

## Original Article

# Children With Vitamin D Deficiency: Is A Wrist X-Ray Necessary?

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## Abstract

**Background:** Rickets is failure in mineralization of growing bone and cartilage due to extreme vitamin D deficiency (VDD). The study aimed to identify rickets among vitamin D deficient children and determine any relationship between clinical findings and paraclinical evidence.

**Methods:** This study was conducted in two stages. In the first stage, blood was drawn from 406 children aged 30–72 months for measurement of 25(OH)D level. Of these children, 108 had 25(OH)D levels of <20 ng/dL and were evaluated physically for signs and symptoms scores (0-1) of VDD and rickets. Biochemical analysis and radiography of the child's left wrist and hand was performed.

**Results:** Of the 119 children (29.67%) with 25(OH)D levels of <20 ng/dL, 42 (10.3%) had vitamin D levels of  $\leq 15$  ng/dL. There was no correlation between serum 25(OH)D level and levels of calcium (Ca) ( $r = -0.16$ ), alkaline phosphatase (ALP) ( $r = -0.12$ ), P ( $r = 0.13$ ), and parathyroid hormone (PTH) ( $r = -0.15$ ) in children with VDD. The mean of signs and symptoms scores had no significant difference between children with ( $1.59 \pm 0.8$ ) and without ( $1.73 \pm 1.01$ ) VDD ( $P = 0.3$ ). None of the children with VDD had radiographic evidence of rickets. Radiographic data showed that 69.2% (72), 10.6% (11), and 20.2% (21) of the children had delayed, normal, and advanced bone age, respectively.

**Conclusion:** Abnormal radiological findings of rickets were not found on wrist X-rays. Thus, this investigation is not necessary within the range of vitamin D levels described in the current study.

**Keywords:** Children, Radiography, Rickets, Vitamin D deficiency

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## Introduction

Nutritional rickets, the most obvious consequence of vitamin D deficiency (VDD), continues to affect children and adolescents worldwide.<sup>1</sup> Rickets has been described as being among the five most common diseases in children in developing countries.<sup>2</sup> Rickets is the failure of mineralization in growing bone and cartilage during childhood<sup>3</sup> that causes considerable lifelong disability.<sup>2,3</sup> Rickets is an example of extreme consequence of VDD.<sup>4</sup> Vitamin D is a prohormone that is vital material for skeletal growth by inducing the absorption of calcium (Ca) from the gut.<sup>3</sup> VDD reduces intestinal Ca and phosphorus (P) absorption, and in response, parathyroid hormone (PTH) levels increase, leading to mobilization of Ca from bone so that serum Ca levels remain normal or are only moderately decreased.<sup>4</sup>

VDD has different causes, including limited exposure to sunlight (type of clothing traditionally worn, use of sunscreen, and amount of indoor activity), seasonal geographic latitude and altitude, diet, age, and the presence of atmospheric pollution.<sup>5</sup> Rickets associated with VDD is preventable with adequate sunshine exposure and nutritional

intake of vitamin D.<sup>4</sup> However, nowadays, despite numerous preventive strategies, a resurgence of VDD and rickets in infants and children remains a concern.<sup>6</sup> Thus, VDD has been and remains as a long-standing public health issue worldwide.<sup>7</sup>

Various studies have shown there is still no agreement on the 25(OH)D level that defines a sufficient level of vitamin D. According to suggestions of the Endocrine Society Clinical Practice Guidelines, 25(OH)D concentrations of  $\geq 75$  nmol/L (30 ng/mL), between 52.5 and 72.5 nmol/L (21–29 ng/mL), and  $< 50$  nmol/L (20 ng/mL) are considered indicative of vitamin D sufficiency, insufficiency, and deficiency, respectively.<sup>1</sup>

Features of radiological rickets include widening of the growth plate, and metaphyseal splaying and cupping. Owing to the effect of vitamin D on the mineralization of the growing bone, radiography of the wrist or knee has been recommended.<sup>8</sup> Some authors have reported that severe VDD can impair mineralization of bone tissue and growth plates.<sup>7</sup> Some previous study results showed that VDD-associated rickets occur when 25(OH)D levels are  $< 5$  ng/

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mL.<sup>9</sup> Therefore, they concluded that radiographic evaluation should be performed for all children with VDD. Thus, this study was conducted to answer the following questions: What are the radiological findings in children with VDD? Are the radiological findings related to vitamin D levels in children with VDD? We aimed to assess any relationship between clinical findings and radiological and biochemical evidence to identify rickets in children with VDD.

### Materials and Methods

The study was conducted between 2015 and 2016 in a tertiary care center (Amirkola children's hospital) in north of Iran. In this study, we evaluated children who were selected via an epidemiological study that examined the prevalence of VDD among children aged 30–72 months.

The eligibility criteria were: children without a history of chronic illness, use of medications known to affect bone metabolism, or use of vitamin D supplements during the 6 recent months. After identification of children with subnormal vitamin D levels (25(OH)D <20 ng/dL), parents were contacted via phone to visit the hospital. Stored blood samples in the initial stage were subjected to biochemical analysis, including Ca, P, PTH, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, fasting blood glucose, blood urea nitrogen (BUN), and creatinine (Cr), via standard methods for VDD cases determined through an epidemiologic study. Serum 25(OH)D and PTH levels were measured by using enzyme-linked immunosorbent assay (ELISA; IDS and EUROIMMUN kits, respectively, both manufactured in England.) The intra- and inter-assay coefficients of variation (CV) were respectively 5.3% and 4.6% for 25(OH)D level and 9.5% and 11% for PTH.

All the children were evaluated by using a questionnaire and medical history. The children with VDD were thoroughly examined by one pediatric gastroenterologist to identify clinical signs and symptoms of VDD based on previous studies, including leg pain with walking, wrist enlargement, muscle weakness, ankle enlargement, frequent falls, inability to walk, bowing of the tibia, bowing of the femur, history of fracture, windswept deformity, irritability, sweating, Harrison's groove, frontal bossing, chest pain, low weight gain, obesity, short stature (SS; high-for-age Z-score, <-2), knee enlargement, anorexia, delayed tooth eruption, bowing of the arms, infection history (pneumonia), delayed motor skill development, and leg pain at rest.<sup>6,10</sup> Any signs and symptoms were scored 0 or 1. Presence of each of the above signs and symptoms received a score of 1 and absence of the above signs and symptoms received a score 0.

Standing height was measured to the nearest 0.1 cm with a Harpenden fixed stadiometer. Body weight (kg) was measured on a Seca balance scale to the nearest 0.1 kg. Body mass index (BMI) was calculated by dividing weight by height (kg/m<sup>2</sup>). Anthropometric weight-for-age, height-for-age, weight-for-height, and BMI-for-age Z-scores were calculated according to the LMS method (L: lambda, M: mu, S: sigma) of Cole and Green<sup>11</sup> by using the World

Health Organization Child Growth Standards 2006.<sup>12</sup> BMI status was categorized according to BMI-for-age Z-scores as normal (2 > Z-scores <-2), overweight and obese (Z-scores >2) and underweight (Z-scores <-2).

In this study, the VDD subgroups were defined as 25(OH)D levels of ≤15 ng/mL for VDD and between 16 and 20 ng/mL for insufficiency. One pediatric radiologist from Amirkola children's hospital observed radiographs for detection of rickets and bone age.<sup>13</sup> Rickets was defined as the presence of cupping of the epiphyses, widening of the wrist, and fraying of the epiphyseal edges.<sup>14</sup>

The Fisher exact test,  $\chi^2$  statistics, *t* test, logistic regression, one-way analysis of variance, Mann-Whitney U test, Kruskal-Wallis test, or Pearson or Spearman rank correlation was used as appropriate to the nature and distribution of the variables used in comparison of the study groups.

### Results

Among the 406 children, 119 (29.67%) had 25(OH)D levels of <20 ng/dL, of whom 42 (10.3%) and 77 (19%) had VDD (≤15 ng/dL) and vitamin D insufficiency (16–20 ng/dL), respectively. During the second admission, 108 children (90.7%) returned for assessment of biochemical and radiographic parameters, but radiographic analysis was not performed for 4 children due to lack of cooperation. Comparison between children with VDD and without vitamin D deficiency (WVDD) is shown in Table 1.

The majority of children (95.3%) had a Z-score height for age between -1 and 1 and 11.4% (40) of the children were classified as underweight. The proportions of overweight children differed between children with VDD and those WVDD (11.1% (12) vs. 3.3% (8), *P* = 0.01). Compared with the children with WVDD, those with VDD had a slightly reduced height for age and had a slightly greater weight for age.

The main chief complaints on physical examination included frequent falls 14.2% (17) vs. 19.9% (54), sweating 14.2% (17) vs 23.6% (64), anorexia in 19.2% (23) vs 17% (46), irritability in 5.8% (7) vs 11.1% (30), and leg pain at rest in 20.8% (25) vs 13.7% (37) in VDD and WVDD, respectively. The frequency of signs and symptoms was not different between VDD and WVDD other than for sweating (*P* = 0.04). The mean of signs and symptoms scores had no significant differences between children with (1.59 ± 0.8) and without (1.73 ± 1.01) VDD (*P* = 0.3).

With regard to serum 25(OH)D and Ca levels, VDDs were classified into 4 groups, defined as the pure VDD (group 1), combined VDD and Ca insufficiency (group 2), pure vitamin D insufficiency (group 3), and combined vitamin D and Ca insufficiency (group 4; Figure 1). Then, biochemical, radiographic, and anthropometrics criteria were compared among the groups (Table 2).

The serum 25(OH)D level showed no correlation with the levels of Ca (*r* = -0.16, *P* = 0.09), ALP (*r* = -0.12, *P* = 0.21), P (*r* = 0.13, *P* = 0.15), or PTH (*r* = -0.15, *P* = 0.11) in the children with VDD. However, the serum 25(OH)D level showed an inverse correlation with age (*r* = -0.18, *P* <

**Table 1.** Study Subject Characteristics

	VDD Children	WVDD Children	P Value
Gender, No.(%)			0.09
Male	51 (13)	141 (36.2)	
Female	68 (13.1)	130 (36.2)	
Age (y)	4.18 ± 0.99	4.06 ± 0.98	0.25
No. of children	1.74 ± 0.78	1.68 ± 0.63	0.44
Height for age z-score	0.39 (0.15–0.94)	0.33 (0.15–0.9)	0.02
Weight for age z-score <sup>a</sup>	0.08 ± 1.25	-0.37 ± 1.1	0.001
Weight for Height z-score <sup>a</sup>	-0.02 ± 1.32	-0.54 ± 1.3	0.001
BMI for age z-score	0.008 (-2.84–3.81)	0.48 (-4.17–3.94)	0.002
Father's age (y)	35.1 ± 6.5	34.25 ± 6.2	0.19
Mother's age (y)	31.07 ± 6.1	30.26 ± 5.5	0.2
Father's education (y)	10.72 ± 3.5	9.86 ± 3.1	0.02
Mother's education (y)	11±2.9	10.55 ± 2.7	0.15
Age of weaning (mon)	22.24±3.3	20.8 ± 4.3	0.002
Age of walking (mon)	11.57±1.6	11.9 ± 2.3	0.21
Age of teething (mon)	8.2±2.3	8.5 ± 2.68	0.01
Time of vitamin D supplement (mon)	16.36 ± 6.5	16.25 ± 7.47	0.89

Abbreviations: VDD, vitamin D deficiency; WVDD, without vitamin D deficiency.

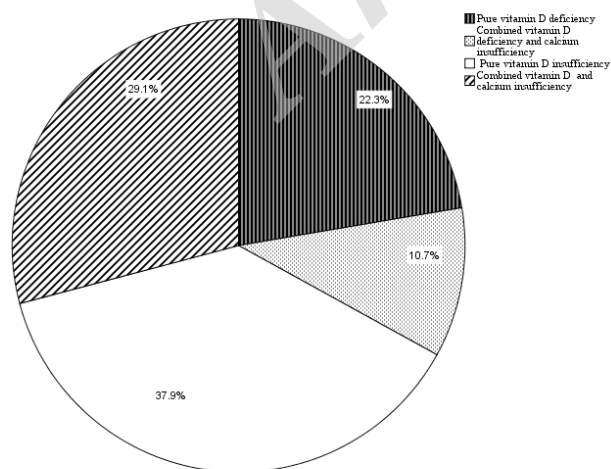
<sup>a</sup> Normally distributed data are shown as mean ± SD, Non-normally distributed data are shown as median (range).

0.001), Z-scores of weight-for-age ( $r = -0.23$ ,  $P < 0.001$ ), weight-for-height ( $r = -0.23$ ,  $P < 0.001$ ), and BMI-for-age ( $r = -0.2$ ,  $P < 0.001$ ), but a direct correlation with the height-for-age Z-score ( $r = 16$ ,  $P = 0.002$ ).

The logistic regression models showed high BMI markedly increased the odds ratio of VDD ( $P = 0.001$ ) and overweight versus normal BMI had more than 3 times the odds ratio of VDD ( $\beta = 3.30$ , CI ( $\beta$ ):1.30–8.34,  $P = 0.0$ .) and underweight versus normal BMI had nearly 30% less odd ratio ( $\beta = 0.31$ , CI ( $\beta$ ): 0.12–0.83,  $P = 0.02$ ).

Regular use of vitamin D supplements did not vary between the children with VDD and vitamin D insufficiency ( $P = 0.42$ ). The proportions of children who received regular prophylactic vitamin D supplementation (400 IU/d) during the first 2 years of life were 69.6%, 81.8%, 69.2%, and 80% in the 4 groups, respectively.

Radiographic data showed that among the 104 children



**Figure 1.** Groups Classified According to Serum 25 OH D and Calcium Levels.

with VDD, 69.2% (72), 10.6% (11), and 20.2% (21) had delayed, normal, and advanced bone ages, respectively. None of the children with VDD had radiographic evidence of rickets.

The distribution of the children according to the presenting complaint is shown in Table 3. The most common complaints were leg pain at rest in 19.4% (21), anorexia in 16.6% (18), sweating in 14.8% (16), frequent falls in 13% (14), leg pain with walking in 9.6% (10), and a history of fracture in 4.8% (5) of the children.

Comparison of the biochemical and anthropometric characteristics demonstrated that weight-for-age ( $P = 0.04$ ) and height-for-age ( $P = 0.02$ ) were significantly different among the bone age groups (Table 4).

## Discussion

The present study had striking findings. First, leg pain at rest was the chief complaint in children with VDD. Second, despite high prevalence of VDD, there was no radiographic evidence to support presence of rickets. Our results showed the most common complaints upon presentation included leg pain at rest, anorexia, sweating, and frequent falls. Leg pain was a common chief complaint in children with combined vitamin D and Ca insufficiencies, but anorexia was the main complaint in children who only had low vitamin D levels. The features of VDD varied according to age. In the study by Torun et al,<sup>10</sup> failure to thrive, muscle weakness, and leg and chest pains were the chief complaints in the 4- to 6-year age groups.

Motaal et al<sup>15</sup> suggested that low serum 25(OH)D levels have little predictive value for severity of rickets in populations with VDD. Some studies reported that VDD-associated rickets usually occurred when serum 25(OH)D levels were  $<5$  ng/mL, whereas other studies reported a cutoff value as high as 12 ng/mL.<sup>16,17</sup> In our study, none of the children had 25(OH)D levels of  $<5$ , but 5 children had 25(OH)D levels of  $<12$  ng/dL. Considering the cost of

**Table 2.** Characteristics of Children Classified According to Serum 25 OH D and Calcium Levels<sup>a</sup>

	Group 1 n = 24	Group 2 n = 11	Group 3 n = 40	Group 4 n = 30	P Value
Age (y)	4.2 ± .93	4.09 ± 1.03	4.05 ± .99	4.15 ± .98	0.94
Female gender, No. (%)	14 (13.6)	5 (4.9)	24 (23.3)	16 (15.5)	0.74
Serum biochemistry					
25 (OH)D (ng/dL)	12.9 (9.5–14.6) <sup>b</sup>	14 (8.9–15) <sup>b</sup>	18.5 (15.2–19.8) <sup>b</sup>	17 (15.3–19.95) <sup>b</sup>	<0.001
PTH (pg/dL)	16.8 (4–35.5) <sup>b</sup>	9.8 (8.6–35.2) <sup>b</sup>	10.2 (4–32) <sup>b</sup>	16.15 (8.8–33.7) <sup>b</sup>	0.002
Ca (mg/dL)	9.5 (8.6–10.2) <sup>b</sup>	8.3 (8–8.5) <sup>b</sup>	9 (8.6–10.7) <sup>b</sup>	8.2 (7.1–8.5) <sup>b</sup>	<0.001
P (mg/dL)	5.1 (4.1–5.9)	4.9 (4.3–5.6)	5 (4.4–5.5)	5.2 (4.6–8.8)	0.26
ALP (U/L)	577 (410–943)	534 (351–923)	560 (352–942)	560 (5.8–845)	0.95
Total P (g/dL)	7.6 (6.6–85)	7.6 (7.2–8.3)	7.8 (6.4–816)	7.65 (6.7–8.3)	0.56
Hb (g/dL)	12.35 (9.6–14.6)	12.5 (10.7–13.4)	12.75 (9.1–14.2)	12.05 (10.1–14.2)	0.31
PLT	284 (220–411)	288 (143–452)	289 (117–556)	305 (158–503)	0.86
FBS	78.5 (51–103)	77.5 (63.5–94)	82 (57–115)	75.25 (62–91)	0.4
Anthropometric characteristics					
Height for age z-score	0.33 (0.16 –0.9)	0.36 (0.29 –0.8)	0.34 (0.16–0.87)	0.34 (0.17–0.85)	0.94
Weight for age z-score	0.01 ± 0.95	-0.045 ± 0.95	0.2 ± 1.5	-0.09 ± 1.3	0.81
Weight for height z-score	-0.52 ± 1	0.11 ± 1.3	0.21 ± 1.3	0.07 ± 1.27	0.29
BMI for age z-score	-0.61 (-2.41–2.14)	0.22 (-1.89–2.48)	-0.65 (-2.84–3.81)	-0.28 (-2.42–3.03)	0.48
Bone age (mon)	57 (30–78)	36 (24–60)	42 (18–84)	48 (24–84)	0.06
Height age (mon)	60.13 ± 14.3	55.6 ± 15.08	56.36 ± 16.09	56.28–16.26	0.78
Chronological age (mon)	57 (31–79)	59 (38–81)	54 (34–77)	54 (36–81)	0.94

<sup>a</sup> Normally distributed data are shown as mean ± SD, Non-normally distributed data are shown as median (range).

<sup>b</sup> P value < 0.001.

**Table 3.** Frequency of Clinical Findings of Classified Children According to Serum 25 OH D and Calcium Levels

Clinical findings No. (%)	Group 1 n = 23	Group 2 n = 11	Group 3 n = 39	Group 4 n = 30
Anorexia	7 (30.5)	1 (9.1)	9 (23.1)	6 (20)
Leg pain at rest	4 (17.4)	5 (45.5)	8 (20.5)	8 (26.6)
Sweating	2 (8.7)	3 (27.2)	8 (20.5)	4 (13.3)
Frequent fall	6 (26.2)	-	6 (15.4)	5 (16.6)
Irritability	1 (4.3)	1 (9.1)	3 (7.7)	2 (6.6)
History of fracture	1 (4.3)	1 (9.1)	2 (5.1)	1 (3.7)
Chest pain	1 (4.3)	-	-	-
Rest of signs and symptoms	1 (4.3)	0	3 (7.7)	4 (13.2)

**Table 4.** Comparison of Characteristics in Different Bone Age Groups<sup>a</sup>

	Normal bone age n = 11	Delayed bone age n = 72	Advanced bone age n = 21	P Value
Age (y)	4.35 ± 1.06	4.14 ± 0.95	4.34 ± 1.1	0.62
Gender (%)				0.19
Female	8 (7.7)	36 (34.6)	16 (15.4)	
Male	3 (2.9)	36 (34.6)	5 (4.8)	
Serum biochemistry				
25(OH)D (ng/dL)	16 (12.8–19.8)	16.6 (8.9–19.5)	16.5 (10.8–19)	0.26
PTH (pg/dL)	14.2 (8.3–22.1)	14.55 (4–35.5)	14.7 (4–30.7)	0.45
Ca (mg/dL)	9 (8–10.2)	8.6 (7.1–10.7)	8.9 (7.8–10.3)	0.18
P (mg/dL)	5 (4.4–48)	5.1 (4.3–55)	5 (4.1–8.9)	0.34
ALP (U/L)	566 (410–861)	561.5 (6.2–943)	606 (5.8–942)	0.36
Total Pr (g/dL)	8 (6.4–9)	7.7 (6.4–816)	7.7 (6.8–85)	0.77
Anthropometric characteristics				
Height for age z-score	0.28 (0.15 –0.86) <sup>b</sup>	0.35 (0.16 –0.87) <sup>b</sup>	0.32 (0.16–0.67) <sup>b</sup>	0.02
Weight for age z-score	1.03 ± -0.13 <sup>b</sup>	-0.13 ± 1.2 <sup>b</sup>	0.65 ± 1.45 <sup>b</sup>	0.04
Weight for height z-score	0.61 ± 1.27	-0.1 ± 1.27	-0.08 ± 1.67	0.32
BMI for age z-score	0.53 (-1.16–2.16)	-0.18 (-2.8–3)	0.07 (-2.4–3.8)	0.7
Height age (mo)	67.8 ± 17.9 <sup>b</sup>	55.45 ± 15.7 <sup>b</sup>	64.47 ± 15.6	0.01
Age of walking(Mo)	12 (12–15)	12 (7–15)	12 (8–15)	0.82
Age of teething(Mo)	9.5 (6–12)	8 (4.5–15)	7 (5–12)	0.33

<sup>a</sup> Normally distributed data are shown as mean ± SD, Non-normally distributed data are shown as median (range).

<sup>b</sup> P value < 0.001.

radiography and the radiation exposure inflicted on children, we clarified whether radiographic assessment is necessary to rule out rickets in children with VDD. In a previous study, only 7.5% of children with VDD were identified based on radiographic findings.<sup>18</sup> Among 500 immigrant children in Scotland, 6% showed radiographic changes indicative of rickets.<sup>19</sup> The mean 25(OH)D level in the children with subclinical rickets was 8.5 ng/mL- lower than the 16.5 ng/mL in the patients without radiographic changes.<sup>20</sup> A study of Australian children showed that 25(OH)D levels did not significantly differ between the children with and without radiological evidence of rickets.<sup>21</sup>

Rickets, as a bone disease, has many morphological and biochemical features, and diagnostic methods have been identified that assess different aspects of these features. ALP has been introduced as the best biochemical marker for confirmation of rickets severity as assessed based on radiological evidence in vitamin D-deficient populations<sup>3,8</sup> because it is an isoenzyme component of osteoblastic proliferation in the rachitic skeleton.<sup>15</sup> In the present study, first, children with VDD and insufficiency had ALP levels within the normal range, without a significant difference between them. Second, ALP levels showed no significant relationship with clinical findings, radiographic evidence, or 25(OH)D level. However, Mark et al.<sup>22</sup> proposed 3 of 5 clinical signs, including wide wrists, frontal bossing, rachitic rosary, Harrison's sulcus, and bowed legs for which ALP level should be measured, and ALP values of  $\geq 250$  IU/L measured by a Chinese bone-specific ALP test kit confirmed active rickets. These children should be given therapeutic doses of vitamin D and Ca supplements and undergo increased exposure to sunlight. In our study, none of these signs were observed. In addition, elevated ALP levels without clinical signs of rickets could not confirm the presence of rickets, but nutritional education should be recommended for adequate vitamin D and Ca intake.<sup>22</sup> If  $>3$  clinical signs on radiography are observed and ALP levels are high, a definitive diagnosis of rickets can be made.<sup>22</sup>

Most of the children (VDD plus WVDD) in our study had normal growth. The results of the study by McGillivray et al<sup>23</sup> were in agreement with our results and showed that the median height-for-age, weight-for-age, and weight-for-height Z-scores were 0.2, -0.1, and -0.3, respectively. A consistent relationship was observed between the height-for-age Z-score and 25(OH)D levels. Hence, the children WVDD were taller. Another study showed that children with nutritional vitamin D-associated rickets had greater weights and shorter heights because of leg deformities associated with lower limb shortening, but we did not find rickets in children in our study.<sup>24</sup> Thus, we can conclude that adequate serum vitamin D levels lead to better skeletal growth. Anthropometric evaluation revealed that low serum 25(OH)D concentration associated with more BMI and weight. Also, high BMI increased risk of VDD. Vitamin D is a fat-soluble vitamin that is possibly stored in adipose tissue and associated with decreased bioavailability.<sup>25,26</sup> These findings are in agreement with reports of some studies.<sup>27-32</sup> For investigation of vitamin D effect on bone

age, a comparison of the radiographs of the left wrist and hand between children with VDD and those with WVDD would be superior, but we could not do this because of ethical considerations. Another interesting point was that the biochemical bone markers in different bone age groups showed no significant difference, but children with delayed bone age were taller and had lower weight than the two other groups. Although delayed bone age can be observed as a consequence of nutritional deficiency in children with SS,<sup>33</sup> there was not any child with SS in the present study. Levels of vitamin D did not relate to bone age but the majority of the children's bone ages in this study were abnormal compared to the reference. Thus, it may be necessary to determine the special radiographic atlas of the hand and wrist in terms of bone age for children in our country.

In conclusion, our study demonstrated that clinical findings have no significant relationship with radiological or biochemical evidence in children with VDD. Abnormal radiological findings of rickets were not obtained on any wrist X-Rays of the children included in this study. Thus, radiological assessment seems unnecessary for the vitamin D levels described.

#### Authors' Contribution

LM contributed to conception, design, data collection, statistical analysis and drafting of the manuscript. ME-D contributed to conception, design and manuscript drafting. AAM, MAA, HA contributed to conception and design. MP and HGN contributed to conception and data collection. AB contributed to design and statistical analysis. All authors approved the final version of the manuscript for submission.

#### Conflict of Interest Disclosures

The authors have no conflicts of interest.

#### Ethical Statement

The study was approved by the ethics committee of Babol University of Medical Sciences (MUBABOL.REC.94.114). Written informed consent was obtained from parents or guardian of all study subjects.

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