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Original Article

The Epidemic of Poor Vitamin D Status among 9-12 Years Old Children in Tehran, 2008, Using HPLC: Need for an Urgent Action

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ABSTRACT

Background and Objectives: Vitamin D deficiency/insufficiency is a global health problem. The importance of this problem is doubled in growing children because of their increased need for skeletal growth. This investigation was performed firstly to assess vitamin D status, and secondly to examine its possible relationship with sex, residing area and duration of sun exposure in 9-12 years old children of Tehran.

Materials and Methods: We studied 257 randomly selected children out of 1111 children of a huge study, titled "Vitamin D and Calcium Deficiency Prevalence of Tehran's Elementary School Children (VDPT)", performed in fall and winter 2008 in Tehran. The children were without the history of diabetes, allergy or autoimmune disease, and any calcium, vitamin D and fish oil supplement use during the last three months. Venous blood samples were taken, and the sera were analyzed by high performance liquid chromatography (HPLC) for measuring 25-hydroxycalciferol (25(OH)D).

Results: The participating children comprised of 53.7% girls and 46.3% boys from different economical areas of Tehran (40.5% poor, 26% middle and 33.5% rich). Their mean age was 10.1±0.7 years, mean duration of sun exposure was 41.2±34.6 min/day, and mean serum 25(OH)D concentration was 21.9±15.6 nmol/L. Duration of sun exposure was not significantly different either between boys and girls (p=0.220), among different residing areas (p= 0.057), or between the girls and boys of different areas. The occurrence of vitamin D deficiency was 72.4% (n=186). Vitamin D status was significantly different between boys and girls (p= 0.01) and among the areas (P= 0.004). There was no significant relation between poor vitamin D status and duration of sun exposure (p=0.411).

Conclusions: The findings showed a noticeable occurrence of vitamin D deficiency/insufficiency among 9-12 years old children in Tehran. The data warrants urgent interventions.

Keywords: Vitamin D, 25-hydroxycalciferol, School children

Introduction

Vitamin D, an essential fat soluble vitamin, plays a critical role in bone growth and development, and in prevention of chronic diseases including cancer, diabetes, autoimmune disorders, and cardiovascular diseases (CVDs) (1-2). This vitamin is synthesized in the skin upon exposure to ultraviolet-B (UVB) radiation from sunlight (at 290-315nm wavelengths), or can be obtained from the diet or supplements (3-8)

Around one million people in the world are vitamin D deficient (9). Breastfed infants whose mothers are vitamin D deficient or exclusively breastfed infants without any supplementation, growing children, women in child bearing years, pregnant women, menopause women, veiled women, elderly, homebound people, and patients using immunosuppressive drugs are at risk of vitamin D deficiency (10-11). Living in cities, especially in the

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industrial ones, air pollution, living in higher latitude, having dark skin, smoking, and high body mass index (BMI), especially in women, increase vitamin D deficiency risk (11-14). Vitamin D deficiency is prevalent in all at risk groups (mentioned above) in Iran (15-18).

Vitamin D deficiency is more prevalent in girls than in boys and in obese children than in non-obese ones (12, 19-21). In Iran, it has been reported that vitamin D deficiency is more prevalent in girls and women than in boys and men and in polluted than in less polluted areas of Tehran (17, 22-23). The first National Investigation for Micronutrient Status (NIMS) revealed the high prevalence of vitamin D deficiency in various age and sex subgroups in Iran (24). Newer studies also showed escalating numbers for vitamin D deficiency/insufficiency across the country (16-18, 22, 25-26).

Sun exposure is the most important source of vitamin D in many countries such as Iran (27), wherein voluntary food fortification with the vitamin is currently implemented.

Skin synthesized vitamin D bonds with vitamin D binding protein (DBP) in the blood stream, and goes to the liver to form 25-hydroxycalciferol [25(OH)D], the major circulating form of the vitamin, which is measured to determine vitamin D status (28-30).

Here, we aimed to determine vitamin D status of Tehranian 9-12 years old children using high performance liquid chromatography (HPLC), which is the gold standard method (31). The relationship of vitamin D status with sex, residing area and duration of sun exposure was also examined.

Materials and Methods

Subjects: In this study, we used the information and serum samples of 257 randomly selected children out of 1111 children of a huge study, namely "Vitamin D and Calcium Deficiency Prevalence of Tehran's Elementary School Children (VDPT)", performed in fall and winter 2008. The study was conducted by the National Nutrition and Food Technology Research Institute (NNFTRI) in cooperation with Iran Ministry of Education (MOE) in Tehran (32). The children were divided into poor (districts 15-19), middle income (districts 8-14) and rich (districts 1-7) according to the MOE economical classification. An informed consent was sent to the parents, and they were asked to announce if their child had a history of diabetes, allergy or autoimmune disease, and if he/she had taken calcium, vitamin D and fish oil supplements during the last three months. The inclusion criteria used in the VDPT were age 9-12 years, having no clinical diseases including diabetes, allergy or autoimmune disorders, and not taking calcium, vitamin D and fish oil supplements since three months prior to the study.

Then demographic questionnaire was used to collect the participants' personal data. Also duration of direct sun exposure was asked from each child. For this purpose, the children were asked to recall the number of minutes/hours they have spent in daylight (32).

Blood Sampling and Handling: Venous blood samples were collected in glass tubes, and transported to the Laboratory of Nutrition Research, NNFTRI, in less than 2 hrs. Sera were separated, aliquoted, and stored at -80 $^{\circ}$C for further analysis by HPLC, as previously described (33).

HPLC Analysis

Equipments: HPLC system equipped with UV detector (Young Lin, Seoul, South Korea). HPLC column was C18 Tracer Excel 120 ODS 15×0.4, 3µm (Teknokroma, Spain).

Solvents: All solvents (methanol, acetonitrile, hexane, isopropanol, and ethanol) were of HPLC grade, and purchased from Romil England. 25(OH)D3 standard was purchased from Sigma-Aldrich.

Procedure: The procedure has been fully described elsewhere (33).

Statistical Analysis: Data were expressed as mean ± standard deviation (SD). The normality of data distribution was checked using Kolmogorov-Smirnov's test. Between-group comparison of values was performed by student t-test (for data with normal distribution) or Mann–Whitney's U test (for data with non-normal distribution). Differences in proportions were evaluated using Chi-square test. All statistical analyses were done by Statistical Package for Social Sciences (SPSS, version 16; SPSS Inc, Chicago, IL). $P<0.05$ was considered significant.

Results

Concentrations of 25(OH)D and duration of sun exposure did not have normal distribution. The children comprised 138 girls (53.7%) and 119 boys (46.3%) from three economically different areas

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(poor, middle, and rich) of Tehran. The mean age was 10.1±0.7 years, and the mean duration of sun exposure was 41.2±34.6 min/day. Serum 25(OH)D concentration was 21.9±15.6 nmol/L.

Data analysis showed that 104 students (40.5%) were from the poor, 67 (26%) were from the middle and 86 (33.5%) were from the rich areas of Tehran.

Mean sun exposure time in girls and boys was 36/0±24.7 and 47.1±42.6 min/day, respectively. There was no significant difference between the boys' and girls' mean sun exposure time (P=0.059).

Table 1. Duration of sun exposure in girls and boys

Sun exposure time	Sex	
	Gilrs No. (%)	Boys No. (%)
≤15 minutes	28 (20.7)	18 (15.3)
15-30 minutes	58 (43.0)	45 (38.1)
≥ 30 minutes	49 (36.3)	55 (46.6)
	135	108

Sun exposure time was not significantly different in boys and girls (P= 0.22).

Duration of sun exposure did not show any significant difference between the different economical areas of Tehran (P= 0.057).

Similarly, no significant difference in duration of sun exposure was observed between the girls and boys of different areas (P= 0.562, P= 0.057).

The concentrations of 25(OH)D in 77 serum samples (29.9%) were below the detection limit of 12.5 nmol/L; so they were excluded to calculate means. Having said that, the mean 25(OH)D in the remaining samples was 29 ± 14.5 nmol/L, with significantly higher concentrations in boys (n_1=84) than in girls (n_2=96) (30.6 ± 16.7 nmol/L vs. 25.9 ± 14.2 nmol/L, respectively; P=0.012).

Table 2. Sun exposure time in different districts between girls and boys

Sun exposure time (min/day)	Poor		Middle		Rich	
	Girls No. (%)	Boys No. (%)	Girls No. (%)	Boys No. (%)	Girls No. (%)	Boys No. (%)
≤15 minutes	13 (21)	10 (24.4)	6 (18.2)	3 (9.4)	9 (22.5)	5 (11.1)
15-30 minutes	26 (41.9)	11 (26.8)	18 (54.5)	18 (56.3)	14 (35)	16 (35.6)
≥ 30 minutes	23 (37.1)	20 (48.8)	9 (27.3)	11 (34.4)	17 (42.5)	24 (53.3)

The occurrence of vitamin D deficiency, insufficiency and sufficiency in our subjects were 72.4% (n=186), 23.3% (n=60) and 4.3% (n=11), respectively.

Comparing vitamin D status between boys and girls showed that vitamin D status was significantly different (p= 0.01) (Table3).

Table 3. Vitamin D status in girls and boys

Vitamin D status	Sex	
	Girls No. (%)	Boys No. (%)
Severe deficiency (<27.5 nmol/L)	109 (79)	77 (64.7)
Insufficient (27.5-50 nmol/L)	27 (19.6)	33 (27.7)
Sufficient (>50 nmol/L)	2 (1.4)	9 (7.6)
Total	138	119
p value (χ^2 = 0.01)		

Comparison of vitamin D status among different areas revealed that severe vitamin D deficiency in poor, middle and rich areas was 59.6%, 80.6% and 81.4%, respectively. Vitamin D insufficiency in the poor, middle and rich areas was 35.6%, 16.4% and 14%, respectively. Sufficient vitamin D concentration was seen only in 4.8%, 3% and 4.7% of the students in the poor, middle and rich areas of Tehran, respectively. Vitamin D status was significantly different among the different areas (P= 0.004).

The occurrence of vitamin D deficiency/ insufficiency in the girls living in different economic areas of Tehran did not show any significant difference (p=0.281) whereas the situation differed significantly in the boys residing in different areas (p<0.001). Further analyses revealed that while the occurrence of poor vitamin D status did not differ significantly between the boys and girls in both the middle (p = 0.760) and rich (p = 0.580) areas, there was a significant difference in the boys and girls residing in the poor areas (p < 0.001). There was no significant relation between poor vitamin D status and duration of sun exposure (p=0.411) (Table 5).

Sun exposure time was not significantly different between the boys and girls (p=0.220).

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Table 4. Vitamin D status of girls and boys in different districts

Vitamin D status \ District	Girls			Boys		
	Poor No. (%)	Middle No. (%)	Rich No. (%)	Poor No. (%)	Middle No. (%)	Rich No. (%)
Severe deficiency (<27.5 nmol/L)	46(73.0)	29(82.9)	34(85.0)	16(39.0)	25(78.1)	36(78.3)
Insufficient (27.5-50 nmol/L)	16(25.4)	6(17.1)	5(12.5)	21(51.2)	5(15.6)	7(15.2)
Sufficient (>50 nmol/L)	1(1.6)	0	1(2.5)	4(9.8)	2(6.2)	3(6.5)
Total	63	35	40	41	32	46

Table 5. Vitamin D deficiency relation with sun exposure in girls and boys

Vitamin D status \ Sun exposure	Girls			Boys		
	<15 min	15-30 min	>30 min	<15 min	15-30 min	>30 min
Deficiency (<27.5 nmol/L)	20(71.4)	48(82.8)	38(77.6)	11(61.1)	31(68.9)	35(63.6)
Insufficient (27.5-50 nmol/L)	8(28.6)	9(15.5)	10(20.4)	7(38.9)	11(24.4)	14(25.5)
Sufficient (>50 nmol/L)	0	1(1.7)	1(2.0)	0	3(6.7)	6(10.9)
Total	28	58	49	18	45	55

Discussion

The research findings showed an alarming prevalence of poor vitamin D status among 9-12 years school children residing in the different areas of Tehran. The HPLC analysis was employed to determine circulating 25(OH)D concentrations. Vitamin D deficiency/insufficiency is one of the major health problems in the world, including Iran. Nearly 70% of the US children and 37% of the European children suffer from vitamin D deficiency or insufficiency(34).

In the current study, the occurrence of poor vitamin D status was significantly higher in girls than in boys supporting the existing data on the gender difference of vitamin D status. NHANES 2001-2004 showed that the prevalence of vitamin D deficiency (25(OH)D < 75 nmol/L) was more prevalent in girls than in boys (21). Also analysis of data from NHANES 2001-2004 and NHANES 2004-2006 showed that vitamin D deficiency was more prevalent in women than men (12). Other studies from the European, Asian and Middle East countries also revealed higher occurrence of vitamin D deficiency in women than in men (35). Vitamin D deficiency is more sever in veiled women, pregnant women, residents of industrial or polluted cities, and old people. It is more prevalent in smaller children than in older children (10). Different kind of cloths in men and women (10, 17), difference in outdoor activities (16, 36), fat mass, and sexual hormones are important in higher 25 (OH) D concentrations in men than in women.

The results further indicated different vitamin D status of boys in different areas of Tehran. There was a significant difference between the boys and girls of poor areas. Puri et al. found that low socioeconomic girls had more sun exposure due to higher outdoor activities and walking to school (37). Factors such as duration and intensity of sun exposure (10, 28-29, 36), sampling season (29, 38), age of the subjects (21, 29), and socioeconomic status could affect vitamin D status(37, 39-40). In this study, duration of sun exposure and vitamin D status were not either different significantly between girls and boys, or related to vitamin D status. This might be due to small sample size and very short duration of sun exposure (21). Air pollution is an important contributing factor of vitamin D deficiency (9-10, 23, 41). In latitudes above 35 degree, there is not enough UV radiation for vitamin D synthesis in winter. Tehran is located in 35.34 latitude (18, 29).

The season of blood sampling is also another important issue as 25 (OH) D concentrations are season-dependent with the maximum and minimum levels in the end of summer and winter, respectively (29). Boston study showed that low serum level of vitamin D is more prevalent in winter and spring than summer and fall (38). A study in Esfahan showed that vitamin D deficiency of 14-18 years old children and adolescents was 46.2% in spring; however, in summer the occurrence rates were smaller (17). Another year-long study showed that the lowest vitamin D level was in October and

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February (22). Our blood samples were all taken during cold seasons; so it is highly probable that circulating 25(OH)D would rise later in summer. However, how much increment in 25(OH)D will occur and if this increment would be able to improve vitamin D status of the children remarkably remain to be elucidated by further studies.

Study of 3-69 years old Tehranian residents showed that vitamin D deficiency was more prevalent in 10-20 years old subjects (27.2% women and 13.6% men)(22). The noticeable difference of these numbers and our report could be due to the season of blood sampling, definition of vitamin D status, and the assay method. Several studies have reported disagreement among the different assay methods for 25(OH)D (33). HPLC is a gold standard method; however, low throughput and need for skilled personnel are the barriers for its use in large-scale studies. Regular quality control of other methods' results by HPLC may help to improve accuracy and precision of the assays in population studies.

This study had some limitations. The narrow age range of the participants makes our results hard to generalize to other children with different ages. However, considering the very limited food sources of vitamin D and similar living conditions (including duration of sun exposure), more or less similar situation can be expected in younger school children. Blood sampling was done just during the cold seasons so the results can hardly be extended to the warm seasons. Some potential associations might be uncovered because of small sample size.

In conclusion, our findings showed alarming rates of poor vitamin D status among the 9-12 years old children in different areas of Tehran. Some interventions like vitamin D-fortified snacks and supplementation have been evaluated earlier (42). The results of these studies could be used for action plan.

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