Vitamin D Status in a Population of Well Children: Amasya Sample

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Objective: The aim of this study was to determine the vitamin D status of children living in Amasya, a city in northern Turkey. Vitamin D is an essential hormone for the health of the musculoskeletal system and acts as a modulator for the immune system, a protector of the cardiovascular system and a regulator of metabolic homeostasis.

Methods: A total of 2551 healthy individuals aged 1 to17 years enrolled in this study. They were divided into 4 groups, according to their ages. Vitamin D status was classified based on the 2015 global consensus statement from the Endocrine Society. The data were collected from the hospital record system, retrospectively, and categorized according to age, gender, and the season in which the blood sample was taken for the measurement of 25 (OH) D levels.

Results: Only 39% of the subjects were found to have sufficient vitamin D levels (>50 nmol/l [i.e., >20 ng/ml]), while the rest had poor vitamin D status. The insufficiency and deficiency problems were greater in females especially in winter and increasing with age.

Conclusion: The high frequency of poor vitamin D status in otherwise healthy children indicates a necessity for supplementation and for implementing lifelong strategies that increase and maintain adequate levels of sun exposure. [*P R Health Sci J 2019;38:255-261*]

Key words: Vitamin D status, Frequency, Well children, Adolescent, Northern Turkey

Ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) are members of "calciferols" a group of fat-soluble secosteroids with the famous name of "vitamin D". Ergocalciferol can be ingested from plants, whereas cholecalciferol is animal derived and can be synthesized in the skin. That cholesterol in the skin is converted to vitamin D by direct exposure to the sun's ultraviolet B (UV-B) radiation was discovered many years ago (1, 2). The main source of vitamin D in the body is the photo conversion of 7- dehydrocholesterol to previtamin D3 in the skin. Processes in the liver and kidneys lead to the formation of active vitamin D; 1, 25 (OH)2 D3; also called calcitriol, under the control of parathyroid glands and some other mediators (3). The active form of vitamin D has a crucial role in the regulation of calcium and phosphorus metabolism which affects bone health throughout life, but especially in childhood. A deficiency of vitamin D can result in bone mineralization defects, such as rickets and osteomalacia. Recent studies have pointed out that vitamin D has significant beneficial effects on extra skeletal systems where its receptors exist, including the heart, central nervous system, gastrointestinal system, gonads, lymphatic system and skin. As an immune modulator, vitamin D improves immune function providing effective immunity against infectious diseases and malignancies and limiting the auto-inflammatory response, thereby leading to the reduction of auto immune disease (3, 4). These findings have led to there being a greater interest in vitamin D status and supplementation, recently.

The main source of vitamin D in the body is its synthesis in the skin under exposure to sunlight and 80 to 90% of serum vitamin D is provided in this way. Natural vitamin D sources in the diet are oily fish, cod liver oil, egg yolks, mushrooms, and liver; the amount of vitamin D in fruit and vegetables is negligible. Having dark skin pigmentation, using sunscreens with sun protection factors 8 or greater, being exposed to the sun only before 10.00 a.m. and/or after 3.00 p.m., being exposed only to indirect sunlight, winter, living in northern latitudes, living at high altitudes, wearing dark clothing, and wearing thick, long garments; all reduce the synthesis of vitamin D in the skin (3, 5-7). Vitamin D levels in human milk are low and are influenced by maternal factors including effective sun exposure, skin pigmentation, clothing, season, latitude and maternal intake (3,

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8). Infant formulas contain approximately 400IU/l (9). Unless there is enough sun exposure, supplementation with drugs and/or fortifying food with this vitamin becomes inevitable (3).

Vitamin D status in childhood has been investigated all around the world, by many studies. It is determined by measuring serum 25(OH) D, the primary metabolite of the active form. The measurement of 25 (OH) D levels is the most reliable way to evaluate vitamin D status because the half-life of 25 (OH) D is about 3 weeks, while the half-life of the active form is 4 to 6 hours. Supplementation programs and enriching food with vitamin D had caused to decrease the rate of nutritional rickets. However, in recent years, changing life styles and nutritional habits have caused vitamin D insufficiency to re-emerge as a public health problem (10). Another challenge related to vitamin D status is the fact that there are different "normal" ranges for people of different ages and who live in different communities (3). Recent studies help clinicians to access national and international data.

Hypovitaminosis D may be a result of inadequate effective sun exposure, poor dietary intake of vitamin D, malabsorption syndromes, conditions or drugs that impair vitamin D metabolism, life style habits, age, low maternal levels of vitamin D, and/or genetic predisposition (3). In this study, we aimed to determine the status of vitamin D in a sample of healthy Turkish children and establish the effects of gender, age, and season on 25 (OH) D levels to offer some advice about the prevention of hypovitaminosis D throughout an individual's life.

Patients and Methods

This study was conducted in pediatric clinics of the Sabuncuoğlu Şerefeddin Hospital, the education and research hospital of Amasya University, Faculty of Medicine. The medical records of the participants were retrieved from the hospital information system, retrospectively. Patients who had health problems requiring regular treatment and/or a special diet, with either or both lasting for 3 months or longer, were excluded from the study. The exclusionary health problems included asthma, DM, epilepsy, and malabsorption syndromes. Additional exclusion criteria were the presence of growth retardation – defined as having a weight and height under the 3rd percentile, according to the growth charts based on age and sex for Turkish children- and suffering from obesity – defined as having a body mass index (weight in kilograms divided by height in meters squared) at or above the 95th percentile, according to the growth curves based on age and sex for Turkish children. A total of 2551healthy children aged 1 to 17 years who were referred to pediatric outpatient clinics at our hospital from January 1, 2016, to December 31, 2017, were enrolled in this study. Their weights were measured with a calibrated digital scale and their heights with a stadiometer, before the physical examination.

Venous blood samples were obtained from all the patients after a physical examination that took place in the morning hours, 8.30 a.m. to 12.00 noon. Serum 25 (OH) D levels (normal values: 20-100 ng/ ml) were measured by electrochemiluminescence method using Roche Modular Analytical E 170® (Roche Diagnostics). The specificity and sensitivity of the method is 98% and 95% respectively with CV value of 6.8.Vitamin D levels were analyzed immediately after the blood samples were taken. For the evaluation of the results the participants were divided into 4 age groups: 1 to 4 years (preschool), 5 to 8 years (middle childhood), 9 to 13 years (early adolescence), and 14 to 17 years (adolescence). They were also grouped according to their vitamin D status, itself based on a consensus statement from the Endocrine Society (2015): deficiency, <30 nmol/ml (<12ng/ml); insufficiency, 30–50 nmol/l (12–20 ng/ml); and sufficiency: >50 nmol/ml (>20 ng/ml) with 1 ng/ml = 2.5 nmol/l (11). The American Academy of Paediatrics (AAP) classified vitamin D status in the pediatric population using the following 25(OH)D concentrations: A value <5 ng/ml is classified as "severely deficient," a value <15 ng/ml is classified as "deficient," a value falling in the range of 15–20 ng/ml is classified as "insufficient," a value falling in the range of 20–100 ng/ml is classified as "sufficient," a value falling in the range of 101–150 ng/ml is classified as "excess" and a value >150 ng/ml is classified as "toxic" (12).

Statistical analyses

Statistical analyses were performed using SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA). The data were presented as frequencies, medians and minimum–maximum, ranges or mean (± SD) by descriptive statistics, when indicated. The vitamin D status of the study population was presented by gender and age, while also considering the season that the blood was sampled. The variables were investigated using visual (histograms, probability plots) and analytical (Kolmogorov Simirnov test) methods to determine whether they were normally distributed or not. As the serum 25 (OH) D levels were not normally distributed, Kruskall Wallis test was conducted to compare the levels between the seasonal and age groups. Mann Whitney U test was performed to test the significance of pair wise differences using the Bonferroni correction to adjust for multiple comparisons. Chi-square test or Fisher's exact test was also used to compare these variables in different groups. The correlation associations were calculated using Spearman's test. A p value lower than 0.05 was considered as statistically significant.

Ethics

On October 19, 2017, this study was approved by the local Committee of the Institute of Amasya University, Sabuncuoglu Serafeddin Education and Research Hospital with decision number: 62949364-000-6222.

Results

In this study, the vitamin D status of 2551 healthy children categorized according to age, gender, and the season in which their blood had been sampled was presented. Of these children

47.3 % (n= 1206) were boys and 52.7% (n=1345) were girls. The mean age of the population was 6.37 (±4.98) years (1–17 years). The number of samples obtained in winter was 658 (25.8 %), in spring 553 (21.7 %), in summer 632 (24.8 %) and in autumn. 708 (27.8%). The mean serum concentration of 25(OH)D in the study population was 18.63 (±10.77) ng/ml (3.5–96.4 ng/ml; median: 16.70 ng/ml). Only 39 % (n=995) of the participants had sufficient vitamin D levels (>20 ng/ml), whereas 27.2 % (n=694) had insufficient levels and 33.8 % (n=862) deficient levels (Table 1). According to the AAP criteria 61 % (n= 1556) of the participants had poor vitamin D levels (<20ng/ ml).

The mean concentrations of serum 25(OH) D changed significantly between age groups (p<0.0001). The preschoolers were the largest group of the study, with 1184 participants; the mean concentration of 25(OH)D in this group was 20.66 (±10.32) ng/ml. (3.50-95.9) There were 546 participants in the middle- childhood group with a mean concentration of 25(OH) D of 18.11 (±9.46) ng/ml (4.2–89.4). In the 474 participants in the early- adolescence group the mean concentration of serum 25(OH) D was 17.30 (±12.12) ng/ml (4.2–96.4). The adolescent group had 347 participants with a mean concentration of 25(OH)D of 14.33 (±10.63) ng/ml (4–66.3) (Figure 1). Correlation analyses revealed that the 25(OH)D levels correlated negatively with age (r=0.21; p<0.0001).

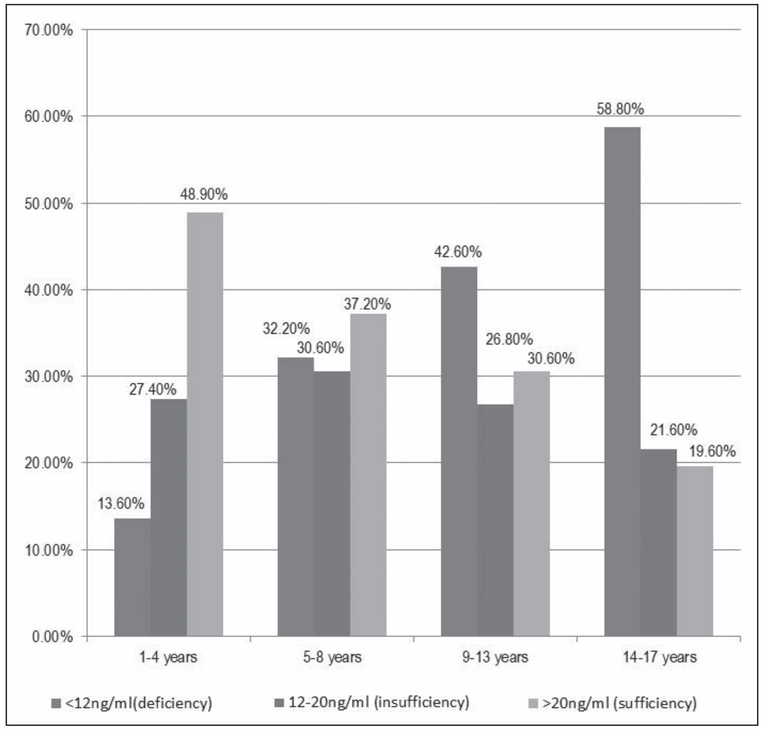

Figure 1. 25 (OH) D status in the different age groups.

Table 1. The summary of grouping variables and results.

		n	%
Gender	Male	1206	47.3
	Female	1345	52.7
	Total	2551	100.0
Age (years)	1–4	1184	46.4
	5–8	546	21.4
	9–13	474	18.6
	14–17	347	13.6
	Total	2551	100.0
Season	Spring	553	21,7
	Summer	632	24.8
	Autumn	708	27.8
	Winter	658	25.8
	Total	2551	100.0
25 (OH) D status (ng/ ml) (AAP)	<5 (severe deficiency)	60	2.4
	<15 (deficiency)	1064	41.7
	15-20 (insufficiency)	432	16.9
	>20 (sufficiency)	995	39
25 (OH) D status (nmol/ l and ng/ ml), Endocrine Society criteria (2015)	>50 nmol/l; >20 ng/ml (sufficiency)	995	39
	30–50 nmol/l; 12–20 ng/ml (insufficiency)	694	27.2
	<30 nmol/l; <12 ng/ml (deficiency)	862	33.8
	Total	2551	100.0

In this study, considering the 2015 consensus statement, 39.2 % (n=527) of the girls had a vitamin D deficiency and 26.3% (n=354) had an insufficiency, whereas 28.2% (n=34 0) of the boys had an insufficiency and 27.8 % (n= 335) had a deficiency. Only 34.5% (n=464) of the girls and 44% (n=531) of the boys had sufficient serum 25(OH)D levels (Figure 2). The mean serum concentration of 25(OH)D was 19.84 (±10.85) ng/ml in the boys and 17.48 (±10.37) ng/ml in the girls; the levels of serum 25(OH)D changed significantly depending on gender (p<0.0001).

Additionally, serum 25(OH)D levels were evaluated considering the season in which blood samples were taken (Figure 3). In winter and spring, the mean serum concentrations were 14.98 (±10.47) ng/ml and 15.68 (±11.27) ng/ ml, respectively. The levels were significantly higher in summer and autumn with the values of 24.27±9.85 ng/ml and 19.28 (±9.07) ng/ml. The mean concentrations of serum 25(OH)D changed significantly from one season to the next (p<0.0001).

Discussion

In this study, a high prevalence of poor vitamin D status was found in a sample of children aged 1to 17 years old. These results show that low vitamin D status is an important and common public health problem in children of Amasya and is related to age, gender and season (worsening in those with less sunlight). The highest frequency of insufficiency was detected

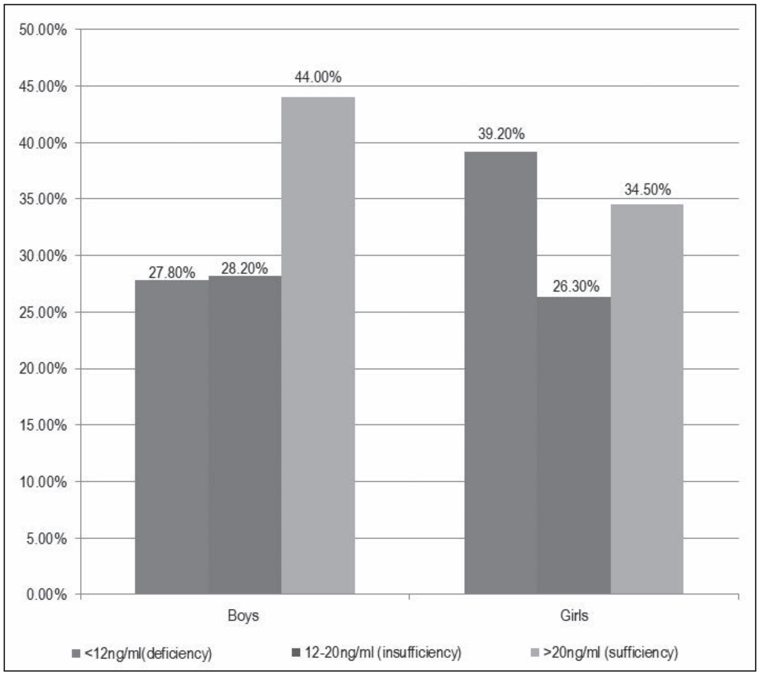

Figure 2. 25 (OH) D status in the two genders.

in adolescent girls and this is compatible with the previous reports in the literature.

Serum concentration of 25(OH)D is presently regarded as the best indicator of vitamin D status, but the diagnosis of vitamin D deficiency is methodologically challenging because of the various measurement techniques and available assays that are used. Moreover, there is lack of consensus about what "adequate" serum 25(OH)D levels consist of, considering the skeletal and non- skeletal effects of vitamin D (3). Various cut- off points for the definition of vitamin D deficiency have been adopted by various organizations and authors (11, 12). In this study, vitamin D status was classified according to the guidelines of the previously mentioned consensus statement and was reported as follows: <12 ng/ml (<30 nmol/l) was considered a deficiency, 12 to 20 ng/ml (30-50 nmol/l), an insufficiency and >20ng/ml (>50 nmol/l), a sufficiency. The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends the pragmatic use of a 25(OH)D serum concentration of >50 nmol/L (20 ng/ml) to indicate a sufficiency and a serum concentration of <25 nmol/L(10 ng/ml) to indicate a severe deficiency (13). As mentioned before, the AAP has classified vitamin D status as deficient for values <15 ng/ml and sufficient for those >20ng/ml in the pediatric population (12). Sufficiency is determined according to serum PTH level which is inversely associated with 25(OH)D. Studies showed that PTH remained within normal ranges when the blood level of 25(OH)D was 30 to 34 nmol/ l and bone remodelling was under control, preventing nutritional rickets (11, 14). According to the AAP classification 39 % (n=995) of the study group had sufficient vitamin D levels whereas 2.4 % (n=60) had severe deficiencies. However, going by the guidelines of the Endocrine Society, it tuns out that 39% (n = 995) of our sample population had a vitamin D sufficiency, while more than 60% had poor vitamin D status.

The main source of vitamin D is sun exposure, approximately 90% of vitamin synthesis is provided in the skin under direct sun light. This process requires UV-B radiation in the 290 to 315 nm waveband. Poor vitamin D status is correlated with insufficient sun exposure and is linked to factors such as dark skin, season (few sunshine hours and/or few sunny days), altitude, latitude, sunscreen, and concealing clothes (6, 15). Amasya is a small city located at 36^0 57' 06"- 36^0 31' 53"eastern meridians and $41^0$04' 54"- 40^0 16' 16" northern parallels, in the middle Black Sea region of northern Turkey, at an altitude of 411 m (16). The average number of rainy days per year is 111.5 (17). The estimated maximum monthly average global solar radiation ranges from 20.05 to 23.71 MJ /m^2, and the estimated maximum monthly average daily sunshine duration ranges from 6.8 to 11 hours, in this region (18). Sixty percent of this study group had poor vitamin D status, whereas in another study from Turkey (a country having a subtropical climate) vitamin D deficiency

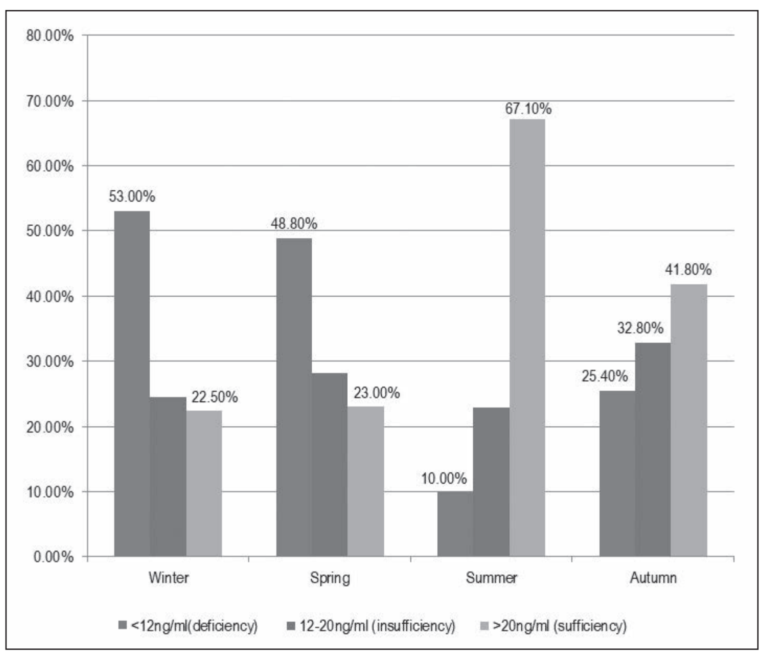

Figure 3. 25 (OH) D status and seasons.

(<12 ng/ml) was observed in 40 to 45% of individuals at early ages, rising to 80 to 90% after the age of 10 (19). In middle Anatolia the prevalence of vitamin D deficiency (<20 ng/ml) was 8% and that of vitamin D insufficiency (20–30 ng/ml) was 25.5% in the population investigated (20). In the western part of Turkey the prevalence of deficiency was reported to be 31 % in a sample of 100 infants aged 1to 24 months (21). The prevalence of vitamin D deficiency in healthy school children was 93% during spring and 71% during autumn (22). Poor vitamin D status seems to be a global health problem, because high prevalences of deficiency and insufficiency (affecting, especially, young people, women—gravid and non—and immigrants) have been reported in Eastern Europe, Italy, Spain, the Middle East, India, Japan, and China (23).

In this study, our finding of an increased frequency of vitamin D deficiency associated with age is compatible with previous reports in the literature (19, 24). The data for this study were obtained from the hospital information system, which accounts for one of the study's limitations: We were unable to obtain data on vitamin D supplementation or habits regarding the consumption of fortified foods; nor could we acquire information about the frequency of outdoor activities—or of any type of physical activity—exposure to sunlight, sunscreen use, clothing habits, or skin color. Nevertheless, the heretofore mentioned increased frequency of age-related vitamin D deficiency can be explained by several clinical observations: During a child's infancy and preschool years, his or her parents and doctors are more willing to give or prescribe multivitamins; furthermore, in this span of time, children tend to spend more time taking part in outdoor activities. In addition, most children's styles of dress allow greater exposure to sunlight. Another factor is that in 2005, Turkey's Ministry of Health initiated a nationwide, free vitamin D supplementation campaign for every child, from birth to the 2nd year of life (25). As the years pass, sedentary activities increase and adolescents, especially, tend to limit their outdoor activities because they prefer screen-based ones, such as watching television or playing computer games. Additionally, their time outdoors is decreased because they have to study to prepare for high school entrance exams; for these kids, physical activity is usually limited to physical education classes at school. Finally, dietary intake changes with age; the ingestion of fast food and soft drinks along with unbalanced diets to lose weight become common, while the consumption of dairy products, fish, and other nutritious foods decreases (3, 20, 24–26).

Poor vitamin D status has been reported to be more prevalent among girls than boys (26.27). In this study, only 44 % (n=531) of the boys and 34.5% (n=464) of the girls had sufficient serum 25(OH)D levels. Additionally, the mean concentrations of serum 25(OH) D changed significantly between genders, and a negative correlation with age was detected. This may be due to the clothing habits, dietary habits, sunscreen use (more) and/or time spent engaging in outdoor activities (less) of girls (24, 26, 27).

Vitamin D deficiency is an important health problem in childhood because this vitamin is necessary not only for normal growth and skeletal development, but also to boost immunity, to regulate metabolic homeostasis, and to protect the cardiovascular system (3). For all age groups this problem can be prevented by vitamin D supplementation. As of 2005 in Turkey, all infants up to 2 years of age are offered vitamin D oral supplements (400 IU/day); such supplements should be provided to all children, without the necessity of screening. The dosage can be decided according to age, gender and season. In 2011 the Institute of Medicine of the National Academia of Sciences, Engineering and Medicine in the United States, proposed a recommended dietary allowance of 400 and 600 IU/day of vitamin D for healthy infants younger than 1 year and for children from 1 to 18 years old, respectively (28). Fortifying some foods with vitamin D is a common strategy for increasing the intake of this important vitamin, but the practice varies widely, depending on nutritional culture and lifestyle habits of the community. Usually, dairy products, cereals, or fruit juice (or a combination of any 2 or all) is fortified with vitamin D in developed countries (29). The content of milk and orange juice after fortification is 400 IU/l. The adequate uptake of vitamin D cannot be reached unless individuals consume the proper foods in sufficient amounts. Tailored strategies for food fortification are necessary due to the cultural and geographical statuses and traditional nutritional habits of every country. Until such strategies are available, vitamin D supplementation seems to be the only way to prevent hypovitaminosis D (3,29). Schools should have curricula that include more outdoor activities and/ or scouting for every student. Parents should also support these activities. Sunscreen usage should be standardized to minimize the adverse effects on cutaneous vitamin D synthesis (3).

This study had several limitations and strengths. Although the results reflect the data of a large population of well children, the conditions associated with vitamin D status and metabolism, such as dietary history, vitamin D supplementation, clothing habits, skin color, sun exposure, and outdoor and physical activity time data, are not available. Moreover, parathormone, calcium, phosphorus and alkaline phosphatase levels are not mentioned. Vitamin D is not only essential for the health of the musculoskeletal system, but also the cardiovascular, central nervous, and gastrointestinal systems; it also has crucial roles in immunity and the metabolism of insulin. It acts as a modulator for homeostasis and improves the function of the systems that contain its receptors (3, 4). As one of the great limitations of the study, this report was not focused on the clinical effects of vitamin D status on the presented population. The clinical follow- ups of the participants were not mentioned. Only 15 participants had "rickets," with the typical radiological signs present in the radiographies of their wrists (confirmed by hospital records) and they were prescribed high- dose vitamin D and calcium supplements. Two hundred and twenty-one had been prescribed vitamin D drops for treatment. Further epidemiologic studies assessing these variables are needed if we are to understand the clinical implications of hypovitaminosis. In addition, the assessment of vitamin D status is possible using radioimmunoassays, competitive protein binding assays (CPBA), high pressure liquid chromatography (HPLC) or liquid chromatography-tandem mass spectrometry; the results of these various methods of ascertaining 25(OH)D levels can vary from 10 to 25% (30). In this study electrochemiluminescence method was used and this limited our ability to generalize our results.

In conclusion, this report shows that poor vitamin D status is a frequent and important health problem in the pediatric population of our city. This poor status may be related to changing lifestyles and poor vitamin D intake. We suggest that the supplementation of vitamin D and the performance of outdoor physical activities, providing sufficient sun exposure be encouraged during childhood and adolescence. To alleviate this important public health problem, individuals of all ages should take vitamin D supplements.

Resumen

Objetivo: El objetivo de este estudio es determinar la presencia de vitamina D en niños que viven en Amasya, una ciudad en el norte de Turquía. La vitamina D es una hormona esencial para la salud del sistema músculoesquelético y actúa como un modulador del sistema inmunitario, el sistema cardiovascular y regulador la homeostasis metabólica. Métodos: Un total de las 2.551 personas sanas de edades comprendidas entre 1 y 17 años fueron sometidos a este estudio y se han dividido en cuatro grupos, según su edad. El estado de la vitamina D fue clasificado teniendo como base la declaración del consenso mundial de 2015. Los datos fueron recogidos del sistema de registro hospitalario y fueron categorizados teniendo en cuenta la edad, el género y el estado de la medición de 25 (OH) en la vitamina D. Resultados: Los resultados del estudio determinaron que sólo el 39% de los sujetos tenía los niveles suficientes de vitamina D (> 50 nmol/l, > 20 ng/ml) mientras que el resto tenía un nivel pobre de vitamina D. Los problemas de escasez y deficiencia fueron superiores en las niñas, especialmente en invierno y aumentaron con la edad. Conclusión: La alta frecuencia de estado deficiente de la vitamina D en niños sanos indica una necesidad de suplementación y adoción de medidaspara aumentar y mantener níveles adecuados de exposición a la luz solar de por vida.

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