of the study children had a vitamin D deficiency, while 15.5% had a vitamin D insufficiency and 68% had a normal vitamin D level.

As mentioned above, the mean value of serum 25 (OH) D_3 levels was significantly higher (P<0.05) in toddlers than in the preschool children (Table 1). In addition, there was a negative correlation between age and serum 25 (OH) D_3 levels (r = -0.3741; P<0.0001).

Anthropometric Indicators

The mean value of serum 25 (OH) D_3 levels was significantly higher (P<0.05) in normal and underweight children than in those children who were overweight or at risk of becoming overweight as shown in Table (3). Moreover, serum 25 (OH) D_3 levels were negatively correlated with BMI (r = -0.4235; P<0.0001).

Life Style Characteristics

Serum 25 (OH) D_3 levels for the children who spent more than one hour at outdoor physical activities were significantly higher (P<0.05) than those for children who spent 30 minutes or less, but not significantly higher (P>0.05) than the children who spent time at outdoor activities between 30-60 minutes as shown in Table 4. The duration of outdoor physical activity showed a positive correlation with serum 25 (OH) D_3 levels (r = 0.3940, P<.0001). In addition, it can be seen that the mean levels of serum 25 (OH) D_3 for the children with outdoor physical activity from 21 - 25 and from 26 - 31 times in the past month were significantly higher (P<0.05) than those for children with outdoor physical activities of less than 20 times in the past month. In addition, serum 25 (OH) D_3 was positively correlated with the monthly frequency of outdoor physical activity (r = 0.61087, P<0.0001). Indoor physical activity and watching TV are also shown in Table (4). The mean value of serum 25 (OH) D_3 levels was significantly lower (P<0.05) for the children who participated in indoor physical activities and watched extensive amounts of TV (> 8 hours per day) than children who participated in indoor physical activities and watched less TV (< 8 hours per day).

Nutritional Data

Regarding the type of feeding during infancy, the mean value of serum 25 (OH) D_3 levels was significantly higher (P < 0.05) for the children who were exclusively formula-fed or those who had mixed feeding (breast and formula feeding) as compared with those who were exclusively breast-fed (Table 5). Moreover, there was a negative correlation between duration of breast feeding and serum 25 (OH) D_3 levels (r =-0.3554, P<0.0001), while there was a positive correlation between duration of formula feeding and serum 25 (OH) D_3 levels (r = 0.4849, P<0.0001).

As mentioned above, there were no significant differences (P>0.05) in the means of vitamin D and calcium intakes among the four study groups; however, calcium and vitamin D intakes were slightly higher in toddler groups than those of the preschool children groups. Moreover, serum 25 (OH) D_3 was positively correlated with intakes of vitamin D (r =0.4678, p<0.0001), calcium (r =0.4951, p<0.0001), milk (r =0.4998, p<0.0001), yogurt (r =0.2580, p<0.0002), cheese (r =0.4469, p<0.0001), egg (r =0.4959, p<0.0001), fish (r =0.4593, p<0.0001) and liver (r =0.4294, p<0.0001).

Table (1): Characteristics of the study group.[1,2]

	Boys		Girls	
Variable	1-3 years	>3-6 years	1-3 years	>3-6 years
Number	50	50	50	50
Age (month)	23.7 ± 1.0	55.4 ± 1.8	23.47 ± 1.0	57.12 ± 1.5
Body mass index (kg/m²)	16.1 ± 0.2	16.2 ± 0.2	15.7 ± 0.2	15.7 ± 0.2
25 (OH) D_3 levels (ng/mL)	27.1 ± 1.3 [a]	21.2 ± 0.9 [b]	25.4 ± 1.3[a]	21.8 ± 1.0[b]
Duration of breast feeding (month)	7.1 ± 0.9	10.6 ± 1.2	6.3 ± 1.0	8.9 ± 1.2
Duration of formula feeding (month)	10.7 ± 1.1	8.3 ± 1.2	9.2 ± 0.9	8.1 ± 1.1
Duration of outdoor physical activity (min./d)	35.6 ± 3.6	45.6 ± 4.7	32.1 ± 3.8	39.8 ± 5.2
Calcium intake (mg)	771.4 ± 59.3	590.1 ± 45.5	764.0 ± 57.6	562.8 ± 44.1
Vitamin D intake (IU)	198.4 ± 17.4	156.1 ± 14.1	208.6 ± 19.9	173.0 ± 21.8

1. Each value is represented as means ± SEM.
2. Means with different superscripts within the same row are significantly different (analysis of variance, P<0.05) using Fischer's Protected Least Significant Differences Test.

Table (2): Distribution of the study groups according to vitamin D status.*

	Vitamin D Status		
Gender	Normal n (%)	Insufficiency n (%)	Deficiency n (%)
Boys	65 (65%)	19(19%)	16(16%)
Girls	71 (71%)	12 (12%)	17 (17%)
Total	136 (68%)	31 (15.5%)	33 (16.5%)

n= number of children involved in the study
****Vitamin D deficiency, insufficiency and normal levels are defined when the level of serum vitamin D is not exceeding 15ng/mL, more than 15 to 20 ng/mL and more than 20 ng/mL respectively.***

Table (3): Vitamin D levels of the study groups according to the body mass index classification. [1,2]

Body mass index classification (BMI) [3]	Number of children n= 200	25 (OH) D_3 Levels (ng/mL)
Under weight	16	23.9 ± 1.9 [a]
Normal weight	160	25.4 ± 0.6 [a]
Risk of becoming overweight	13	15.1 ± 2.2 [b]
Overweight	11	13.0 ± 2.4 [b]

1- Each value is represented as means ± SEM.
2- Means with different superscripts within the column are significantly different (analysis of variance, P<0.05) using Fischer's Protected Least Significant Differences Test.
3- BMI= Weight (kg) / (Height (m))^2, according to CDC classification, BMI less than 5th percentile indicates underweight, BMI between 5th percentile to less than the 85th percentile indicates healthy weight, BMI between 85th to less than the 95th percentile indicates risk of overweight and BMI equal to or greater than the 95th percentile indicates overweight.

Table (4): Vitamin D levels of the study groups according to life style factors. [1,2]

Independent variable	Number of children n= 200	25 (OH) D_3 Levels (ng/mL)
Duration of outdoor physical activity		
< 15 min	29	18.2 ± 1.6 [c]
≥ 15 – 30 min	63	24.6 ± 1.4 [b]
> 30 – 60 min	66	27.8 ± 0.9 [a]
> 60 min	42	28.4 ± 1.1 [a]
Monthly frequency of outdoor physical activity		
None	18	15.6 ± 1.4 [c]
1-5	20	15.3 ± 1.1 [c]
6-10	43	23.8 ± 1.4 [b]
11-15	34	24.0 ± 1.6 [b]
16-20	35	25.1 ± 1.0 [b]

21-25	*31*	*30.1 ± 1.1* [a]
26-31	*19*	*33.0 ± 1.5* [a]
Intensity of indoor activities and watching TV		
To a great extent (> 8 hours per day)	*50*	*16.7 ± 0.8* [c]
To a moderate extent (4 - 8 hours per day)	*72*	*19.9 ± 1.0* [b]
To a lesser extent (< 4 hours per day)	*78*	*27.2 ± 0.8* [a]

n= number of children involved in the study
1- Each value is represented as means ± SEM.
2- Means with different superscripts within the column are significantly different (analysis of variance, P<0.05) using Fischer's Protected Least Significant Differences Test.

Table (5): Vitamin D levels of the study groups according to type of feeding during infancy. [1,2]

Independent variable	***Number of children n= 200***	***25 (OH) D_3 Levels (ng/mL)***
Type of feeding		
Exclusive breast feeding	*64*	*17.0 ± 0.9* [b]
Exclusive formula feeding	*64*	*27.4 ± 0.8* [a]
Breast and formula feeding	*72*	*26.7 ± 0.9* [a]

n= number of children involved in the study
1- Each value is represented as means ± SEM.
2- Means with different superscripts within the column are significantly different (analysis of variance, P<0.05) using Fischer's Protected Least Significant Differences Test.

Discussion

Serum Vitamin D Level

There has been consensus that most children should be able to synthesize sufficient vitamin D by brief exposure to sunlight and that only children living in northern or southern latitudes may require supplementation with vitamin D. Examination of recent evidence on vitamin D deficiency leads to different conclusions. Several studies have shown a high prevalence of vitamin D deficiency in tropical and sunny countries such as Turkey, India, Iran, Lebanon and Saudi Arabia. [14, 23-26] In Jordan, similar to other Middle Eastern countries, there have been no governmental regulations mandating vitamin D fortification of food products. The main source of vitamin D is, therefore, through skin synthesis in response to sun exposure. Information about vitamin D status in Jordan is scarce. Furthermore, vitamin D status in healthy preschool children from a sunny country like Jordan and its association with nutrition and lifestyle has not been objectively studied. Hence, the current study was planned to assess vitamin D status in preschool children in Jordan.

Our study demonstrates that 16.5% of the study sample had vitamin D deficiency, 15.5% had vitamin D insufficiency and 68% of them had normal vitamin D. The present finding is in agreement with the results of the studies from sun-rich countries like Lebanon, Egypt, Turkey and India. [14, 24, 27]

Age and Gender Groups

In this study, the mean value of serum vitamin D for the toddler groups was significantly higher (P<0.05) than those of the preschool children groups. The explanation for this result may be due to higher vitamin D and calcium intake in toddler groups than in those of the preschool children groups as toddlers are more likely to consume more milk and vitamin D fortified foods. However, there were no significant differences (P>0.05) in mean serum vitamin D between the gender groups. This result was similar to that reported by Nicolaidou et al. and Gordon et al., who found that there were no significant differences in serum vitamin D between boys and girls. [15, 21]

Anthropometric Indicators

In the present study, the mean value of serum vitamin D was significantly higher (P<0.05) in normal and underweight children than in those who were overweight or at risk of becoming overweight. This result is consistent with the results of many studies that have investigated the prevalence of vitamin D deficiency among overweight and obese people, suggesting that the larger body fat compartments in the obese individuals sequester vitamin D. [21- 22, 28-29]

Life Style Characteristics

Another possible determinant of low vitamin D status in this study could be low sunshine exposure which may be explained by less time spent on outdoor physical activity and greater indulgence in indoor activities like watching television, computer gaming and other recreational activities. [18] Our study demonstrates that the mean value of serum 25 (OH) D_3 levels was significantly lower (P<0.05) for the children who participated in indoor physical activities and spent extensive time watching TV (> 8 hours per day) than children who participated in indoor physical activities and watched less TV (< 8 hours per day). However, serum 25 (OH) D_3 levels were positively correlated with the duration of outdoor physical activity (r = 0.3940, P<0.0001) and the monthly frequency of outdoor physical activity (r = 0.61087, P<0.0001). This is in agreement with the fact that the main source of vitamin D is that produced by the action of solar ultraviolet B (UVB) radiation acting on 7-DHC in the skin. [10]

Nutritional Data

The results of the present study are in agreement with those proposing that 25(OH) D_3 levels are influenced by the type of feeding during infancy. These studies have shown that breast milk typically contains about 25 IU or less of vitamin D per liter, which when taken alone is insufficient for the prevention of rickets. [7] In addition, as the breast-feeding period is extended (i.e. more than 6 months), the incidence of vitamin D deficiency-caused rickets is expected to rise. [30] On the other hand, all infant formulas have a minimum vitamin D concentration of 40 IU/100 mL. Thus, if an infant is taking at least 500 mL per day of formula (vitamin D concentration of 40 IU/100 mL), he or she will receive the recommended vitamin D intake of 200 IU per day. [30]

Based on the food frequency table, there were no significant differences (P>0.05) detected in the vitamin D or calcium intake among the four study groups. However, as expected, calcium and vitamin D intakes were slightly higher in toddlers than those of the preschool children groups. This may be due to high intake of dairy products, especially fortified milk, in the toddler age group. In addition, the present study showed significant positive correlation between serum vitamin D and intakes of vitamin D, calcium, milk, yogurt, cheese, eggs, fish and liver. These results are in agreement with the results of the study conducted by Fuleihan and her colleagues. [14] These authors found that vitamin D intake was significantly correlated with 25 (OH) D. Furthermore, Scragg and Camargo showed that serum vitamin D levels were positively associated with milk intake, but not with cereal intake. [31]

It is concluded from these results that even in a sunny country like Jordan, a significant proportion of preschool children suffered from vitamin D deficiency and insufficiency and that lifestyle conditions influenced the vitamin D serum levels of the studied sample of children.

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الوضع التغذوي لفيتامين "د" في عينة من الأطفال بعمر 1–6 سنوات من مراجعي عيادة الأطفال في مستشفى الجامعة الأردنية

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الملخص:

هدف الدراسة: أجريت هذه الدراسة بهدف معرفة الوضع التغذوي لفيتامين د في عينة من الأطفال بعمر 1–6 سنوات يراجعون عيادة الأطفال في مستشفى الجامعة الأردنية ، ويهدف تقدير العلاقة بين الخصائص الإجتماعية وأسلوب الحياة والعادات الغذائية من جهة والوضع التغذوي لفيتامين د لهؤلاء الأطفال من جهة أخرى.

منهجية البحث: قسّمت عينة الدراسة إلى مجموعتين: مجموعة بعمر 1–3 سنوات والأخرى بعمر 4–6 سنوات، وجمعت معلومات حول نمط الحياة والأغذية المتناولة والوزن ومنسب كتلة الجسم وتركيز 25–هيدروكسي فيتامين د.

النتائج: أظهرت النتائج أن 16.5 % من الأطفال كانوا يعانون من نقص فيتامين د، بينما عانى 15.5% منهم من عدم كفاية الفيتامين. وكان مستوى فيتامين د في الأطفال من الجنسين كليهما في الفئة العمرية 1–3 سنوات أعلى معنوياً ($P<0.05$) منه في الفئة العمرية 3–6 سنوات (26.25±1.16 مقابل 21.49± 1.16). وأظهرت النتائج أن مستوى فيتامين د في دم الأطفال الذين رضعوا رضاعة صناعية (27.42± 0.86) والأطفال الذين رضعوا خليطا من الرضاعة الطبيعية والصناعية (26.69±0.92) كان أعلى معنوياً ($P<0.05$) من الأطفال الذين رضعوا رضاعة طبيعية (17.02±0.90) . كما بينت النتائج وجود علاقة إيجابية بين مستوى فيتامين د في الدم مع مدة الرضاعة الصناعية (ر= 0.4849 ، $P\leq0.0001$). ولوحظ وجود علاقة إيجابية بين مستوى فيتامين د في الدم مع مدة اللعب (ر=0.3940 ، $P\leq0.0001$) وعدد مرات اللعب خارج المنزل خلال الشهر الواحد (ر= 0.6109 ، $P\leq0.0001$). وبين مستوى فيتامين د في الدم وكمية فيتامين د وكمية الكالسيوم المتناولة من الطعام(ر=0.4678، $P\leq0.0001$ و ر=0.4951 ، $P\leq0.0001$ على التوالي). وقد أظهرت النتائج وجود علاقة سلبية بين مستوى فيتامين د مع مدة الرضاعة الطبيعية (ر= 0.3554 – ، $P\leq0.0001$) و بين زيادة منسب كتلة الجسم ومستوى فيتامين د (ر= 0.4235 – ، $P\leq0.0001$).

الاستنتاجات: يستنتج من هذه الدراسة وجود نسبة جيدة من أطفال الأردن بعمر 1–6 سنوات يعانون من نقص فيتامين د أو عدم كفايته. كما ثبت وجود علاقة بين الوضع التغذوي للفيتامين د والظروف المتعلقة بنمط الحياة في الأردن.

الكلمات الدالة: نقص فيتامين د، الأردن، 25–هيدروكسي فيتامين د .