Vitamin D Insufficiency and Its Association with Biochemical and Anthropometric Variables of Young Children in Rural Southwestern China

Shan-Shan Geng[1,2], Jing-Qiu Ma[1,2], Shan-Shan Liu[1,2], Jie Zhang[3], Xiao-Yang Sheng[1,2]

[1]Department of Children and Adolescents Health Care, Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China
[2]MOE-Shanghai Key Laboratory of Children's Environmental Health, Shanghai Institute for Pediatric Research, Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China
[3]Department of Anesthesia, The Children's Hospital, School of Medicine, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310003, China

Abstract

Background: With recognition of the important roles of Vitamin D (VitD) in various physiological processes, increasing attention has been drawn to the status of VitD in early life. However, the VitD status of young children and the related factors in rural areas of Southwestern China remain unclear. This study aimed to explore VitD status and its seasonal variation in 18-month-old children living in rural Southwestern China. The association of VitD with biochemical and anthropometric variables was also investigated.
Methods: A total of 177 18-month-old children in a rural area of Yunnan Province, Southwestern China, were enrolled. Serum concentrations of 25-hydroxy Vitamin D (25(OH)D) were measured through high-performance liquid chromatogram-tandem mass spectrometry. Parathyroid hormone (PTH) levels were measured with a chemiluminescence assay. Serum concentrations of calcium, phosphorus, and alkaline phosphatase (ALP) were also measured. Anthropometric data and the outdoor activity time of each participant were collected.
Results: The serum 25(OH)D concentration was 26.61 ± 7.26 ng/ml; concentrations lower than 30 ng/ml accounted for 70.6% of the participants and concentrations lower than 20 ng/ml accounted for 16.4%. The level of serum 25(OH)D was not significantly different among four seasons ($P > 0.05$). A positive relationship was found between 25(OH)D concentration and the time of outdoor activities ($r = 0.168$, $P < 0.05$). Serum PTH concentration was negatively correlated with 25(OH)D concentration ($r = -0.163$, $P < 0.05$). A positive relationship was found between the serum concentrations of 25(OH)D and calcium ($r = 0.154$, $P < 0.05$). No significant association was observed between 25(OH)D and ALP, phosphorus, or anthropometric variables.
Conclusions: The prevalence of VitD insufficiency is high among young children in the rural Southwestern China regardless of the seasons. VitD supplementation is still essential to maintain VitD sufficiency for children living in rural area.

Key words: 25-Hydroxy Vitamin D; Infant; Rural Area; Vitamin D; Vitamin D Deficiency

Introduction

Vitamin D (VitD) is an essential nutrient with hormone-like activity that regulates calcium and bone metabolism throughout life. Adequate VitD is required among children for effective bone mineralization and normal growth. Reduced intestinal calcium and phosphate absorption and increased bone resorption might happen when the levels of VitD are too low. Furthermore, VitD insufficiency not only influences the bone formation of children, but it is also related to many other diseases, including respiratory infection, nocturnal enuresis, diabetes, and asthma in children.[1-8] The serum concentration of 25-hydroxy Vitamin D (25(OH)D) has been routinely used to assess the VitD status of children because it is the primary form that exists in the circulation.

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Address for correspondence: Dr. Xiao-Yang Sheng, Department of Children and Adolescents Health Care, Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China
E-Mail: shengxiaoyangcn@126.com

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However, the serum concentration of 25(OH)D has also been reported to be related to parathyroid hormone (PTH), calcium, phosphorus, and alkaline phosphatase (ALP).[9,10]

To keep the adequate serum levels of 25(OH)D, the US Endocrine Committee has suggested the intake of 400–1000 U/d under 1 year of age and 600–1000 U/d from 1 to 18 years of age.[11] However, most parents are not aware of VitD supplementation because of a lack of information with the high rates of reported suboptimal VitD levels among children.[12] Hence, VitD insufficiency or deficiency remains as an important public health problem in children, with a reported prevalence of 15%–80% worldwide.[13-15]

Many studies observing the VitD status of young children have been conducted in the developed countries.[16-18] Similarly, in the developing countries, almost all studies have been conducted in major cities where the living standard and socio-economic status are as high as developed countries.[13,19,20] However, few studies investigated the VitD status of young children in poor rural areas where less or no VitD supplementation were given to children. VitD status of young children and the potential factors related to VitD status in rural areas remain unclear.

The VitD status of young children without VitD supplementation in the poor rural area has been investigated in Xichou, which is a poor county in a rural area of Yunnan Province, Southwest China (located at 104°41′E, 23°25′N). The average altitude of this area is higher than 1000 m, and this area receives adequate sunlight. The participants enrolled in this study were all from the countryside and not given any VitD supplements. The serum concentrations of 25(OH)D, calcium, phosphorus, PTH, and ALP were measured to explore the VitD nutritional status of 18-month-old children in the rural Southwestern China. We also investigated the seasonal variation of VitD status and its relationship with biochemical indices and anthropometric data.

Methods

Study design and subjects

This study is a part of our large study entitled "Development and Health of Rural Chinese Children Fed Meat as a Daily Complementary Food from 6 to 18 Months of Age", which was conducted in Yunnan Province. This is a cross-sectional study conducted in Xichou County, Yunnan Province of China, from May 2010 to April 2011. The eligibility criteria for infants were as following: healthy-term singleton infants, 17–19-month-old, a birth weight over 2500 g born between 37 and 42 weeks of gestational age, no metabolic or physical problems, breastfeeding for at least 6 months, living in Xichou County, and a willingness to follow the study protocol. The exclusion criteria included any preterm/low birth weight infant, use of VitD supplements, or medication that might affect VitD metabolism. Written informed consent was obtained from the mother of each infant. The study protocol was approved by the Ethics Committee of Xinhua Hospital, Shanghai Jiao Tong University School of Medicine. According to the prevalence of VitD deficiency reported in domestic studies and the following equation, the minimum sample size is estimated as 96 cases (equation 1).[13]

$$n = \frac{U_{1-\alpha/2}^{2} p_0 (1 - p_0)}{d^2} \quad (1)$$

Where, U is the statistic of confidence level; p_0 is the estimated prevalence of VitD deficiency (22%);[13] and d is the investigation error (20%).

Biochemical measurements

Nonfasting blood samples (5 ml) were obtained using venipuncture by a medical doctor. The serum was separated, aliquoted, and stored at −80°C until analysis. Serum 25(OH)D was measured using high-performance liquid chromatography-tandem mass spectrometry (AB Sciex, USA). The serum concentrations of 25(OH)D_2 and 25(OH)D_3 were successively measured. The serum concentration of PTH was assayed with a chemiluminescence assay (automatic chemiluminescence analyzer, Siemens, Germany). Serum concentrations of calcium, phosphorous, and ALP were determined using the ortho-cresolphthalein complexone, phosphomolybdic acid ultraviolet (UV), and French Society for clinical Biochemistry (SFBC) rate method, respectively.

Anthropometric measurements

All anthropometric measurements were carried out by two well-trained researchers. Height and weight were measured with a standard clinical Seca stadiometer (Seca, Germany) to the nearest 0.1 cm and a standard body electronic measuring scale (Seca) to the nearest 0.005 kg, respectively, without shoes and in light clothing. Body weight was measured twice consecutively; if the difference between the two measurements was less than 0.01 kg, the first measurement was recorded. Height was also measured twice consecutively, if the difference between the two measurements was <0.4 cm, the mean height of the two measurements was recorded. If the difference was more than 0.01 kg (0.4 cm), continuous measurements were conducted again until the difference between the two measurements was in the permissible range, and then the datum in the last measurement was recorded. The body mass index (BMI) was calculated according to the following equation: BMI = weight (kg)/(height [m])^2. Z-scores were calculated using the WHO Anthro 3.0.1 software program (WHO Anthro, Geneva, Switzerland), including the weight-for-age Z-score (WAZ), weight-for-height Z-score (WHZ), and length-for-age Z-score (LAZ). Underweight, stunting, and wasting were defined as WAZ <−2, LAZ <−2, and WLZ <−2, respectively. Overweight was defined as WLZ >+2. Questionnaire about basic information, dietary intake, and the time for outdoor activities per week were collected in the face-to-face interviews.

Definition of Vitamin D status

In this study, VitD deficiency was defined as a serum 25(OH)D level <20 ng/ml, while VitD insufficiency was in the range of 20–30 ng/ml, and normal values were defined as >30 ng/ml.[14,21]

Statistical analysis

Continuous data were shown as mean ± standard deviation (SD). The frequency of subjects in each group of VitD status was identified. The distributions of all variables were checked for normality. Among the variables with normality, differences between two groups were examined using the independent samples *t*-test. When more than two groups were considered, one-way analysis of variance (ANOVA) was used. Pearson correlation coefficients were calculated to investigate the correlations between VitD status and other normal variables. A significance level of 0.05 (*P* value) was selected for the test. Statistical analyses were performed using SPSS 17.0 software (IBM Corp., Chicago, IL, USA).

Results

Sample characteristics

One hundred and eighty-four young children were enrolled in this study. Among these children, seven subjects were excluded for unavailable biochemical data. Totally, 177 children were enrolled into the final data analysis. The participants included 85 boys (48.0%) and 92 girls (52.0%) with a mean age of 18.0 months (range: 17.8 − 18.1 months). The mean time of outdoor activities for all children was 21.3 h/week, and there was no significant difference between boys and girls ($P > 0.05$). According to the WHO Child Growth Standards, there were 57 children (32.2%) exhibiting signs of stunting (LAZ <−2), 17 underweight (9.6%) (WAZ <−2), and two wasting (1.1%) (WLZ <−2). In this study, the height and weight of the boys were significantly higher than those of the girls, as same as the BMI ($P < 0.05$). The difference of the LAZ between the boys and girls was also significant ($P < 0.05$). However, there was no significant difference in age, WHZ, or WAZ between the two genders [Table 1].

Measurements of 25-hydroxy Vitamin D concentration

25(OH)D exists in two forms in serum, namely 25(OH)D_2 and 25(OH)D_3. In this study, the levels of both 25(OH)D_2 and 25(OH)D_3 were measured; however, 25(OH)D_2 was detected in only eight cases. The peak serum concentration of 25(OH)D_2 measured was 12.60 ng/ml, accounting for 38.2% of the 25(OH)D in one case, whereas 25(OH)D_2 only accounted for approximately 10.0% of the 25(OH)D in all of the other seven cases. Serum 25(OH)D levels ranged from 7.70 to 58.50 ng/ml, with a mean concentration of 26.61 ± 7.26 ng/ml. Sufficient concentrations of 25(OH)D were found in 29.4% of all cases. A total of 125 (70.6%) children presented with low blood levels of 25(OH)D, with 29 (16.4%) children exhibiting 25(OH)D concentration of <20 ng/ml. No significant difference in the 25(OH)D concentration was found between the boys and girls ($P > 0.05$) [Table 1].

Seasonal variation of 25-hydroxy Vitamin D concentration

According to different blood collection times, the subjects were divided into four groups: spring (March to May), summer (June to August), autumn (September to November), and winter (December to February). The serum 25(OH)D concentration was 25.28 ± 7.14 ng/ml, 26.71 ± 6.47 ng/ml, 28.51 ± 8.38 ng/ml, and 25.99 ± 6.43 ng/ml in spring, summer, autumn and winter, respectively. The seasonal variations in the serum of 25(OH)D concentrations are shown in Figure 1. There was no significant difference of 25(OH)D concentrations among these four seasons ($P > 0.05$).

Table 1: Characteristics of anthropometric and biochemical measurements of 177 18-month-old children in a rural area of Yunnan Province, Southwestern China, enrolled in this study

Items	Boys ($n = 85$)	Girls ($n = 92$)	t	P
Age (months)	18.0 ± 0.3	18.0 ± 0.2	0.715	0.476
Weight (kg)	9.90 ± 0.94	9.33 ± 0.91	4.076	0.000
Height (cm)	77.69 ± 2.62	76.77 ± 2.63	2.327	0.021
BMI (kg/m²)	16.37 ± 1.04	15.80 ± 1.04	3.645	0.000
WHZ	−0.19 ± 0.79	−0.23 ± 0.76	0.323	0.747
WAZ	−0.94 ± 0.86	−0.80 ± 0.81	−1.090	0.277
LAZ	−1.70 ± 0.99	−1.34 ± 0.89	−2.497	0.013
25(OH)D (ng/ml)	27.41 ± 7.59	25.88 ± 6.90	1.407	0.161
Calcium (mmol/L)	2.39 ± 0.10	2.41 ± 0.11	−1.201	0.231
ALP (U/L)	214.98 ± 66.26	222.99 ± 73.50	−0.760	0.449
PTH (pg/ml)	15.11 ± 8.12	17.47 ± 10.21	−1.690	0.093
Phosphorus (mmol/L)	1.77 ± 0.24	1.74 ± 0.26	0.715	0.476
Outdoor time (h)	21.34 ± 0.98	20.96 ± 0.92	0.430	0.673

Values were shown as mean ± SD. BMI: Body mass index; WAZ: Weight-for-age *Z*-score; WHZ: Weight-for-height *Z*-score; LAZ: Length-for-age *Z*-score; 25(OH)D: 25-hydroxy Vitamin D; PTH: Parathyroid hormone; ALP: Alkaline phosphatase; SD: Standard deviation.

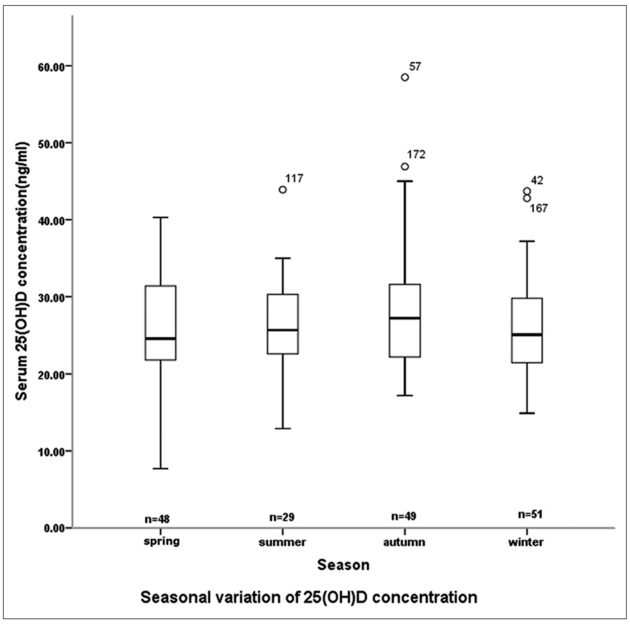

Figure 1: Serum 25-hydroxy Vitamin D concentration among different seasons.

Measurement of biochemical variables

The concentrations of calcium, phosphorus, ALP, and PTH were 2.40 ± 0.11 mmol/L, 1.75 ± 0.25 mmol/L, 219.14 ± 70.03 U/L, and 16.34 ± 9.32 pg/ml, respectively. There was no significant difference in the serum PTH levels between boys and girls ($P > 0.05$), and neither were the differences of calcium, ALP, or phosphorus between the two genders ($P > 0.05$ for all).

Relationship of 25-hydroxy Vitamin D concentration with biochemical and anthropometric variables

The correlation coefficients between the 25(OH)D concentration and biochemical variables are presented in detail in Table 2. There was a significant difference among the concentration of PTH in different 25(OH)D status ($P < 0.05$) [Figure 2]. A significant negative correlation was found between the 25(OH)D concentration and serum PTH level ($r = -0.163$, $P = 0.030$) [Figure 3]. The 25(OH)D concentration was positively correlated with the serum calcium concentration ($r = 0.154$, $P = 0.040$) [Figure 4]. However, the 25(OH)D concentration was not found to be significantly related to the serum levels of ALP or phosphorus ($P > 0.05$ for both). No significant correlation was found between the 25(OH)D concentration and several anthropometric variables (WAZ, WHZ, LAZ, and BMI) ($P > 0.05$ for all) [Table 2].

Discussion

VitD deficiency is regarded as the most common nutritional deficiency and also one of the most common undiagnosed medical conditions worldwide.[22] It has been estimated that one billion people worldwide have VitD deficiency or insufficiency. Though the optimal level of VitD is still controversial, it has been widely adopted in the clinic that VitD insufficiency is defined as a serum 25(OH)D level <30 ng/ml and deficiency is defined as serum levels of 25-(OH)D <20 ng/ml. The prevalence of VitD deficiency varies among different populations and regions.[16] In this study, 70.6% (125 subjects) of all the participants were

Table 2: Correlation between 25(OH)D concentration and several variables by Pearson correlation test

Variables	Correlation coefficient	P
PTH	−0.163	0.031
ALP	−0.004	0.956
Calcium	0.154	0.041
Phosphorus	0.062	0.416
WAZ	0.135	0.073
WHZ	0.110	0.147
LAZ	0.108	0.152
BMI	0.085	0.258
Outdoor time	0.168	0.029

25(OH)D: 25-hydroxy Vitamin D; PTH: Parathyroid hormone; ALP: Alkaline phosphatase; WAZ: Weight-for-age *Z*-score; WHZ: Weight-for-height *Z*-score; LAZ: Length-for-age *Z*-score.

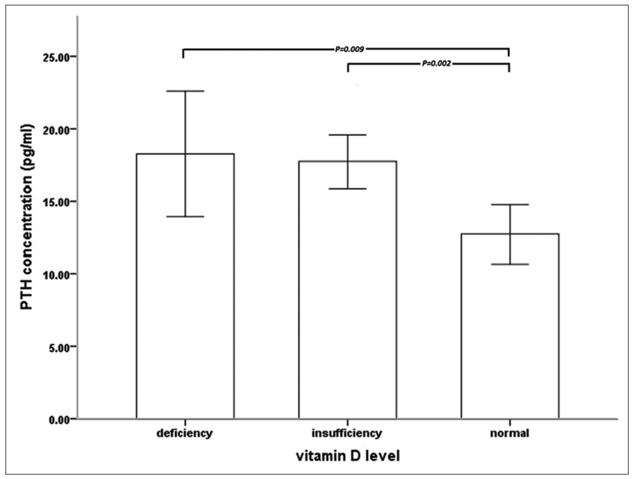

Figure 2: Serum parathyroid hormone (PTH) concentration among different levels of 25-hydroxy Vitamin D concentration.

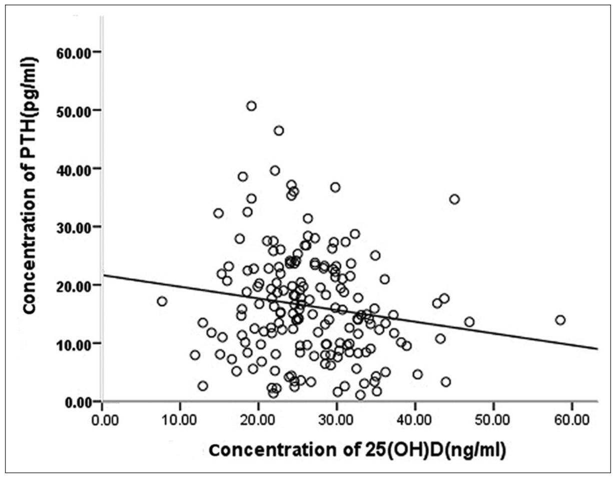

Figure 3: The relationship between 25-hydroxy Vitamin D (25(OH)D) concentration and serum parathyroid hormone (PTH) concentration by Pearson correlation test. $r = -0.163$, $P = 0.030$.

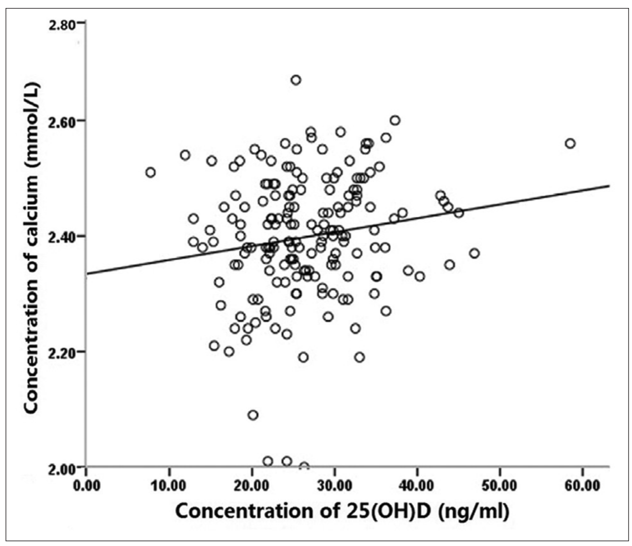

Figure 4: The relationship between 25-hydroxy Vitamin D (25(OH)D) concentration and calcium concentration by Pearson correlation test. $r = 0.154$, $P = 0.040$.

observed to have VitD insufficiency and 16.4% (29 subjects) with VitD deficiency. Compared with domestic studies, the children in our study had a much lower prevalence of VitD deficiency (89.2% in Beijing, 58.6% in Shanghai, and 22% in Hangzhou).[13,19,20] In our study, 29 (16.4%) children presented with 25(OH)D concentration <20 ng/ml, which was similar to the study performed by Saintonge *et al*.[16] However, in the study performed in Britain, 35% of all subjects had serum 25(OH)D concentration <20 ng/ml, so were the studies conducted in Belgian and Tehran.[17,18] Several factors might account for the relatively lower prevalence rate of VitD deficiency in this study. The present study was conducted in Xichou County, which is at a lower latitude (23°25′N) and higher altitude (more than 1000 m). Children living in this area are exposed to UV irradiation with stronger UV intensity for the latitude- and altitude-influenced solar exposure. Furthermore, participants in our study had long time of outdoor activities with a mean time of 21.25 h. The children were not given proper protection from the sun exposure due to lack of awareness. Thus, the sunlight-induced VitD synthesis was higher among our participants compared with that in other reports.[13,19,20] Besides that, this study was conducted in community rather than in hospitals. The children have better general health compared with the hospital-based population.

It is worth noting that the prevalence of VitD insufficiency is still higher compared with other studies in which the children received VitD-fortified diets.[9] As we know, there are two main sources of VitD for humans. One source is the cutaneous synthesis of VitD (mainly $VitD_3$) and the other source is dietary intake (mainly $VitD_2$). VitD from either source is efficiently converted to 25(OH)D. In our study, the children took no VitD supplements, and the VitD intake from diet was rare. The lack of VitD supplementation might be the reason for the high prevalence of VitD insufficiency in this rural area, even though the latitude of this area is low and the solar exposure is ample.

In this study, no significant difference in the serum 25(OH)D concentrations among the four seasons was found. However, Kemp *et al*. reported that the serum 25-OH-D concentrations in summer were significantly higher than those in winter.[23] This discrepancy may be mainly caused by the different study sites. Xichou County, where the present study was conducted, is located at 23°25′N, within the plateau area. The UV intensity varies slightly throughout the entire year. Hence, there was no significant difference among four seasons. However, the level of VitD in this plateau area is still varied in the four seasons. In summer, children were exposed to UVR with less clothes. Therefore, the serum 25(OH)D concentration in autumn reached to the peak after accumulation during summer. In winter, children wore more and exposed less to UVR for the coldness. The 25(OH)D level of Spring was the lowest after being consumed during winter. It is worthwhile noting that although the level of 25(OH)D in autumn was the highest among the four seasons, the mean 25(OH)D concentration was still insufficient (28.51 ± 8.38 ng/ml), which implies that insufficient VitD status is common in this area.

The anthropometric characteristics of the participants demonstrated that the growth and development status of the children in this study are lower than the WHO standard. This lower status is mainly because our subjects were all from one rural area in Southwest China where socio-economic levels are low. Razzaghy-Azar and Shakiba reported that the 25(OH)D level in males is significantly greater than that in females.[24] However, significant difference in the 25(OH)D concentrations between boys and girls was not observed in this study. This discrepancy might be caused by the age differences of the subjects recruited in the studies. This study enrolled a much younger population with a mean age of 18 months. Whereas, some subjects recruited in other studies were in puberty, which exerts significant differences in development between boys and girls. These developmental status subsequently resulted in different requirements for 25(OH)D. Furthermore, compared with boys, adolescent girls are more reluctant to bask in the sun. VitD is more easily deposited and stored in the fat.[16,25,26] However, in this study, there was no difference in the time of outdoor activities between the boys and girls.

The small intestine absorbs no more than 10%–15% of dietary calcium without VitD. However, the absorption efficiency of dietary calcium in the small intestine is 30% on average if the person has sufficient VitD, increasing to as high as 80% during growth, lactation, and pregnancy.[27] In the present study, a positive correlation was found between the serum concentration of calcium and 25(OH)D, revealing that VitD deficiency can reduce the absorption of calcium. VitD supplement is required for children in this rural area to increase the calcium absorption. VitD has also been reported to promote the absorption of phosphorous in the gastrointestinal tract.[28] However, no significant associations of the 25(OH)D concentration with the levels of phosphorous or ALP was found in this study. The result was consistent with other studies conducted in New Zealand, Tehran, or California.[29,30]

The serum calcium concentration decreases when the level of 25(OH)D concentration is low. Meanwhile, the decrease in serum calcium concentration promotes the secretion of PTH to maintain the calcium concentration. The negative correlation between the concentration of 25(OH)D and PTH was found in this study, which is consistent with previous studies.[29-32] Weng *et al*. have estimated the 25(OH)D concentration required for maximal suppression of PTH in children and adolescents according to the inverse relationship between 25(OH)D and PTH.[25] The inverse relationship between serum 25(OH)D and PTH concentration reveals that a poor VitD status can be defined as the 25(OH)D concentration below which the PTH concentration will rise. It is presumed that at this concentration of 25(OH)D, the supply of substrate for $1,25(OH)_2D$ production is inadequate to meet its requirements for function in the parathyroid gland. A high serum concentration of PTH may have adverse effects on bone health. Therefore, VitD supplementation is requisite for young children in this rural area.

There are some limitations in this study: the sample size in this study was relatively small; however, to the best of our knowledge, it is larger than other studies conducted in this rural area of Southwestern China to assess VitD status. The optimal 25(OH)D concentration for the children in this area was not determined in this study. Great caution should be noted when interpreting the seasonal variation of 25(OH)D because this is a cross-sectional study, where each participant only had one observation.

In conclusion, the majority of young children in the rural Southwest China had VitD insufficiency regardless of the season. VitD supplementation might still be essential to young children in this rural area because ample solar exposure alone does not enable children to maintain VitD sufficiency.

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Conflicts of interest

There are no conflicts of interest.

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