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Investigation of Vitamin D Levels in Children Aged 0–4 Years in Yunnan Province

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Abstract

Aim: To investigate the vitamin D levels in children aged 0–4 years in Yunnan Province.

Methods: This study selected children aged 0 to 4 years who underwent physical examination in the special needs clinic of the Hospital from October 2019 to December 2020 as subjects to analyze serum 25(OH)D levels.

Result: Vitamin D deficiency was more common in girls than in boys. There was no significant difference in serum vitamin D levels between boys and girls at any age. However, there was a significant decrease in vitamin D levels after 2 years old in all chidren at all ages. The levels of vitamin D in children were highest in summer, which were significantly higher than other seasons, and were lowest in winter. At the same time, vitamin D levels were significantly different based on the economic level of cities. The serum vitamin D contents of infants and young children in the top five cities with the highest economic levels were significantly higher than in lower-ranked cities. There was a significant correlation between vitamin D content and serum calcium.

Conclusion: This study preliminarily determined a reasonable reference range for serum 25 hydroxyvitamin D content in infants aged 0–4 years in Yunnan Province. Which will be of significance for the establishment of official guidelines on vitamin D supplementation in infants and young children in Yunnan and for policy formulation.

1. Introduction

Vitamin D is an essential nutrient that regulates the activity of calcium and bone hormones throughout life. A study has suggested that higher vitamin D levels can protect the body from multiple diseases [1]. Additional research over the last 20 years has shown that vitamin D plays an important role in a variety of physiological processes. Long-term vitamin D deficiency not only causes abnormal bone development and impedes the absorption of calcium and phosphorus, but also increases the risk of chronic diseases such as infection [1], cancer [2, 3], autoimmune diseases [4], asthma [5] and infectious diseases [6], as well as cardiovascular and vascular diseases [7, 8]. Vitamin D deficiency is common worldwide and recent studies have suggested that vitamin D may be involved in the development of the nervous system because its deficiency may lead to the occurrence of depression [9]. Infants and young children (0–4 years old) are at a critical stage of physical growth and development. Vitamin D deficiency may lead to metabolic syndrome in childhood and adverse effects into adulthood [10]. Therefore, screening for vitamin D levels in children, especially infants aged 0–4 years, is particularly important.

Receptors for vitamin D are found in almost all organs of the body, with various studies showing them present in the brain, heart, skin, reproductive organs, small intestine, colon, and even in activated T and B lymphocytes and B-islet cells [11]. More than 90% of the body's own vitamin D is produced by the skin, while dietary supplements may contribute 5–10% of total vitamin D [12]. Under ultraviolet radiation (UV-B), 7-dehydrogenated cholesterol, the precursor of vitamin D, is transformed into vitamin precursor D3 through the skin and isomerized into vitamin D3, which is transported to the liver through the blood, where it is synthesized into 25(OH)D and finally converted to the active form 1, 25 (OH)2D in the kidney catalyzed by 1α1-hydroxylase [13]. Vitamin D enters cells through receptors on the cell surface and binds to retinol X receptors and the vitamin D receptor complex in the nucleus, where it regulates target genes to produce calcium-binding proteins, which bind calcium ions transported out of intestinal epithelial cells and released into the blood. If the blood calcium concentration is high, calcium is deposited in bone for storage or excreted out of the body through the kidney in urine. If the blood calcium concentration is low, calcium is supplemented by bone calcium [14]. Lack of vitamin D leads to abnormal calcium metabolism and eventual changes in bone, resulting in deformities. Diet alone cannot meet daily vitamin D needs because vitamin D levels in natural foods are limited. Children who spend less time outdoors are more likely to have lower serum vitamin D levels.

Although there is no standard on the optimal level of serum 25-hydroxyvitamin D, the general consensus has generally emphasized that 25-hydroxyvitamin D levels above 50 nmol/L are normal [15, 16]. Serum 25(OH)D levels are classified into five categories by the American Academy of Pediatrics [17]: severely deficient, < 12.5 nmol/L; deficient, > 12.5 and < 25 nmol/L; insufficient, > 25 and < 50 nmol/L; sufficient, > 50 nmol/L and < 250 nmol/ L; and toxic, > 250 nmol/L. However, recommendations for vitamin D do not take into account the optimal level of serum 25(OH)D required for general health in infants and young children, whose threshold level is even more controversial than in adults because less data are available. A retrospective study showed that the most advantageous serum concentration of 25 (OH)D for bone mineral density, lower extremity function, dental health and reduced risk of fractures from falls in infants and young children should be approximately 90 to 100 nmol/L [17]. The results of this study also suggested an infant serum vitamin D concentration threshold of 75 nmol/L to avoid the sequelae caused by vitamin D deficiency [18, 19].

2. Subjects And Methods

2.1 Subjects

Children aged 0 to 4 years old who underwent physical examination in the special needs clinic of the Hospital from October 2019 to December 2020 were selected as the research subjects. Cases with recent infection, various chronic medical histories and related clinical symptoms and signs such as rickets in physical examination were excluded. A total of 378 cases were screened, including 214 boys and 164 girls. The subjects were divided into four groups according to the time of blood sampling. Blood samples drawn from March to May were classified as the Spring group; blood samples obtained from June to August were classified as the Summer group; blood samples drawn from September to November were classified as the Autumn group; and blood samples obtained from December to February were classified as the Winter group. At the same time, we also recorded the gender, age and home address of children according to a questionnaire survey in which the collected data were divided into two categories based on city. The top five economic cities in Yunnan Province (Kunming, Qujing, Honghe, Yuxi and Dali) were classified as category 1 while the other cities were classified as category 2. Data collected in this study were also divided into four categories according to age. The frequency of subjects in the different study groups is shown in Table 1.

Table 1
Frequency of subjects in different study groups.

Season	n	Gender	n	Age years	n	Location	n
Spring	87	Male	214	0-1	128	Top 5 City	239
Summer	248	Female	164	1-2	129		
Autumn	10			2-3	78	Other	137
Winter	33			3-4	43		

2.2 Methods

All subjects were measured for height and weight, and 2 mL of venous blood drawn after morning fasting were placed at room temperature for 20–40 min and then centrifuged to separate serum. The contents of 25 hydroxyvitamin D in serum were determined by Waters-ACQUITY-UPLC ultra performance liquid chromatography. The concentration of blood calcium, blood phosphorus and alkaline phosphatase were determined by Hitachi7600 automatic biochemical analyzer. The contents of lead, zinc, copper and iron were determined by PinaACLE900 atomic absorption spectrometer.

2.3 Data processing and analysis

SPSS version 19.0 software was used for statistical analysis. Measurement data were expressed as the mean ± standard deviation (x ± SD). Independent sample *t*-tests were used for comparisons between any two groups, and analysis of variance was used for comparisons between multiple groups. $P < 0.05$ was considered statistically significant. A P-P plot was used to verify the correlation between the data distribution and Pearson test variables.

3. Results

3.1 Frequency of subjects in different vitamin D groups.

A total of 378 healthy infants (214 boys and 164 girls) aged 0–4 years were enrolled in this study. According to the American Academy of Pediatrics, vitamin D deficiency accounted for 1.06% of the total cases, with 0.47% of boys and 1.83% of girls having vitamin D deficiency (Table 2). The experimental evidence suggested that vitamin D deficiency was more common in girls than in boys ($P < 0.001$).

Table 2
Frequency of subjects in different vitamin D groups.

Vitamin D Group	Male % (n)	Female % (n)	Total % (n)
Severely Deficient	0(0)	0(0)	0(0)
Deficient	0(0)	0(0)	0(0)
Insufficient	0.47(1)	1.83(3)	1.06(4)
Sufficient	99.53(213)	98.17(161)	98.94(374)
Toxic	0(0)	0(0)	0(0)
Total	100(214)	100(164)	100(378)

2.2 Laboratory data in different groups according to age, sex, time of blood draw and city.

Data on vitamin D, serum calcium, serum phosphorus as well as alkaline phosphatase in 378 infants are summarized in Table 3. There was no significant difference in serum vitamin D levels between boys and girls at any age. However, in both boys and girls at all ages, there was a significant decrease in vitamin D levels after 2 years old ($P < 0.05$). The levels of vitamin D in children were highest in summer, which were significantly higher than other seasons, and were lowest in winter ($P < 0.05$). At the same time, vitamin D levels were significantly different based on the economic level of cities. The serum vitamin D contents of infants and young children in the top five cities with the highest economic levels were significantly higher than in lower-ranked cities ($P < 0.05$).

Table 3
Laboratory data in different groups according to age, sex, time of blood draw and city.

		Vitamin D nmol/L		**Serum Calcium** mmol/L		**Serum Phosphor** mmol/L		**Alkaline Phosphatase** U/L	
Age	n	Male	Female	Male	Female	Male	Female	Male	Female
0-1	128	137 ± 31.3*	142 ± 28.5*	2.6 ± 0.10	2.6 ± 0.17	1.8 ± 0.13	1.8 ± 0.24	267 ± 62.9	251 ± 56.6
1-2	129	133 ± 33.0	129 ± 30.5	2.5 ± 0.11	2.6 ± 0.18	1.8 ± 0.16	1.8 ± 0.17	308 ± 198.4	270 ± 66.6
2-3	78	115 ± 29.1	112 ± 35.1	2.5 ± 0.10	2.5 ± 0.10	1.7 ± 0.13	1.7 ± 0.14	256 ± 62.7	284 ± 103.5
3-4	43	107 ± 32.6	112 ± 24.9	2.4 ± 0.14	2.4 ± 0.08	1.7 ± 0.25	1.7 ± 0.14	241 ± 49.8	226 ± 48.9
Season									
Spring	87	127 ± 29.8	123 ± 27.9	2.5 ± 0.13	2.5 ± 0.11	1.8 ± 0.16	1.7 ± 0.27	321 ± 221.5	283 ± 94.8
Summer	248	132 ± 33.4*	132 ± 32.3*	2.5 ± 0.12	2.5 ± 0.15	1.8 ± 0.17	1.8 ± 0.16	258 ± 63.9	256 ± 64.5
Autumn	10	116 ± 42.9	106 ± 25.7	2.6 ± 0.14	2.4 ± 0.08	1.7 ± 0.09	1.6 ± 0.12	243 ± 39.3	254 ± 55.0
Winter	33	105 ± 29.9	118 ± 43.5	2.4 ± 0.13	2.5 ± 0.41	1.8 ± 0.20	1.7 ± 0.18	270 ± 53.0	252 ± 54.6
Location									
Top 5 City	239	131 ± 31.8*	132 ± 33.2*	2.5 ± 0.13	2.6 ± 0.19	1.8 ± 0.16	1.7 ± 0.21	280 ± 144.6	268 ± 78.7
Other	137	121 ± 35.4	124 ± 30.7	2.5 ± 0.12	2.5 ± 0.12	1.7 ± 0.17	1.7 ± 0.16	263 ± 60.1	255 ± 60.3
*P < 0.01 (two-tailed)									

2.3 Correlation between vitamin D content and other biochemical indicators.

This study first verified that the data were normally distributed using a p-p plot while Pearson correlation tests were conducted between vitamin D content and other biochemical indicators. $P < 0.05$ was considered a significant correlation. The results (Table 4) showed a significant correlation between vitamin D content and serum calcium ($P < 0.01$).

Table 4
Correlation between vitamin D content and other biochemical indicators.

Correlation	Alkaline Phosphatase	Blood Calcium	Blood Phosphorus	Lead (Pb)	Zinc (Zn)	Iron (Fe)	Copper (Cu)
Pearson correlation	0.029	0.277**	0.069	-0.058	-0.051	0.004	-0.062
Significant (bilateral)	0.579	0	0.183	0.262	0.325	0.935	0.227
N	378	378	378	378	378	378	378
**P < 0.01 level (two-tailed)							

2.4 Reference range for normal serum vitamin D content in boys and girls aged 0–4 years in Yunnan Province.

This study preliminarily determined a reasonable reference range for serum 25 hydroxyvitamin D content in infants aged 0–4 years in Yunnan Province (Fig. 1). According to our calculations, the reference range for serum vitamin D content in 0–4-year-old boys in Yunnan Province should be set at 123.033–131.967 nmol/L, while the reference range for serum vitamin D content in girls should be set at 123.656–133.544 nmol/L.

4. Discussion

In this study, the vitamin D content of children aged 0–4 years was measured and the results showed no gender bias in vitamin D content. In contrast, vitamin D content tended to decrease with increasing age, which was also shown in a previous study by Challa [20]. The Challa study indicated that 2 years old was a critical time, which may be related to the nutrition and health care recommendations in China [21]. According to those recommendations, infants (including exclusively breast-fed infants) should receive 400 U/day of vitamin D from 2 weeks after birth until 2 years of age. In general, Chinese children (especially those between the ages of 1 and 3 years) are gradually shifting their vitamin D supplementation from breast milk to natural foods and fortified infant formula because of their lifestyle (such as more active, which was also leading them to spend less time outdoors.

Children are the future of humankind and an important resource for the sustainable development of society. Child development is an important part of national economic and social development and the progress of civilization. Promoting children's development is of strategic significance for comprehensively improving the quality of the Chinese nation and building a healthy country with sustained human resources [22]. The ages of 0–4 years are the most critical and important period in children for life development. During this period, vitamin D, calcium, phosphorus and trace elements are major nutrients that play a very important role in the growth of children. Imbalance or lack of these essential nutrients may result in infantile vitamin D-related diseases including respiratory infections and rickets, which have always been a key focus of research on childcare [7, 23].

Because Yunnan Province is located on a plateau where sunshine time and intensity are stronger than in other areas due to its unique geographical environment, vitamin D content produced by ultraviolet radiation from the sun may generally be higher than in other areas. The results of this study showed that the levels of vitamin D in children in this region are significantly higher than the levels reported in other studies [24, 25]. The main reason for this finding may on one hand be that the subjects of our survey were infants aged 0–4 years, and according to Chinese eating habits and parenting experience, their parents may be more willing to expose infants in this age group to the sun. On the other hand, our study found that the vitamin D content in the body tends to decrease with increasing age. In these comparative studies, the subjects were more likely to be pre-school, adolescents and adults.

In this study, there was a significant correlation between vitamin D content and serum calcium concentration. The decrease in vitamin D content may lead to a decrease in calcium ions entering the blood, which results in supplementation of bone calcium to blood calcium. Long-term vitamin D deficiency may thus lead to a decrease in calcium content in bone and induce abnormal bone development. Therefore, in this study we established a preliminary reference value for serum vitamin D content in children from this region. These data will be important for the future establishment of vitamin D supplementation guidelines for infants and young children in Yunnan Province and in the formulation of relevant policies for child health in highland areas of China [7, 23].

Declarations

Ethics approval, guidelines and consent to participate

This study was approved by the Kunming Children's Hospital ethics committee. The procedures of the present study were all performed following the ethics standards of the responsible committee on human experimentation and Declaration of Helsinki. Participants under 18 years of age obtained informed consent from their parents and/or legal guardians.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

We all declare that we have no conflict of interest.

Finding

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Authors' contributions

All the authors participated in the experiment .Yanfei-Yang and Xiao-Xiao acquired the data. Lin-Wang and Zheng-Yin analyzed and interpreted the data. Yuqin-Wu and Yangfang-Li designed the project and wrote the paper.

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Not applicable.

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Figures

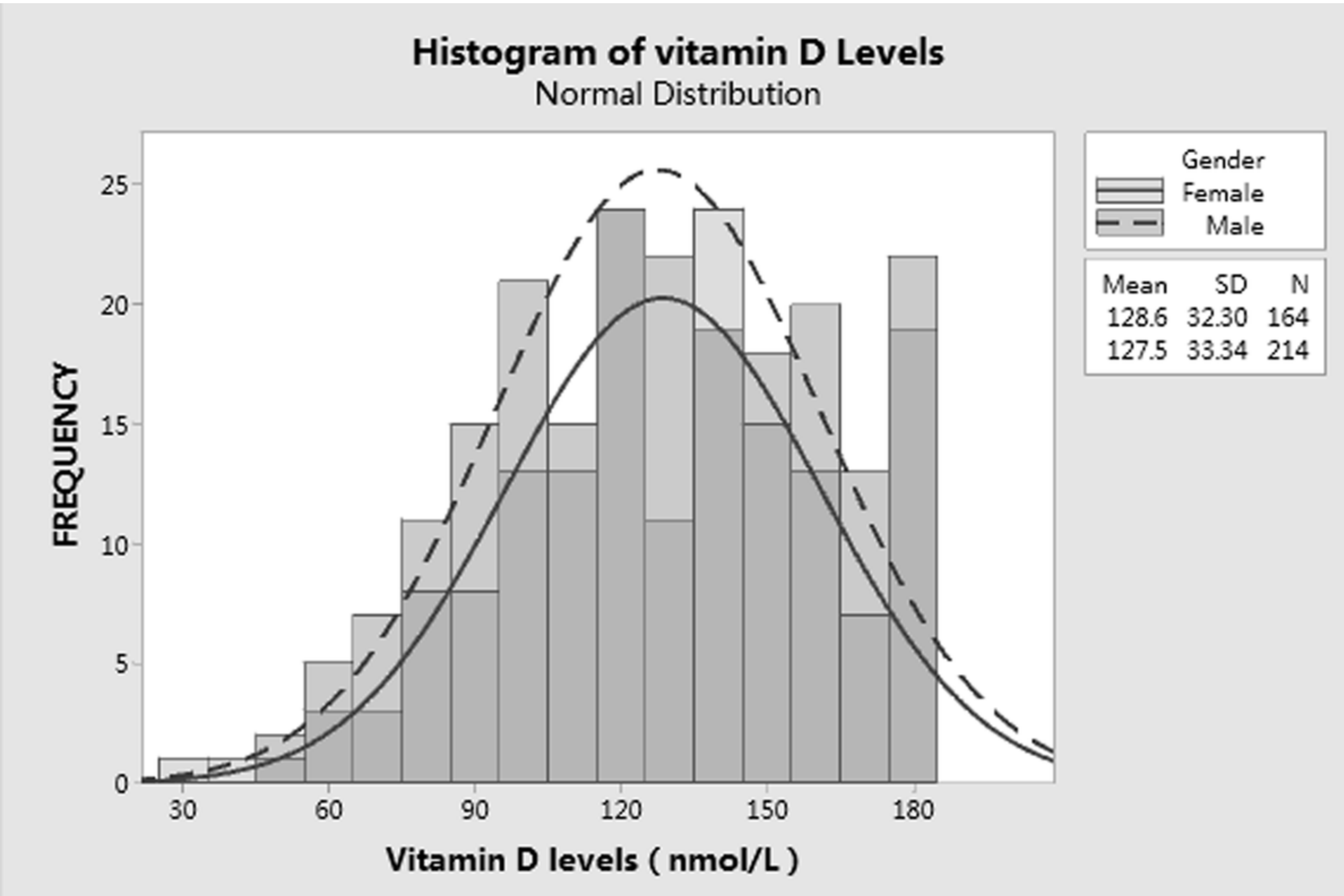

Figure 1

Reference range for normal serum vitamin D content in boys and girls aged 0–4 years in Yunnan Province.