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Serum Vitamin D Levels in Children by Seasons of the Year

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ABSTRACT

Different reference values are used for 25-OH vitamin D in children than in adults. There are no cut-off values determined by seasons in children. This study aimed to determine 25-OH vitamin D plasma cut-off values according to seasons in children.
A total of 1237 children, including 614 girls and 623 boys, aged 0-18 years were included in the study. The children were divided into 11 different groups according to the three main age groups (infancy (0-36 months), prepubertal and pubertal) and other age groups in the summer and winter seasons. Serum calcium, phosphorus, alkaline phosphatase, magnesium, parathormone, and 25-OH vitamin D levels were determined in all cases.
The values for serum 25-OH vitamin D levels were >18,7 ng/ml in summer and ≤18,7 ng/ml in winter in infants, >21,8 ng/mL in summer and ≤21,8 ng/mL in winter in prepubertal children, >22,2 ng/mL in summer and <22,2 ng/mL in winter in pubertal children.
Very different results regarding vitamin D in age groups and high deficiency rates even in summer indicate a serious problem. There is a need to redefine the 25-OH vitamin D reference values in children by seasons. We think that if we stick to the calculated values, the treatment doses to be given could be reduced and perhaps not given at all. This could eliminate lengthy treatment protocols and hospital visits and avoid the use of unnecessary vitamin D.

Keywords: child, vitamin D, seasons, reference values

Introduction

The main source of 25-OH vitamin D in humans is sunlight. The angle of incidence of the sun's rays to the earth in winter and summer , the difference in duration of daylight and night, the frequency of sun exposure, any factor that prevents ultraviolet rays from reaching the skin (indoor air, polluted air, indoor work, increased skin pigmentation, veiling for religious or social reasons, not letting children out in the cold or overdressing against the cold in children who go outside, use of sunscreens, etc.) reduces the synthesis of vitamin D in the skin. Exclusive breastfeeding, dietary habits such as an insufficient vegetarian diet, fat malabsorption, use of anticonvulsant medications, and genetic traits may also lead to vitamin D deficiency. The socioeconomic level of the society and the level of use of health services in the region are also crucial. Since many factors affect vitamin D levels, the incidence of rickets also shows regional variations (1, 2).

Reference values of 25-OH vitamin D depending on seasons and age groups are under discussion. If reference values for Vitamin D are redefined about seasons, can unnecessary treatments be avoided? As far as we know, there are no studies on plasma values depending on seasons in childhood.

The standard for determining whether adequate vitamin D is present in the body is the normality of levels of bone turnover markers such as calcium, phosphorus, alkaline phosphatase, magnesium, parathormone, and 25-OH vitamin D. The Vitamin D level that has started to increase the parathormone level is accepted as the deficiency limit (3). The vitamin D level is lower in winter than in summer, but the lack of rickets symptoms suggests that the 25-OH vitamin D normal level should be considered lower in winter.

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In this study, our primary objective was to determine the values of 25-OH vitamin D in the age groups of children (0-3 years, prepubertal and pubertal) and by seasons winter (December, January, February) and summer (June, July, August).

Materials and Methods

This prospective study included children aged 0-18 years who admitted at the General Polyclinics of Child Health and Diseases between December 1 and August 31. All subjects were of Turkish nationality and lived in the geographical location of the region at latitude 37-39°N and longitude 42-44 °E. A total of 1237 children, including 614 girls and 623 boys, aged 0-18 years were included in the study.

Those who had rickets treatment, those who had a chronic disease, those who were taking anticonvulsant medications, and those who were taking vitamin D supplements and calcium tablets were excluded. Study participants were divided into two groups depending on the season of the year when they enrolled: the summer group (between June 1 and August 31) and the winter group (between December 1 and February 28). March, April, May, September, October, and November were considered intermediate months, and cases enrolling during these months were not included. Blood was drawn once from each patient. In this study, the value of vitamin D was not taken. Children were divided into three main groups according to their chronological age and puberty: Group 1: Children aged less than 3 years (0-6 months (Group 1A), 6-12 months (Group 1B), 12-24 months (Group 1C), 24-36 months (Group 1D)); Group 2: Children aged 3-11 years (3-6 years (Group 2A), 6-9 years (Group 2B), 9-11 years (Group 2C)); Group 3: Children aged 11-18 years (depending on Tanner stage: T2 (Group 3A), T3 (Group 3B), T4 (Group 3C), T5 (Group 3D)). Those who had obtained written and verbal informed consent from their families were enrolled in the study. After, their physical examinations were performed. Children's pubertal development was determined according to Tanner's pubertal developmental stages. The same person performed all measurements. The venous blood collected was analyzed in the biochemistry and hormone laboratories of University Faculty of Medicine. Routine biochemistry analyses of calcium, phosphorus, alkaline phosphatase, and magnesium were determined using routine analytical procedures and Roche (Germany) brand commercial kits in Hitachi PP Modular Autoanalyzer. Parathyroid Hormone measurement was performed with the Architect i 4000 SR device of Abbott brand, USA, using chemiluminescence. Serum 25-OH Vitamin D level was measured with Agilent Technologies 1200 Series (Germany) instrument and Chromsystems kit using the high-performance liquid chromatography (HPLC) method.

Our study was approved by the Ethics Committee of the Faculty of Medicine, Research and Application Hospital, with decision number 201009.

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Statistical Analysis: Statistical analyses were performed using the SPSS statistical package (v. 26.0 for Windows). Kolmogorov Simirnov test was used for testing normality. Descriptive statistics were expressed as median, interquartile range (IQR), minimum and maximum values. Comparison of medians between two groups (Winter-Summer) was examined by using Mann-Whitney U test, where the data were nonnormal distributions. Receiver operating characteristic curve (ROC) analysis was used to determine the power of 25-OH vitamin D level to differentiate winter and summer groups, and the area under the curve was calculated; significant cut-off levels were calculated using a Youden index. A p value of < 0.05 was seemed to indicate statistical significance.

Results

Of 1237 children included in the study, 614 (49.6%) were girls, and 623 (50.4%) were boys. The age of our cases was calculated in years. The youngest participant was 1 month old, and the oldest was 18 years old. The mean age of the study patients was 6.6 ± 5.2 years. Of the children, 636 (51.5%) were recruited in the summer and 601 (48.5%) in the winter season. Among the children enrolled during the summer, the youngest was 5 years old and the oldest was 17.9 years old, with a mean age of 7.1 ± 5.3 years. In winter, the youngest age was 3 years, while the oldest age was 18 years, and the mean age was 6.1 ± 5.2 years. 504 (40.7%) of the children were infants, 321 (26%) were prepubertal, and 412 (33.3%) were pubertal. The mean age was 1.15±0.85 years for infants, 7±2.1 years for prepubertal children, and 12.8±1.83 years for pubertal children. There was no statistical difference between girls and boys in serum calcium (P=0.502), magnesium (P=0.106) and phosphorus (P=0.570) levels. Serum alkaline

Table 1: Comparison of Serum Calcium, Phosphorus, Magnesium, Alkaline Phosphatase, Parathormone, and Vitamin D Levels By Seasons

Parameters	Summer (n=636)	Winter (n= 601)	Overall (n= 1237)	P**
Calcium (mg/dL)	9,60 (0,60) (7,40 - 11,40)	9,50 (0,70) (6,20 - 11,60)	9,60 (0,70) (6,20 - 11,60)	0,450
Phosphorus (mg/dL)	4,60 (1) (2,40 - 6,80)	5 (1,20) (1 - 8,70)	4,80 (1,10) (1 - 8,70)	<0,001
Alkaline phosphatase (U/L)	535 (255) (104 - 1283)	545 (292) (104 - 4015)	539 (267) (104 - 4015)	0,001
Parathormone (pg/mL)	40 (24) (6,30 - 262)	46 (33) (7,40 - 610)	43 (29) (6,30 - 610)	<0,001
Magnesium (mg/dL)	2 (0,2) (1,50 - 3,10)	2 (0,3) (1,20 - 4,50)	2 (0,2) (1,20 - 4,50)	<0,001
25-OH Vitamin D (ng/mL)	27,9 (22,5) (4,60 - 311)	18,9 (14,2) (0,80 - 105)	23,5 (17,7) (0,80 - 311)	<0,001

***: Mann Whitney U test, Median (IQR), (Minimum-Maximum)*

phosphatase (P=0.004) and parathormone (P=0.016) levels were higher in boys than girls. The difference between serum 25-OH vitamin levels of children by gender was not statistically significant (P=0.23).

In general evaluation, there was no significant difference between serum calcium levels by season (P=0.450). However, serum phosphorus, alkaline phosphatase, parathormone and magnesium levels were higher in winter (P <0.001) than in summer, while serum 25-OH vitamin D levels were lower (P <0.001). These situations are shown in Table 1.

The levels of serum calcium, phosphorus, alkaline phosphatase, magnesium, parathormone, and 25-OH Vitamin D found in all groups according to seasons and whether they were statistically significant or not are shown in Table 2.

The cut-off value for serum 25-OH vitamin D level in infants in winter was set at ≤18,7 ng/ml, the sensitivity and specificity of 25-OH vitamin D were 50,6%, 83,6%, respectively. Positive predictive value was 77,6%, negative predictive value was 60,1% and AUC was 0,730 (P<0.001). The cut-off value for serum 25-OH vitamin D level in prepubertal in winter was set at ≤21,8 ng/ml, the sensitivity and specificity of 25-OH vitamin D were 66,7%, 76,2%, respectively. Positive predictive value was 71,8%, negative predictive value was 71,5% and AUC was 0,781 (P<0.001). The cut-off value for serum 25-OH vitamin D level in pubertal in winter was set at <22,2 ng/ml, the sensitivity and specificity of 25-OH vitamin D were 61,1%, 66,4%, respectively. Positive predictive value was 58,2%, negative predictive value was 69% and AUC was 0,0,689 (P<0.001). (Table 3)

Discussion

There is still no consensus on the cut-off value of vitamin D in children. It has been reported that parathormone levels are elevated in children when serum 25-OH-vitamin D3 levels are 18 ng/mL (4) or 30 ng/mL (5). Therefore, two cut-off values are currently in use. Vitamin D deficiency, insufficiency, and sufficiency are defined as serum 25-OH vitamin D levels of <15 ng/mL, 15-20 ng/mL, and >20 ng/mL, respectively, at first presentation (6) and serum 25-OH vitamin D levels of <20 ng/mL, 20-30 ng/mL, and >30 ng/mL, respectively, at second presentation (7). However, the National Academy of Medicine (USA) considers levels of 25-OH vitamin D below 12 ng/ml to be a sign of vitamin D deficiency (8).

It should be noted that none of these cut-off values have been calculated for seasons. If these limits are taken without regard to seasons and age groups, they show a high rate of vitamin D deficiency. For example, in a study conducted in Greece, vitamin D deficiency/insufficiency was 75% in winter (9). Even in a sunny place like Kuwait, vitamin D insufficiency was appointed at a rate of 81.21% (10). The fact that vitamin D deficiency is appointed at such a high rate and yet does not cause high parathyroid hormone symptoms draws attention to the need for updating vitamin D cut-off values in children.

Table 2: Comparison of Serum Calcium, Phosphorus, Magnesium, Alkaline Phosphatase, Parathormone, and 25-OH Vitamin D Levels By Seasons

Parameters		Group 1A (0-6 months) (n= 144)		Group 1B (6-12 months) (n= 135)		Group 1C (12-24 months) (n= 152)		Group 1D (24-36 months) (n= 73)		Group 2A (3-6 years) (n: 116)		Group 2B (6-9 years) (n: 142)	
		Median (IQR)	*P***	Median (IQR)	*P***	Median (IQR)	*P***	Median (IQR)	*P***	Median (IQR)	*P***	Median (IQR)	*P***
Calcium (mg/dL)	Winter	9,75(1,1)	<0,001	9,75(0,7)	<0,001	9,7(0,7)	<0,001	9,5(0,5)	0,066	9,6(0,85)	0,022	9,5(0,6)	0,001
	Summer	10,05(0,8)		10(0,75)		9,6(0,7)		9,6(0,6)		9,6(0,6)		9,5(0,7)	
	Overall	9,90 (0,97)		9,9(0,8)		9,6(0,7)		9,5(0,6)		9,6(0,7)		9,5(0,7)	
Phosphorus(mg/dL)	Winter	5,55(1,68)	0,001	5,3(1,13)	0,006	5,2(1,2)	0,618	4,9(1,1)	0,711	4,9(1,05)	0,384	4,9(0,8)	0,001
	Summer	4,7(1,25)		4,7(1,35)		4,7(1,2)		4,6(1,1)		4,6(1,2)		4,5(1)	
	Overall	5,15(1,57)		5(1,3)		4,9(1,2)		4,75(1)		4,7(1,17)		4,7(0,9)	
Alkaline phosphatase	Winter	774(472,3)	0,033	509(267,3)	0,751	507(226)	0,772	515(176)	0,969	472(178)	0,517	533(148)	0,001
	Summer	658,5(315,5)		499(227,5)		516(207)		515(232)		483(174)		543(208)	
	Overall	708(433,8)		501(247)		508(208)		515(215,5)		477,5(181,8)		540,5(185,8)	
Parathormone	Winter	45,5(58,75)	0,005	37(45,25)	0,415	44(34)	0,002	44(25)	0,355	40(20)	0,580	48(24)	0,001
	Summer	34,6(28,1)		35,1(17,05)		35(24,2)		37,2(20,3)		39(20,2)		42(20,2)	
	Overall	40(36,6)		36,4(30)		39(28,38)		43(21,38)		39,1(19,88)		43,75(21,08)	
Magnesium (mg/dL)	Winter	2,1(0,4)	0,846	2,2(0,2)	0,020	2,2(0,2)	0,031	2,1(0,3)	0,007	2(0,2)	0,272	2(0,2)	0,001
	Summer	2,1(0,3)		2,1(0,3)		2,1(0,3)		1,9(0,3)		2(0,2)		2(0,3)	
	Overall	2,1(0,3)		2,1(0,3)		2,1(0,2)		2(0,3)		2(0,2)		2(0,2)	
25-OH Vitamin D (ng/mL)	Winter	15,15(14,2)	<0,001	20,2(20,73)	0,001	18,4(15,05)	<0,001	21,7(12,6)	0,001	18,4(12,2)	<0,001	18,1(10,8)	0,001
	Summer	31,9(34,67)		26,6(21,6)		27(21,5)		37(23,95)		29,3(14,4)		28,9(26,1)	
	Overall	20,8(21,9)		24,7(20,7)		23,6(16,25)		26,9(21,25)		22,05(15,58)		23,05(16,98)	

Parameters		Group 2C (9-11 years) (n= 63)		Group 3A (Tanner Stage 2) (n= 98)		Group 3B (Tanner Stage 3) (n= 92)		Group 3C (Tanner Stage 4) (n: 94)		Group 3D (Tanner Stage 5) (n: 128)	
		Median (IQR)	P**	Median (IQR)	P**	Median (IQR)	P**	Median (IQR)	P**	Median (IQR)	P**
Calcium (mg/dL)	Winter	9,4(0,5)	0,454	9,3(0,6)	0,554	9,4(0,5)	0,003	9,5(0,4)	0,002	9,45(0,5)	0,728

	Summer	9,55(0,47)		9,5(0,5)		9,4(0,55)		9,45(0,65)		9,4(0,5)	
	Overall	9,5(0,4)		9,4(0,5)		9,4(0,5)		9,5(0,45)		9,4(0,6)	
Phosphorus (mg/dL)	Winter	4,9(1)	0,197	4,7(0,8)	0,493	4,9(0,8)	0,545	4,9(0,8)	0,583	4,55(0,97)	0,215
	Summer	4,6(0,6)		4,7(0,9)		4,5(1,05)		4,5(0,97)		4,5(1,1)	
	Overall	4,6(0,8)		4,7(0,85)		4,65(0,8)		4,6(0,95)		4,5(1)	
Alkaline phosphatase (U/L)	Winter	615(273,5)	0,043	641(264)	0,210	609(287)	0,290	717(295)	0,007	527(443,5)	0,007
	Summer	550,5(157)		579(224)		584(198,5)		511(361)		354(325)	
	Overall	569(210,5)		595(239,3)		606,5(235,3)		583(361,5)		441(419)	
Parathormone (pg/ml)	Winter	55(30)	0,012	44(30)	0,604	60(45)	0,025	55(46)	0,157	61(49,25)	0,017
	Summer	39,85(18,27)		42,4(24,9)		46,3(25,2)		51,55(25,15)		49,6(28)	
	Overall	45(25,4)		43,5(25,57)		51,55(27,2)		52(31,5)		53(32,6)	
Magnesium (mg/dL)	Winter	2(0,2)	0,696	2(0,2)	0,642	2(0,2)	0,866	2(0,2)	0,059	2(0,2)	0,804
	Summer	2(0,3)		2(0,2)		2(0,1)		1,9(0,3)		2(0,3)	
	Overall	2(0,2)		2(0,2)		2(0,2)		1,9(0,3)		2(0,2)	
25-OH Vitamin D (ng/ml)	Winter	20,3(13,6)	0,008	21,6(16,3)	0,001	18,8(10,1)	0,004	21,2(14,95)	0,008	18,4(14,1)	<0,001
	Summer	25,25(17,77)		26,8(31,6)		24,9(22,35)		26,35(15,93)		26,8(25,6)	
	Overall	23,9(15,2)		24,95(23,9)		21,8(16,93)		23,5(17,65)		23,3(16,2)	

**: Mann Whitney U test, Median (Interquantile Range)

Table 3: Diagnostic Performance of 25–OH Vitamin D Values in Infancy, Prepubertal, and Pubertal Periods by Season

	Cut-off	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Infancy						
Winter	≤18,7	0,730 (0,69-0,77)	50,6 (44,4-56,7)	83,6 (78,3-88,1)	77,6 (71,7-82,5)	60,1 (56,9-63,3)
Summer	>18,7	0,730 (0,69-0,77)	83,6 (78,3-88,1)	50,6 (44,4-56,7)	60,1 (56,9-63,3)	77,6 (71,7-82,5)
Prepubertal						
Winter	≤21,8	0,781 (0,73-0,83)	66,7 (58,6-74,1)	76,2 (69-82,4)	71,8 (65,5-77,4)	71,5 (66,4-76,1)
Summer	>21,8	0,781 (0,73-0,83)	76,2 (69-82,4)	66,7 (58,6-74,1)	71,5 (66,4-76,1)	71,8 (65,5-77,4)
Pubertal						
Winter	<22,2	0,689 (0,64-0,73)	61,1 (53,6-68,3)	66,4 (60-72,4)	58,2 (52,9-63,3)	69 (64,5-73,2)
Summer	>22,2	0,689 (0,64-0,73)	66,4 (60-72,4)	61,1 (53,6-68,3)	69 (64,5-73,2)	58,2 (52,9-63,3)

AUC: Area Under Curve, PPV: Positive Predictive Value, NPV: Negative Predictive Value

For vitamin D, very significantly different results by age groups and seasons have been reported in the literature (9, 11-16). When these studies were examined, serum 25-OH vitamin D levels were highest in the summer months (after September in some studies) or lowest in the winter months (after March in some studies). Also, in our study, in agreement with the literature, serum 25-OH vitamin D levels were lower in winter than in summer.

A study conducted in Greece found vitamin D deficiency/insufficiency of 87.8% in winter and 56.6% in summer. They divided the study participants into three groups such as 1-4 years, 5-12 years, and 13-18 years, similar to us in terms of 25-OH vitamin D and found the highest mean 25-OH vitamin D levels in September 34.6 ± 8.7 and the lowest in January with 17.9 ± 6.8 ng . However, their cases were not vitamin D deficient and had no signs of rickets (9).

In a study conducted in Italy (the cut-off value for deficiency was 20 ng/dl, and the cut-off value for insufficiency was 30 ng), vitamin D deficiency was found to be vitamin D deficiency was found to be 21.2% and insufficiency 46.2% in the summer months while 63.8% and insufficiency 25.1% in the winter months. When considered by age, vitamin D deficiency and insufficiency were appointed at rates of 35.3% and 39.1% below 2.79 years of age, 53.05% and 35% between 2.79-11.3 years of age, and 54% and 35.4% between 11.3-18 years of age (11).

A Korean study found that in the group not taking vitamin supplements, vitamin D levels were lowest in March (17.2±8.6 ng/mL), highest in August (30.8±9.0 ng/mL), and high in September (30.5±5.8 ng/mL). Moreover, in the group taking vitamin supplements, these levels were 30.0±23.9 ng/mL in March and 33.2±11.0 ng/mL in September (12).

In another study from Ukraine, the lowest vitamin D levels were found to be 16.60 ng/mL in February and the highest 32.53 ng/mL in August (14). In a study of obese and healthy controls in Spain, serum 25-OH vitamin D 25.3 ± 8.7 ng/mL were found in obese children in summer and 18.7 ± 7.6 ng/mL in winter. It has been shown that there are seasonal variations of vitamin D in normal children, with levels of 34.4 ± 8.2 ng/mL in summer and 25.7 ± 6.2 ng/mL in winter , even in obese children (15).

In our study, we appointed values of serum 25-OH vitamin D levels in infants as 10.85 ng/mL in summer and 7.05 ng/mL in winter, in prepubertal children as 20.55 ng/mL in summer and 12.10 ng/mL in winter, and in pubertal children as 20.65 ng/mL in summer and 12.35 ng/mL in winter.

In the literature, a study in healthy children recommended an individualized treatment dose depending on the seasons due to insufficient vitamin D levels (<30 ng/mL), which were appointed in >70% of cases even after vitamin D supplementation, and insufficient monthly mean levels except in summer (June-August) (12). In another study, vitamin D deficiency was found in more than 80% of pediatric cases (17). Again, in a previous study, vitamin D deficiency was observed in more than 80% of cases in winter; moreover, the percentage of children with normal vitamin D levels was reported to be 32.6%. This study also suggested that the current situation should be re-evaluated so that the pediatric cut-off levels should be re-examined depending on the seasons, as the prevalence of vitamin D deficiency is high even in areas where there is sufficient sunlight during most months of the year (11).

In the study conducted on adults, it was stated that considering seasonal differences should be mandatory, not recommended (3). When, as in the examples in these studies, the differences between the two seasons are so great and there is no evidence of vitamin D deficiency, failure to adjust values according to the seasons can be a serious problem in children.

Considering the age groups of the cases, vitamin D deficiency in Greece was found mainly in adolescents aged 13-18 years (75%), followed by children aged 5-12 years (71.2%), and least in infants aged 1-4 years (55.2%) (9). In Qatar, it was most commonly appointed in adolescents aged 11-16 years (61.6%), second most commonly in children aged 5-10 years (28.9%), and least commonly in children younger than 5 years (9.5%) (18). In Italy, it was found mainly in adolescents aged 11.3-18 years, while it was also found in children aged 2.79-11.3 years or older (11). In our study, vitamin D deficiency was most observed in infants aged 0-3 years, followed by pubertal children aged 11-18 years, and least commonly in prepubertal children aged 3-11 years.

As in the examples above, children are generally divided into age groups in the literature. Moreover, different results were obtained in each age group. Since the vitamin D cut-off that increases parathormone levels is different in each age group, it has been shown that it is necessary to act according to age groups when setting cut-off values. Rutigliano et al. (11) stated that age is an important parameter affecting vitamin D status, which we also agree with.

This study has some limitations. First, blood was drawn from the participants only once. Second, blood was drawn in only two seasons, winter and summer. Blood was not drawn from the same participants in both winter and summer. In addition, data on residential areas (rural/urban), dietary habits, body mass index, physical activity, sun exposure, and sunscreen use were not included in the study.

In conclusion, the values for serum 25-OH vitamin D were different in different age groups. The values for serum 25-OH vitamin D levels were determined higher in summer and lower in winter. There were very different results in the age groups and high deficiency rates even in summer concerning vitamin D show that there is a serious problem. The vitamin D differences between seasons suggest that normal vitamin D levels may be lower than we think depending on age and during winter months. Therefore, there is a need to re-assess normal 25-OH vitamin D levels in children by age group and for summer/winter seasons. In terms of practical use of seasonal normal vitamin D levels in children; we support 10 ng/ml in summer and 8 ng/ml in winter for 0-36 months infants, 20 ng/ml in summer, and 12 ng/ml in winter for older children. We think that if the vitamin D levels of patients are treated according to seasons, the treatment doses that need to be given to improve biochemical parameters could be reduced or perhaps not given at all, thus avoiding long treatment protocols and unnecessary hospital visits and preventing unnecessary vitamin D supplementation.

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