

Effect of Serum 25-OH D Levels on Vitamin B12 and Folic Acid Levels in Healthy Preschool Age (3-6 Years Old) Children

Özlem Kemer Aycan

Department of Pediatrics, Balıkesir University Faculty of Medicine, Balıkesir, Türkiye

Abstract

BACKGROUND/AIMS: This prospective research aimed to evaluate the effect of serum 25-OH D levels on vitamin B12 and folic acid levels in healthy preschool age (3-6 years old) children.

MATERIALS AND METHODS: The research was conducted as a cross-sectional and descriptive study. In this context, 130 healthy children, aged 3-6, who applied to the Balıkesir University Health Practice and Research Hospital Child Health and Diseases General Pediatrics polyclinic, were included during working hours. Children who came for routine healthy child examination, and had blood taken for control purposes were included in the study.

RESULTS: The frequency of smoking at home, vitamin B12 levels greater than 200, the rate of mothers' diets primarily consisting of meat and vegetables during pregnancy, and the frequency of the child going outside every day were statistically significantly higher in the group with vitamin D levels greater than 20 ($p < 0.05$). The mean folic acid level was significantly higher in the Vitamin D (> 20) group, whereas the mean homocysteine level was significantly lower ($p < 0.05$). All other laboratory parameter differences between Vitamin D groups were not statistically significant ($p > 0.05$). 77.8% of participants with < 20 ng/mL Vitamin D had a Vitamin B12 level between 0-200 pg/mL, whereas 22.2% in the Vitamin D > 20 ng/mL group had a Vitamin B12 level between 0-200 pg/mL, with a statistically significant difference ($p < 0.05$). Correlation analysis results showed that Vitamin D was significantly correlated with homocysteine ($r = -0.2$; $p < 0.05$), Vitamin B12 ($r = 0.3$; $p < 0.01$), and folic acid ($r = 0.3$; $p < 0.01$).

CONCLUSION: There was a significant positive relationship between vitamin D levels and blood B12 and folic acid levels in healthy preschool children. In cases of B12 and folate deficiency in healthy children, measuring serum 25-OH-D levels and correcting the deficiency, if present, may have positive effects on serum B12 and folate levels. New supportive studies are needed on this subject.

Keywords: Vitamin D, vitamin B12, folic acid, children

INTRODUCTION

Vitamins are essential for various biochemical functions in the body.¹ Vitamins are molecules that the body must obtain from the outside, and a deficiency in them leads to hypovitaminosis.² Among them are

vitamin D, a fat-soluble secosteroid prohormone produced in the skin as a result of exposure to sunlight, as well as ergocalciferol, which is found in plants, and cholecalciferol, which is more common in animal sources.³⁻⁵ It is converted into a hormone known as calcitriol, which plays an important role in the metabolism of calcium and phosphorus

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ORCID IDs of the authors: Ö.K.A. 0000-0002-2462-0939.



Corresponding author: Özlem Kemer Aycan
E-mail: ozlem.aycan@balikesir.edu.tr
ORCID ID: orcid.org/0000-0002-2462-0939

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through various metabolic changes in the body.⁶ There are three main target organs for vitamin D. These are the intestine bone, and the parathyroid gland. In the intestine, vitamin D enters enterocytes and induces the synthesis of calbindin, an intestinal calcium-binding protein.⁷⁻⁹ In the bone, it was shown that the 24R,25-dihydroxyvitamin D₃ is an essential hormone in the healing process of bone fractures.¹ In the parathyroid, the levels of parathyroid hormone, blood levels of 1,25 dihydroxyvitamin D, and serum calcium and phosphorus all tightly control the bioconversion of 25-OH D to 1,25 dihydroxyvitamin D₃. Parathyroid hormone and 1,25 dihydroxyvitamin D are major hormonal regulators of Ca homeostasis.⁸

Vitamin B12 is derived primarily from cobalamins found in animal products. Cobalamins are released in the acidic environment of the stomach, and enter the duodenum by binding to R and IF proteins, and the B12-IF complex is absorbed via the cubilin receptor in the distal ileum.¹⁰ This process is calcium dependent. Megalin binds several structurally unrelated ligands, and the IF-vitamin B12 complex enters the intestine via receptor-mediated endocytosis.^{11,12} The complex first binds to a receptor anchored in the outer layer of the plasma membrane, followed by endocytosis of the cargo mediated by cubilin and megalin.¹³ Megalin deficiency indirectly results in cubilin deficiency and impaired vitamin B12 absorption.¹⁴

1,25 dihydroxyvitamin D and serum ionized calcium regulate calcium transport at the bone, gut, and kidney.⁶ In addition, vitamin D is necessary for adequate bone growth.⁸ Folic acid and Vitamin B12 have essential roles in cellular processes, and prevent megaloblastic anemia.¹⁴ Vitamin B12 as a hydrogen receptor, regulates metabolic processes.¹² Some studies have reported a relationship between vitamin D, vitamin B12, and folate, and these studies suggest that vitamin D₃ influences the absorption of B12 and folate.¹⁵⁻¹⁷ This effect may be mediated directly through vitamin D receptors or indirectly through calcium-dependent absorption, but no clinical studies have examined this relationship in children. This research aimed to evaluate the effect of serum 25-OH D levels on vitamin B12 and folic acid levels in healthy preschool age (3-6 years old) children.

MATERIALS AND METHODS

Research Model

The research was designed as a cross-sectional, descriptive study. In this context, 130 healthy children aged 3-6 years, who visited the General Pediatric Pathology and Child Health Outpatient Clinic of Balikesir University Health Practice and Research Hospital during working hours, were included. The study included children who came for a routine healthy child examination and from whom blood samples were collected as controls. Detailed information was provided to the children's parents, and informed consent was obtained. The parents were informed that no additional blood sampling would be performed for this research.

Patients

Children aged 3-6 years without any diagnosed chronic diseases who applied to the Child Health and Diseases Outpatient Clinic of Balikesir University Health Practice and Research Hospital with specific complaints between October 2019 and August 2020 were included in the thesis research. The number of participants included in the

study was determined using G-Power analysis. The aim was to include at least 127 participants with a confidence level of 95%, a power of 80%, and an effect size of 0.¹⁵ A total of 130 children participated in our study. The total number of applications over a 10-month period was 12,015 children, approximately 6% of whom were aged 3-6 years. The participants to be included in the study were randomly determined at the beginning of each week. Complaints of the study participants included: 53% loss of appetite, 15% short stature, 12% constipation, 4% rash, and 16% cough.

Criteria for inclusion of volunteers in the study:

- Age 3 to 6 years
- No chronic diseases and/or irregular medication intake
- Do not take any medications that affect the metabolism of vitamins D, B12, and folic acid

Inclusion criteria for the study:

- Excluding children aged 3 to 6 years
- Presence of a chronic disease and regular medication intake

During the selection phase of the children, random numbers were generated in the SPSS program, and children were selected based on this order.

Of the children who presented to our clinic, 127 were deemed sufficient based on a power analysis; however, 130 were included in the study. During this process, 145 children who met the inclusion criteria and applied within a week were randomly contacted, and 15 of these were excluded because they met the exclusion criteria.

Laboratory

A 6 mL blood sample was taken from the patient for routine biochemical and hormonal analyses. The blood samples were centrifuged at 3000 rpm to separate serum and plasma. Folic acid, vitamin B12, and 25-OH vitamin D from these sera were analyzed by the chemiluminescence immunoassay method in the biochemistry laboratory of Balikesir University Health Practice and Research Hospital using the Beckman Coulter DXI 600/800 instrument. Excess serum, which could become medical waste, was stored at -40 degrees until analysis in three separate Eppendorf tubes. Homocysteine level, which is directly related to vitamin B12 and folate metabolism, was measured by electrochemiluminescence immunoAssay method, on the Abbott Architect instrument, in the microbiology laboratory of Balikesir University Health Practice and Research Hospital.

Normal values for folate were determined to be in the range of 2.33-17.24 ng/mL. Normal values for vitamin B12 were determined to be 126.5-505 pg/mL and for 25-OH vitamin D to be 30-70 ng/mL.

After taking samples for calcium, phosphorus, sodium, potassium, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase, the samples were analyzed spectrophotometrically on the same day using a Beckman Coulter DXI 800 instrument in the biochemistry laboratory of Balikesir University Health Practice and Research Hospital.

Statistical Analysis

SPSS, version 23.0 (IBM INC., Armonk, NY, USA). The SPSS 23.0 software package was used for statistical analysis of the study. Descriptive statistics of continuous variables are displayed using mean, standard deviation, median, minimum and maximum values, while categorical variables are displayed using frequency and percentage. The suitability of continuous variables for normal distribution was tested using the Shapiro-Wilk test. One-way analysis of variance was used when comparing three or more groups of continuous variables with normal distribution. For variables that do not have a normal distribution, the Mann-Whitney U test was used to compare two groups, and the Kruskal-Wallis test was used to compare three or more groups. Pearson's chi-square test, Yates's chi-square test, and Fisher's exact chi-square test were used for group comparisons of categorical variables.^{18,19} In all statistical comparisons in the study, a p-value below 0.05 was considered statistically significant.

Ethical Approval

The study was conducted at the Ethics Committee Balikesir University Health Practice and Research Hospital between October 2019 and August 2020 (approval number: 2019/44, date: 09.10.2019).

RESULTS

Participants were 47% female and 53% male. 92.3% of the couples in the families were married, and the majority of the mothers and fathers had primary school education or below. The majority of mothers (75.4%) were unemployed, while the majority of fathers (98.5%) were employed. Monthly income was mostly at a medium level, and the majority of participants were predominant in the central category. The rate of chronic disease was 11.5%; the number of family members was generally 4 or fewer. The rate of families stating that the mother and father smoked was 54.6%, 48.5% of families had heart disease; and 13.8% used vitamins. The majority of children were outside fewer than 4 days a week, and 69.2% of mothers had a diet rich in meat and vegetables during pregnancy (Table 1).

Statistically significant factors for the vitamin D >20 group ($p<0.05$) included Smoking at home, vitamin B12 levels >200, mothers' adherence to a diet mainly of meat and vegetables during pregnancy, and the child's daily outdoor activities (Table 2).

Folic acid mean was significantly higher in Vitamin D >20 group, whereas Homocysteine mean was significantly lower ($p<0.05$). All other laboratory parameter differences between Vitamin D groups were insignificant ($p>0.05$) (Table 3).

77.8% of participants in the <20 ng/mL Vitamin D group had Vitamin B12 levels between 0-200 pg/mL, whereas 22.2% in the >20 ng/mL Vitamin D group had Vitamin B12 levels between 0-200 pg/mL, and a statistically significant difference was observed ($p<0.05$) (Table 4).

Correlation analysis results showed that Vitamin D was significantly correlated with homocysteine ($r=-0.2$; $p<0.05$), Vitamin B12 ($r=0.3$; $p<0.01$), and folic acid ($r=0.3$; $p<0.01$) (Table 5). Although correlations were significant, correlation coefficients were low.

DISCUSSION

According to the socio-demographic factors that influence vitamin D levels, there were found to be weak associations with ethnicity,

maternal age, married parents, education, and employment status. In a study in the Netherlands, 4167 children were examined, and vitamin D deficiency was found to be more common in children of Western descent.²⁰ It was also found to be associated with birth weight and maternal age. In another study, maternal employment status was associated with vitamin D.²¹⁻²⁴ In our study, no association was found between vitamin D levels and parental employment status, education level, and number of people living with the study participants.

Table 1. Baseline characteristics of patient groups

Parameters		n %
Gender	Female	47.0 (61)
	Male	53.0 (69)
Marital status	Married	92.3 (120)
	Separated	7.7 (10)
Mother education	Primary school or no education	41.5 (54)
	High school	40 (52)
	Bachelor's/master's degree	18.5 (24)
Father education	Primary school or no education	41.6 (54)
	High school	38.5 (50)
	Bachelor's/master's degree	20 (26)
Mother working status	Working	24.6 (32)
	Not working	75.4 (98)
Father working status	Working	98.5 (128)
	Not working	1.5 (2)
Income	0-4999 TL	36.9 (48)
	5000-9999 TL	40.8 (53)
	10000 TL and above	22.3 (29)
Location	Central	86.2 (112)
	Rural	13.8 (18)
Chronic illness	Yes	11.5 (15)
	No	88.5 (115)
Number of people living in the house	3 or less than 3	34.6 (45)
	4	47.7 (62)
	5 and above	17.7 (23)
Smoking at home	Mother and father	54.6 (71)
	Mother and father	10 (13)
	Never smokes	35.4 (46)
Heart disease in the family	Yes	48.5 (63)
	No	51.5 (67)
Vitamin usage history	Yes	13.8 (18)
	No	86.2 (112)
The duration of the child's stay outside	Everyday	16.2 (21)
	4-5-6 days a week	32.3 (42)
	Less than 4 days a week	51.5 (67)
Mother's diet during pregnancy	Meat and vegetable based	69.2 (90)
	Meat based	2.3 (3)
	Vegetable based	28.5 (37)

TL: Turkish lira

Vitamin D plays a role in calcium transfer within the body for the kidneys, gut, and bones.²⁻⁴ Vitamin B12, along with folic acid, is vital for cellular processes.¹⁰⁻¹² These vitamins play a vital role in the body's homeostasis, growth, and basic functions. Many studies have

Table 2. Comparison of sociodemographic data with vitamin D

Parameters		Vitamin D ≤20 ng/mL	Vitamin D >20 ng/mL	p value
Age	36-48 months	14 (41.2)	20 (58.8)	0.480
	49-60 months	13 (56.5)	10 (43.5)	
	61-83 months	37 (50.7)	36 (49.3)	
Gender	Female	30 (49.2)	31 (50.8)	0.990
	Male	34 (49.3)	35 (50.7)	
Marital status	Married	60 (50.0)	60 (50.0)	0.540
	Divorced	4 (40.0)	6 (60.0)	
Mother education	Uneducated or primary	27 (50)	27 (50)	0.930
	High school	26 (50)	26 (50)	
	College/ undergraduate	11 (45.8)	13 (54.2)	
Father education	Uneducated or primary	27 (50)	27 (50)	0.970
	High school	24 (48)	26 (52)	
	College/ undergraduate	13 (50)	13 (50)	
Mother job	Not working	47 (48)	51 (52)	0.610
	Working	17 (53.1)	15 (46.9)	
Father job	Not working	1 (50)	1 (50)	0.980
	Working	63 (49.2)	65 (50.8)	
Income	0-4999 TL	25 (52.1)	23 (47.9)	0.750
	5000-9999 TL	24 (45.3)	29 (54.7)	
	10000 TL and above	15 (51.7)	14 (48.3)	
Location	Rural	9 (50)	9 (50)	0.940
	Central	55 (49.1)	57 (50.9)	
Smoking at home	Yes	14 (30.4)	32 (69.6)	0.020
	No	50 (59.5)	34 (40.5)	
Chronic disease	Yes	9 (56.3)	7 (43.8)	0.540
	No	55 (48.2)	59 (51.8)	
B12 groups	≤200	21 (77.8)	6 (22.2)	0.010
	>200	43 (41.7)	60 (58.3)	
Hearth disease in family	Yes	39 (61.9)	24 (38.1)	0.050
	No	25 (37.3)	42 (62.7)	
Mother's diet during pregnancy	Meat-vegetables**	37 (41.1)	53 (58.5)	0.020
	Meat	2 (68.7)	1 (33.3)	
	Vegetables	25 (67.6)	12 (32.4)	
The duration of the child's stay outside	Everyday	4 (19)	17 (81.0)	0.000
	4-5-6 days a week	8 (19)	34 (81.0)	
	Less than 4 times	52 (77.6)	15 (22.4)	

**Difference group.

TL: Turkish lira

highlighted the importance of diet as a source of vitamin D, especially in countries with little sunlight, such as the Scandinavian countries. In our study, we examined the frequency of consumption of red meat, milk and dairy products, and vegetables, in relation to vitamin D levels and found a particular association with the consumption of red meat, eggs, milk and dairy products with vitamin D levels. When we created a dietary index, we showed again that diet was associated with vitamin

Table 3. Mean values of laboratory parameters and comparison with vitamin D groups

Parameters	Vitamin D	$\bar{x} \pm SD$	p
ALP	<20	240.37±92.84	0.490
	>20	250.69±78.76	
Fosfor	<20	4.79±0.59	0.060
	>20	4.97±0.53	
Hemoglobin	<20	12.15±0.75	0.060
	>20	12.42±0.92	
RBC	<20	4.68±0.31	0.290
	>20	4.75±0.43	
Hematocrit	<20	37.09±2.36	0.790
	>20	37.21±2.77	
Folic acid	<20	11.10±4.34	0.002
	>20	13.41±4.13	
Calcium	<20	9.78±0.40	0.250
	>20	9.86±0.41	
Magnesium	<20	1.94±0.33	0.660
	>20	1.96±0.30	
MCV	<20	79.34±4.21	0.590
	>20	79.73±4.34	
Homocysteine	<20	7.48±2.55	0.030
	>20	6.57±1.66	
Ferritin	<20	19.24±16.26	0.630
	>20	18.23±13.54	
Age	<20	62.80±15.18	0.520
	>20	61.12±15.66	
WBC	<20	8.11±2.39	0.970
	>20	8.17±2.44	
PLT	<20	337.10±113.07	0.160
	>20	350.39±88.22	
MCV	<20	79.34±4.21	0.730
	>20	79.73±4.34	
AST	<20	30.30±11	0.980
	>20	31.40±13.63	
ALT	<20	15.35±5.97	0.140
	>20	17.24±7.37	
TSH	<20	2.87±1.26	0.370
	>20	2.67±1.19	
sT4	<20	0.93±0.22	0.070
	>20	1.04±0.41	

ALP: Alkaline phosphatase, RBC: Red blood cell, MCV: Mean corpuscular volume, WBC: White blood cell, PLT: Platelet, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, TSH: Thyroid stimulating hormone, sT4: Free thyroxine (Free T4), SD: Standard Deviation

Table 4. Comparison of grouped B12 levels with dichotomized vitamin D

		Vitamin D		Total
		<20 ng/mL	>20 ng/mL	
B12	0-200 pg/mL	21 (77.8%)	6 (22.2%)	27 (100%)
	200 pg/mL and over	43 (41.7%)	60 (58.3%)	103 (100%)

p=0.010.

Table 5. Correlation between participants' vitamin D, B12, homocysteine and folic acid levels

Parameters	Homosistein	B12	Folic acid
Vitamin D	r=-0.2 (p=0.03)	r=0.3 (p<0.001)	r=0.3 (p<0.001)
B12	r=-0.5	-	r=0.3
Folic acid	r=0.3	r=0.3	-
Homocysteine	-		r=0.2

D. The study found that mothers' knowledge of vitamin D and of which foods contain vitamin D was poor.²⁵⁻²⁷

Upon comparing dietary patterns in our study, we found that the effect of red meat, egg, milk, and dairy consumption on vitamin D levels was statistically significant ($p<0.05$). However, the frequency of chicken, vegetable dishes, and fish consumption did not have a statistically significant effect on vitamin D levels ($p>0.05$). Fatty fish is mentioned in the literature as one of the best dietary sources of vitamin D.²⁸ However, in our study, we did not find an association between the frequency of fish consumption and vitamin D. We explained this by the fact that 60% of our patients had not developed the habit of eating fish. Although nutrition is important, the majority of children were outside less than 4 days a week, and this may affect their vitamin D levels. Thus, this may be a confounding factor. Further research may be needed on sunlight to understand this factor.

The mean vitamin B12 in our study participants was 303.7 ± 134.4 pg/mL, and the mean folate were 12.2 ± 4.3 ng/mL. Looking at the study, the levels of vitamin B12 and folate were similar in a study conducted among an adolescent group.²⁷ In the study by Öncel et al.²⁷, the levels of vitamin B12 and folate were studied in 889 students aged 12 to 22 years, and vitamin B12 deficiency was 2.2% and folate deficiency was 21.8%.

In a study involving 280 children aged 3-17 years who attended the Child Health and Diseases Outpatient Clinic of Bağcılar Training and Research Hospital, 25-OH vitamin D levels were defined as follows: deficiency <15 ng/mL, insufficiency 15-20 ng/mL, and >20 ng/mL as normal values. Insufficiency was found in 80.36% (n=225) of the patients and deficiency in 11.79% of the patients. In this study, 25-OH vitamin D levels were found to be lower in late winter than those in late summer. Age was negatively correlated with both 25-OH vitamin D levels in late winter ($r=-0.203$, $p=0.001$) and 25-OH vitamin D levels in late summer ($r=-0.184$, $p=0.008$). When comparing levels in late summer and late winter, a positive correlation was found between vitamin D and Ca ($r=0.508$, $p=0.001$).²⁸

In a study, 171 patients (83 girls, 88 boys) aged 3-18 years who attended the pediatric endocrinology outpatient clinic had 25-OH vitamin D levels assessed as <12 ng/mL, deficiency; 12-20 ng/mL, insufficiency;

and >20 ng/mL, normal.²⁹ Of the patients included in the study, 40 had type 1 diabetes mellitus, 47 were obese, and 84 were observed for other endocrine diseases. The study found that vitamin D deficiency was 51.5%, and insufficiency was 35.1%.²⁹

In a study where serum 25-OH D levels were examined in 640 healthy primary school children aged 6-9 years, the median level was found to be 25.95 ng/mL.²⁷ Vitamin D deficiency was defined as serum 25-OH D levels less than 12 ng/mL, and such deficiency was found in 36 children (5.62%).³⁰

There was not a significant difference between children whose family uses vitamins and those whose family does not. The main reason may be that the usage level of vitamins varies and may cause variation in effects. Further research may be conducted on the effect of family vitamin usage.

Study Limitations

Our study is important because it is the first to report that vitamin D is associated with B12 and folate levels in healthy children of this age range. For this reason, comparisons were made with other studies that were partially similar to our study, but the interpretation of the results was very limited. Prospective studies in this area involving larger patient groups, with more detailed information on seasonal characteristics, daily food intake, daily sun exposure, and other sociodemographic characteristics, are needed. In addition, we believe that further large-scale studies are needed to examine polymorphisms in vitamin D hydroxylase and receptor enzymes, which could help clarify the details of the associations we found. Since the study is cross-sectional, results cannot prove a cause-effect relationship. On the other hand, it may be a source for further research. This is another limitation of the study.

CONCLUSION

It is evident that there is a significant positive association between vitamin D levels and serum B12 and folate levels in healthy preschool children. This association is consistent with the notion that vitamin D affects the absorption of both vitamins, as reported in some studies. We believe that in cases of vitamin B12 and folate deficiency in healthy children, measuring serum 25-OH D levels and correcting the deficiency, if any, may have a positive effect on serum vitamin B12 and folate levels. Further confirmatory studies are needed on this issue.

It is evident that vitamin D has calcemic and noncalcemic effects, as well as pleiotropic effects in many tissues. At the same time, there are many studies indicating that the common genetic polymorphism observed in the vitamin D receptor is important in determining the effects of vitamin D on the body. This may have influenced the associations found in our study between vitamin D, B12, and folate levels.

MAIN POINTS

- Consumption of red meat, eggs, milk, and dairy products in particular was associated with vitamin D levels.
- There is a significant positive association between vitamin D levels and serum B12 and folate levels in healthy preschool children.
- Vitamin D affects the absorption of certain vitamins in children.
- Vitamin D has calcemic and noncalcemic effects, as well as pleiotropic effects in many different tissues.

ETHICS

Ethics Committee Approval: The study was conducted at the Ethics Committee Balikesir University Health Practice and Research Hospital between October 2019 and August 2020 (approval number: 2019/44, date: 09.10.2019).

Informed Consent: Detailed information was provided to the children's parents, and informed consent was obtained.

Note: Produced from author's thesis data.

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