Progress in Nutrition 2019; Vol.21, N. 4: 762-768 DOI: 10.23751/pn.v21i4.6850

Assessment of vitamin D status in turkish adolescents: its relation to obesity, cardiometabolic risk factors and nutritional status

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Summary. Studies on effects of vitamin D on human body have indicated that vitamin D deficiency may contribute to an increased risk of obesity and cardiometabolic risk factors, during childhood; however, these relationships remain unclear. The aim of this study is to determine the influences of adiposity, dietary and environmental factors on vitamin D status and the relationship between 25(OH)D and cardiometabolic risk factors in adolescents. The research was carried out in 69 adolescents (60.9% female, 39.1% male) who applied to the Istanbul Marmara University Pendik Educational Research Hospital Pediatric Endocrinology Department, aged between 12 and 17 years. Data was collected in three stages; in the first stage, the questionnaire was developed including the demographic status, nutritional status and physical activities of those adolescents. In the second stage, anthropometric measurements were taken. In the third and final stage, biochemical analysis was measured. There were no statistical differences between groups (Vitamin D deficient and sufficient) in dietary and environmental factors that may have affected serum 25(OH)D. Serum 25(OH) D level was not inversely correlated with anthropometric measurements ($p>0.05$). While there were no significant differences between serum 25(OH)D levels and HOMA-IR; there was a positive correlation between serum calcium and HOMA-IR ($r=0.276$, $p<0.05$) independent of body adiposity. There was a positive correlation between serum 25(OH)D levels and amount of daily dietary protein ($r=0.344$) and fat intake ($r=0.286$) ($p<0.05$), but there was no correlation between serum 25(OH)D and dietary vitamin D and calcium intake ($r=-0.022$, $r=0.235$; respectively). Because foods are not primary sources of vitamin D, this correlation should be assessed with further research on population who consume fortificated food. The results from this study suggest the importance of vitamin D supplementation and food fortification in Turkish adolescents. This study didn't show relationship between vitamin D status and obesity, insulin resistance. Effects of vitamin D deficiency on chronic diseases need to be assessed with prospective studies.

Key words: Adolescent, Insulin Resistance, Obesity, Vitamin D

Introduction

The known task of vitamin D is to provide mineral homeostasis and skeletal function. There are two sources of vitamin D, cholecalciferol (vitamin D3) synthesized at the skin and ergocalciferol (vitamin D2) taken with food.[1]

Vitamin D deficiency, defined as a decrease in serum 25-hydroxyvitamin D level, is a common problem for all age groups.[2] Lack of exposure to direct sunlight, changes in the climate, clothing preferences and inadequate vitamin D content in the diet may result in a deficiency of vitamin D intake.[3] Vitamin D deficiency causes metabolic bone diseases and may increase the risk of chronic diseases such as type 2 diabetes and cardiovascular diseases.[2,3]

Obesity is defined by World Health Organization as abnormal or excessive fat accumulation that may impair health. Centers for Disease Control and Prevention (CDC) assess pediatric obesity with age- and sex-specific growth charts.[4] Between 85th and 95th percent is considered at risk of overweight; 95th percentile is considered obese.[5] In obese children and adolescents, cardiovascular diseases such as hypercholesterolemia, hypertension, dyslipidemia and endocrine system diseases such as hyperinsulinemia, insulin resistance, impaired glucose tolerance, type 2 diabetes mellitus are common.[6]

The aim of this study was to determining the influences of gender, adiposity, dietary and environmental factors on vitamin D status and the relationship between 25(OH)D and cardio metabolic risk factors in adolescents.

Subjects and Methods

Study Population

The research was carried out in 69 adolescents who applied to participate in the study at İstanbul Marmara University Pendik Educational Research Hospital Pediatric Endocrinology Department aged between 12 and 17 years). Children with acute or chronic infections, genetic syndromes, cancers, auto immunologic diseases, hepatic or renal dysfunction, hormonal abnormalities or diabetes who are using any drug that may affect the blood and urine parameters (especially drugs including vitamin D and calcium and anticonvulsant therapy), and adolescents with eating disorders were excluded. The protocol of the study was approved by the Ethical Committee of the Marmara University.

Study Design

Data was collected in three stages. In the first stage, the questionnaire was developed including the demographic status, nutritional status and dietary habits as well as physical activities of adolescents under study. In the second stage, anthropometric measurements (weight, height, body mass index, waist circumference) were taken. In the third and final stage, biochemical analysis was measured. Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR).

Anthropometric Measurements

Weight was measured provided that the subjects were lightly clothed and without any shoes using a digital scale to the nearest 0.1 kg, and height was measured with a calibrated wall-mounted stadiometer to the nearest 0.1 cm (Seca, Hamburg, Germany). Body mass index (BMI) [weight (kg)/height (m)^2] was calculated using gathered data. The minimal abdominal circumference between the xiphoid process and iliac crest was measured to determine the waist circumference.

Dietary assessment

Participants recorded the foods and beverages as well as the amount of consumption during a period of three days (Thursday, Friday, Saturday or Sunday, Monday, Tuesday). The nutrient composition of the food was computed by using a nutrition software (Ebispro for Windows, Stuttgart, Germany; Turkish version BeBiS, Version 6.1) which uses data source 97% the BLS (Bundeslebensmittelschluessel; German Food Code and Nutrient Data Base; Version II.3). The nutrient values for the remaining food items were taken from the US Department of Agriculture (USDA) data base.

Biochemical Analysis

Blood samples were taken in the morning (following an overnight fasting). Serum 25(OH)D, parathormone (PTH), calcium (Ca), phosphorous (P), hemoglobin A1C, insulin and glucose were analyzed by the Biochemistry laboratory of Faculty of Medicine in Marmara University.

Vitamin D insufficiency was defined as serum 25-hydroxyvitamin D concentrations of <20 ng/mL. Serum 25-hydroxyvitamin D levels of >20 ng/mL were considered optimal.[7] The homeostasis model was used for assessing insulin resistance (HOMA-IR) using the following formula: HOMA-IR = fasting blood glucose (mmol/L) ~ fasting insulin (μU/mL)/ 22.5.[8] For adolescents HOMA-IR ≥ 3.16 was considered insulin resistance.[9]

Statistical analysis

Statistical analysis was performed using the SPSS version 17.0. Data are expressed as mean or as percentage. Means and standard deviations were used to summarize continuous variables that were normally distributed. Kolmogorov-Smirnov test was used to check for normality of distribution; $p < 0.05$ was considered evidence of abnormality. Student's t -test was used to compare mean values between men and women. The X^2 test was used to analyze both differences in proportion and distributions of obese and non-obese. Pearson or Spearman coefficient of correlation was used to assess linear correlation between 25(OH)D and other variables.

Results

The demographic characteristics based on serum vitamin D levels are summarized in Table 1. The mean age (range) of the adolescents was 13.3±1.5 years (12-17), 60.9% were female (n=42) and 39.1% (n=27) male. Participants were divided into broadly defined subsets based on serum vitamin D levels as vitamin D insufficient and sufficient. The mean BMI was 26.45±6.15 in the vitamin D insufficient group, 27.25±6.26 in the vitamin D sufficient group. There was no statistically difference between groups in term of BMI.

Serum levels of 25(OH)D did not differ according to gender, weight, and waist circumference. None of participants were taking vitamin D containing supplements. Prevalence of vitamin D deficiency in females is more common and there was no effect of wearing concealing on serum vitamin D level. Daily time spend watching TV was 2.37±1.29 hours in the vitamin D insufficient group while it is 2.09±1.2 hours in the vitamin D sufficient group however, there was no statistically difference between groups. Time spent in outdoor exercise was 3.15±2.24 hours a week in the vitamin D insufficient group whilst the quantity is 2.91±1.75 in the vitamin D sufficient group and did not differ on groups (p>0.05). Similarly average of duration of time spent under the sun did not differ on groups (p>0.05).

Biochemical variables are presented in Table 2. In this study, there were no differences in lipid metabolic profile, HbA1c, fasting blood glucose and HOMA-IR.

Serum vitamin 25(OH)D was positively correlated with dietary intake of proteins (r= 0.344, p< 0.01)

Table 1. Assessment of characteristics according to vitamin D status

Characteristic	Vitamin D insufficient (<20 ng/mL) (n=48)	Vitamin D sufficient (≥20 ng/mL) (n=21)	p Value
	Mean ± SD (median)	Mean ± SD (median)	
[a]Age	13.33±1.56	13.19±1.25	0.713
[b]Gender	n (%)	n (%)	
Female	32	10	0.221
Male	16	11	
[a]Body Mass Index	26.45±6.15	27.25±6.26	0.624
Waist Circumference (cm)	87.0±15.61	90.5±14.43	0.249
Waist to Hip Ratio	0.87±0.1	0.92±0.09	0.67
[d]Wearing Concealing Clothing			
Yes	3 (%12.5)	0	0.539
No	21 (%87.5)	10 (%100)	
[c]Time Spend Watching Tv (day)	2.37±1.29 (2)	2.09±1.22 (2)	0.527
Outdoor Exercise and Active Play in a Week (hours)	3.15±2.24	2.91±1.75	0.787
Taking Vitamin D Containing Supplements	n (%)	n (%)	
Yes	-	-	1.00
No	-	-	
Vitamin D Dietary Intake (μg)	1.45±1.21	1.34±0.58	0.672
[c]Average of Duration of Time Spent Under the Sun (min)	25.10±13.51 (20)	29.52±25.78 (20)	0.995

[a]*Student t test;* [b]*Yates Continuity Correction;* [c]*Mann Whitney U test*

Table 2. Comparison of biochemical parameters of vitamin D insufficient and vitamin D sufficient adolescents

Biochemichal Parameters	**Vitamin D insufficient (<20 ng/mL) (n=48)**	**Vitamin D sufficient (≥20 ng/mL) (n=21)**	**[a]p Value**
	Mean ± SD (median)	**Mean ± SD (median)**	
HbA1C %	5.04±0.43	5.22±0.33	0.109
[c]Triglyceride (mg/dl)	112.04±70.88	108.90±58.79	0.86
Total Cholesterol (mg/dl)	171.37±42.71	154.85±25.50	0.105
HDL (mg/dl)	52.56±15.02	46.62±11.45	0.111
LDL (mg/dl)	96.52±37.88	85.71±20.73	0.224
Serum Ca (mg/dl)	9.94±0.45	10.13±0.55	0.15
PTH (pg/ml)	50.57±16.38	44.27±17.59	0.155
Fasting Blood Glucose (mg/dl)	88.41±9.21	83.41±11.54	0.059
[c]Fasting Insulin	20.13±14.16 (16.5)	26.24±30.71 (14.6)	0.597
[c]Ca/Creatining (mg/g)	43.96±49.25 (30.96)	70.55±94.41 (50)	0.129
[c]HOMA-IR	4.46±3.48 (3.30)	5.16±5.97 (3.21)	0.527

[a]Student t test; [c]Mann Whitney U test

and fats ($r= 0.286$, $p< 0.05$), as listed in Table 3. There was no relation between serum 25(OH)D levels and fasting plasma glucose, HOMA index, serum PTH and insulin ($p>0.05$) (Table 3). Serum calcium level was positively correlated with HOMA index and insulin levels ($r=0.247$; $p<0.05$, $r=0.267$; $p<0.05$ respectively). Serum 25(OH)D, PTH, and calcium levels were not significantly correlated with any anthropometric measurements in adolescents ($p>0.05$) (Table 3).

Table 3. Correlation between 25(OH)D, PTH and serum Ca levels and various other variables

	PTH		**Vitamin 25(OH)D**		**Serum Ca**	
	r	***p***	**r**	***p***	**r**	***p***
Daily dietary nutrients						
Carbohydrate (g)	-0.163	**0.181**	0.188	**0.123**	0.008	**0.945**
Protein (g)	-0.275	**0.022***	0.344	**0.004****	0.227	**0.060**
Fat (g)	-0.135	**0.267**	0.286	**0.017***	0.108	**0.379**
Vitamin D (µg)	0.056	***0.648***	-0.022	***0.860***	0.195	***0.108***
Calcium (mg)	-0.235	***0.052***	0.235	***0.052***	0.110	***0.367***
Biochemichal Parameters						
Serum Ca	*-0.076*	***0.533***	*0.214*	***0.077***	*1*	***1***
PTH	*1*	***1***	*-0.167*	***0.170***	*-0.076*	***0.533***
Fasting Blood Glucose (mg/dl)	0.130	***0.289***	-0.101	***0.410***	0.221	***0.068***
[+]HOMA - IR	0.232	***0.055***	-0.013	***0.914***	0.247	***0.041****
25(OH)D	*-0.167*	***0.170***	*1*	***1***	*0.214*	***0.077***
[+]Insulin	0.203	***0.094***	-0.082	***0.504***	0.267	***0.026****
Anthropometric Measurement						
Weight						
BMI (kg/m^2)	0.094	***0.445***	-0.006	***0.962***	0.178	***0.144***
Waist Circumference (cm)	0.048	***0.694***	0.096	***0.430***	0.232	***0.055***

*r:Pearson Korelasyon; r:+Spearman's Correlation; *p<0.05; **p<0.01*

Discussion

Hypovitaminosis D is a problem in both adults and children.[10] In the United States, 27% of adolescents have vitamin D deficiency, which came to light from the result of the data in the National Health and Nutrition Examination Survey (NHANES).[11] Similarly, in the UK between the ages of 4 and 18 years, D vitamin insufficiency was found to be 35%.[12] According to the Endocrine Society Clinical Practice Guidelines, serum 25-hydroxyvitamin D level is the best indicator of vitamin D levels.[7] In our study vitamin D insufficiency was defined as plasma 25(OH)D concentration below 20 ng/ml.

Exposure to sunlight, measured on the basis of the time spent outdoors, the wearing of ultraviolet radiation-blocking clothing was also assessed in this study. As reported by Demirçeken, half of the girls in our country prefer concealing clothing and most of the adolescents' time spend outside are limited.[13] It is stated as the prevalence of low vitamin D in western-style women is 31%; 55% for hijab wearers; 83% in neqab wearers.[14] There are no differences between groups in terms of their clothing preference. Also, our study was conducted during winter, so results may be different in summer season.

Lack of physical activity can be independent risk factor for vitamin D deficiency because of limited exposure to sun light.[15] Children with deficiency of vitamin D have less physical activity (60.6%), compared with normal children (49%).[16] In a pediatric population aged 4-18 years, doing outdoor physical activity for less than half an hour a day or watching TV more than 2.5 hours a day increases vitamin D deficiency.[17] No differences in exposure to sunlight, outdoor activities under the sun, and physical activity among groups were found in our study ($p>0.05$).

Effects of vitamin D on obesity, cardiovascular disease and diabetes are contradictory in childhood.[18] Data obtained from the National Health and Nutrition Examination Survey (NHANES) indicate that serum 25(OH)D levels are lower in obese children.[19] Drincic et al showed that there is an inverse relationship between vitamin D levels and anthropometric measures.[20] In this study, serum levels of 25(OH)D did not differ in weight, BMI, and waist circumference and there was not a significant correlation among them.

There may be a link between low vitamin D levels and cardiovascular disease, type 2 diabetes.[21] Hypovitaminosis D has long been suspected as a risk factor for glucose intolerance. Ayesha showed that insulin secretion decreased significantly in vitamin D-deficient rats compared to control rats.[22] Zhang et al. found that serum 25 (OH) D levels were negatively correlated with insulin resistance in type 2 diabetic subjects.[23] Alemzedah et al. indicated that there is a relationship between serum vitamin D levels and insulin resistance, glucose intolerance in children and adolescents.[24] In a study conducted in high school in Turkey, no correlation was found between D vitamin deficiency and insulin measurements during oral glucose tolerance test.[25] Likewise, in this study there was no relationship could be established between serum 25(OH)D levels and fasting plasma glucose, HOMA-IR index and insulin ($p>0.05$).

Few studies have examined the relationship between 25(OH)D and cardiovascular risk factors in children and adolescents. Kumar et al., indicate that 25(OH)D deficiency was associated with elevated parathyroid hormone levels, lower serum calcium and high-density lipoprotein cholesterol levels in US pediatric population.[19] Liu et al., found that 25(OH)D levels are correlated with lower non-HDL cholesterol in children.[26] This finding is important because of the fact that non-HDL cholesterol is an important marker for cardiovascular disease risk. In our study there were no correlation between serum 25(OH)D levels and triglyceride ($r=0.062$), HDL-cholesterol ($r=-0.124$), LDL-cholesterol ($r=-0.093$), total cholesterol ($r=-0.105$ ($p>0.05$).

The main source of vitamin D is sunlight. Dietary intake alone doesn't meet vitamin D requirements to prevent deficiency states and associated problems.[27] Recommended daily allowances for vitamin D for adolescents aged 12-17 is 15 mcg.[28] Studies conducted with the children and adolescent implies that daily vitamin D intake is not adequate.[15,29,30] In this study daily dietary vitamin D intake did not meet RDA and there was no correlation between serum 25(OH)D and dietary vitamin D intake. Similarly milk and milk products, fish, and fortified foods are the main dietary sources of vitamin D.[31] Similar with Bezrati[32] et al found, our results showed that dietary intake of proteins were positively correlated with serum vitamin D levels. Because foods are not primary sources of vitamin D, this correlation should be assessed with further research on population who consume fortificated food.

In conclusion, the present study highlighted the high prevalence of vitamin D insufficiency among Turkish adolescents. Because time spend outside is limited and the weather was winter in this study, it was difficult to assess the effect of sun light on serum vitamin D level. Nonetheless in Turkey, vitamin D fortification of food and preference is limited. Because of these reasons vitamin D supplementation should be recommended especially for this age group. On the other hand vitamin D fortification of food which preferred frequently could be helpful to maintain vitamin D levels. The another important result is about chronic diseases which could be related with vitamin D. This study didn't show relationship between vitamin D status and obesity, insulin resistance. Effects of vitamin D deficiency on chronic diseases need to be assessed with larger prospective studies.

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