


Factors Associated with Vitamin A and Vitamin D Profiles among Stunted Children in Bogor, Indonesia

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Abstract

Background: Stunting or stunted is a condition of chronic nutritional deficiency that remains a problem to this day. Vitamin A and D deficiencies can be one of the causes, although there are still differing findings regarding it. This study aims to investigate the levels of vitamins A, D, and their carrier proteins in relation to the characteristics of the subject.

Methods: This study is an exploratory analytical research with a cross-sectional design from 80 samples of venous blood from children aged 24 to 36 months in Bogor Regency, West Java, Indonesia. Retinol concentrations were quantified utilizing High-Performance Liquid Chromatography (HPLC), whilst retinol binding protein (RBP), 25-hydroxyvitamin D (25OH(D)), and vitamin D binding protein (DBP) were assessed by enzyme-linked immunosorbent assay (ELISA). Data was analyzed using SPSS software with independent t-test/ Mann-Whitney, one-way analysis of variance (ANOVA)/ Kruskal-Wallis, and chi-square tests. Statistical significance was considered at 0.05.

Results: The findings revealed no substantial differences in retinol ($P=0.24$), RBP ($P=0.492$), and DBP ($P=0.332$) between stunted and control children. However, 25(OH)D was markedly elevated in the stunted group relative to the control group ($P=0.007$). High vitamin D status may correlate with currently breastfeeding and consumption of vitamin D supplements in stunted children. Conversely, the levels of vitamins A and D were significantly lower in the control group who continued to receive breast milk compared to children who had been weaned.

Conclusion: Vitamin A in the stunted group tends to be the same as that of the control group. High levels of vitamin D were found in the group of stunted children. Additional investigation is required into the mechanism of vitamin D metabolism in stunted children.

Keywords: 25-hydroxyvitamin D, 25OH(D), D Binding Protein, Retinol Binding Protein, Retinol serum, Stunting

Conflicts of Interest: None declared

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Introduction

Stunting or linear growth failure (stunted) is a condition characterized by a decrease of at least -2 SD below the median of the WHO Growth Standards (1). This phenomenon generally can be detected in children after two

years old (2). Stunting is still a major global health problem, including in Indonesia, with a prevalence of 21.5% in 2023 (3). The adverse effects of this problem are not limited to childhood and future development subjects

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↑What is “already known” in this topic:

Stunting is still a major global health problem, including in Indonesia. Nutritional intervention does not completely reduce the incidence of stunting. This fact indicates the possibility of other factors contributing to the role. Micronutrients such as vitamins A and D are very important for growth, but their status in previous studies has varied.

→What this article adds:

The current study addresses this issue and provides a comprehensive information of the status of vitamins A and D and their carrier proteins in stunting, especially in children aged 2-3 years. Therefore, these results can be very helpful for evaluating the intake vitamin and also breastfeeding in this age group.

but also to the next generation (4). As stunting is often approached by anthropometric measurements (5), intervention is based mainly on nutrition (6). However, nutritional intervention only reduces stunting by approximately 12% (7). This fact indicates the possibility of other factors contributing to the role, such as pathophysiological mechanisms (8). Some previous studies reported that the average intake of macronutrients and micronutrients in stunted children was lower than in control children (9), although other studies stated that the average intake was adequate (10).

Insufficient food consumption and persistent infectious diseases mutually influence one another, resulting in malnutrition in children and directly contributing to stunting. Social, economic, and residential environmental factors are indirect contributors to stunting. An unhealthy environment can expose children to chronic and recurrent infections (11, 12). The body's defence system will respond with a low-level systemic inflammatory reaction that is activated when facing a threat and continuously active in maintaining various functions and interacting with its environment (internal and external) (13–15). An optimal immune response depends on adequate nutrition, including micronutrients (minerals and vitamins) (16). Vitamins A and D are key micronutrients that enhance the body's immune function (17–19). Vitamin A deficiency has become a public health issue in more than half of the countries, especially in Southeast Asia and Africa (20). Even in nations with plenty of sunshine, vitamin D insufficiency is prevalent among children (21). But no research has revealed the vitamin A and D profiles with their carrier proteins in stunted children, especially in Indonesia. This study aims to investigate whether there are changes in the levels of micronutrients, specifically vitamins A and D, and their carrier proteins in stunted children and to explore the correlation between the characteristics.

Methods

This study is an exploratory analytical research with a cross-sectional design involving two groups: stunted children and control children. The study was carried out in five community health centre areas through integrated service posts in Bogor Regency, West Java, Indonesia. Research subject with inclusion criteria: children aged 24–36 months, anthropometric measurements of height-for-age (WHO growth curve) $<-2SD$ for the stunting group and $>-2SD$ to $+3SD$ for the control group, parents who are willing to sign the informed consent form and participate in the study. The exclusion criteria for both are being clinically ill and undergoing treatment related to infectious diseases during blood sampling. The researchers conducted the measurement of children's height, accompanied by trained health workers using a stadiometer (Serenity), with an accuracy of up to 0.1 cm. These instruments have been standardized and calibrated by the district health office.

The sample used was computed using the sample formula of the difference in the means of two unpaired populations, using the power of the study to ascertain a sufficient sample size. The level of significance (α) was established at 0.05

with the intended statistical power of 0.80. From this formula, the minimum number of samples for each group was 32. The Health Research Ethics Committee approved this research - Faculty of Medicine Universitas Indonesia and Dr Cipto Mangunkusumo National Hospital (HREC FMUI-CMH) with Registration Number KET-363/UN2.F1/ETIK/PPM.00.02/2024. Due to ethical considerations in research to limit the number of samples in the control child group, the calculation was adjusted using a formula for unequal sample sizes for each group with a 1:2 ratio (22). Thus, the number of samples in the control child group was 27, and the number in the stunted child group was 53. The sampling technique used was consecutive sampling. The sample ratio comparison was adjusted to ensure that both categories of samples were collected from the same location.

Venous blood samples are technically withdrawn, considering safety protocols. A total of 3 mL of blood specimen was obtained in a clot activator vacuum tube to generate serum and subsequently frozen at -80°C for future analysis. The vitamin A profile is seen from the levels of retinol and retinol binding protein (RBP), while the vitamin D profile is measured through the levels of total 25(OH)D and vitamin D binding protein (DBP). The quantification of retinol was conducted utilizing the High-Performance Liquid Chromatography (HPLC) method with a UV detector (Waters e2695) based on its affinity difference towards the stationary phase in a Novapack C18 column using a mixed mobile phase at a wavelength of 325 nm, according to the principles at the SEAMEO RECFON Laboratory, Jakarta, which is accredited ISO/IEC 17025:2017. Measurement of RBP, total 25(OH)D, DBP using standard commercial Enzyme-Linked Immunosorbent assay (ELISA) kits according to the instructions supplied by the manufacturer (BT Lab Human RBP-4 ELISA Kit (E1206Hu); MyBioSource Human 25(OH)D ELISA Kit (MBS268910); BT Lab Human DBP ELISA Kit (EA0045Hu). All subject characteristics were obtained from questions listed in the questionnaire and asked by the research team through interviews.

The SPSS 27 software (IBM Corporation, Armonk, NY, AS) was utilized to evaluate the data and perform statistical analysis. Data normality is seen from the p-value in the Kolmogorov-Smirnov and Shapiro-Wilk tests (p-value >0.05 indicates normal data distribution). Furthermore, data normality will determine the difference analysis test. Hypothesis testing is conducted to examine group differences using an independent t-test and one-way ANOVA for normally distributed data, or the Mann-Whitney and Kruskal-Wallis tests for non-normally distributed data. Chi-square tests are employed to verify the relationship between parameters. A p-value ≤ 0.05 is considered significant.

Results

Subject characteristics including gender, history of exclusive breastfeeding, current breastfeeding status, and children's habits, were presented in Table 1. There is no significant difference in characteristics between the stunting group and the control group.

Table 1. Characteristics of Research Subjects

Characteristic	Stunting group n (%)	Control group n (%)	Pvalue
Gender			
Boy	30 (56.6%)	18 (66.7%)	0.530
Girl	23 (43.4%)	9 (33.3%)	
Current breastfeeding status			
Yes	16 (30.2%)	3 (11.1%)	0.106
No	37 (69.8%)	24 (88.9%)	
Children are routinely given a vitamin A supplementary program twice a year through integrated services or community health centers.			
No	2 (3.8%)	3 (11.1%)	0.210
Yes	51 (96.2%)	24 (88.9%)	
Children consume foods such as milk, cereal, margarine, cheese, eggs, liver, and sardines			
Never	2 (3.8%)	1 (3.7%)	0.104
1 to 3 times per month	6 (11.3)	2 (7.4%)	
Once each week	13 (24.5%)	1 (3.7%)	
Once per day	19 (35.8%)	17 (63%)	
More than once per day	13 (24.5%)	6 (22.2%)	
Children consume supplements now.			
No	30 (56.6%)	18 (66.7%)	0.382
Vitamin D	3 (5.7%)	0 (0%)	
Multivitamin	20 (37.8%)	9 (33.3%)	
Children are exposed to sunlight from 09.00-15.00			
Never or less than one hour per month	9 (17%)	2 (7.4%)	0.078
1 to 3 hours monthly	0 (0%)	1 (3.7%)	
One hour weekly	3 (5.7%)	0 (0%)	
2 to 4 hours each week	7 (13.2%)	0 (0%)	
5 to 6 hours each week	0 (0%)	1 (3.7%)	
0.5 to 1 hour weekly	30 (56.6%)	20 (74.1%)	
More than two hours each week	4 (7.5%)	3 (11.1%)	
Children are given/applied sunscreen/sunblock.			
Never	33 (62.3%)	17 (63%)	0.993
Occasionally	13 (24.5%)	6 (22.2%)	
Often	5 (9.4%)	3 (11.1%)	
Always	2 (3.8%)	1 (3.7%)	
Child skin color			
Pale white skin	0 (0%)	1 (1.3%)	0.330
White skin	14 (26.4%)	5 (18.5%)	
Light brown skin	20 (37.7%)	9 (33.3%)	
Brown skin	19 (35.8%)	11 (40.7%)	
Dark brown skin	0 (0%)	1 (1.3%)	
Mothers taking vitamin D while pregnant/ breastfeeding.			
Yes	9 (17%)	7 (25.9%)	0.344
No	44 (83%)	20 (74.1%)	

Analyzed using a chi-square test

In this study, the differences in retinol and RBP between the stunted children group and the control children group were analyzed and presented in Figure 1. The mean concentration of retinol in stunted children was lower than that of the control group of children (1.79 ± 0.59 vs 1.89 ± 0.64). RBP in stunted children was lower than the control group of children (155.53 ± 127.13 vs 171.24 ± 155). There was no statistically significant difference (retinol P value = 0.24; RBP P value = 0.492).

This study used an independent t-test or one-way ANOVA test to obtain further information about the relationship between retinol as a vitamin A profile and the characteristics of subjects. The findings indicated that in the group of control children, there was a significant difference in retinol serum between current breastfeeding status. The retinol serum in the group of children who were no longer breastfed was higher than in children who still breastfed.

But the difference was not seen in the stunting group (Table 2).

Based on Figure 2, there is a significant difference of 25OH(D) between the stunted children group and the control children group ($P = 0.007$). The mean concentration of 25OH(D) in stunted children was higher than that of the control group of children (26.67 ± 5.29 vs 23.34 ± 4.17). DBP in stunted children was higher than the control group of children (8.12 ± 3.75 vs 7.41 ± 2.57), but there was no statistically significant difference ($P = 0.332$).

Next, this study conducted further tests to obtain further information about the relationship between 25OH(D) as a vitamin D profile and the characteristics of subjects (Table 3). The findings indicated that in the group of control children, there was a significant difference of 25OH(D) serum between current breastfeeding status, the habit of

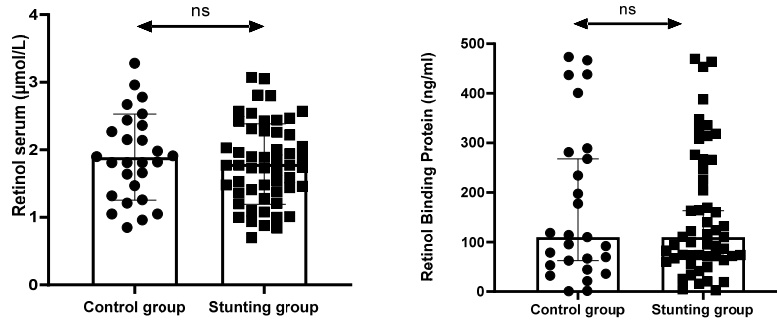


Figure 1. Retinol and RBP between the stunting and control groups

Table 2. Retinol serum based on the characteristics of subjects

Variable	Stunting group		Control group	
	Retinol, Mean (SD)	p value	Retinol, Mean (SD)	P value
Gender				
Boy	1.76 (0.66)	0.670 ^a	1.89 (0.58)	0.960 ^a
Girl	1.83 (0.51)		1.90 (0.78)	
Currently breastfeeding				
Yes	1.62 (0.52)	0.165 ^a	1.09 (0.15)	0.017 ^{**}
No	1.86 (0.61)		1.99 (0.6)	
Children are routinely given a vitamin A supplementary program twice a year through integrated services or community health centers				
No	2.32 (0.67)	0.198 ^a	2.02 (0.71)	0.713 ^a
Yes	1.77 (0.6)		1.87 (0.64)	
Children consume supplements now.				
No	1.7 (0.55)	0.375 ^b	1.79 (0.58)	0.285 ^a
Vitamin D	2.1 (0.92)		-	
Multivitamin	1.88 (0.62)		2.08 (0.74)	

^a Analyzed using independent t-test, ^b Analyzed using one-way ANOVA test, LSD Post hoc analysis: no group was statistically significant, ^{*}Statistically significant at $P<0.05$

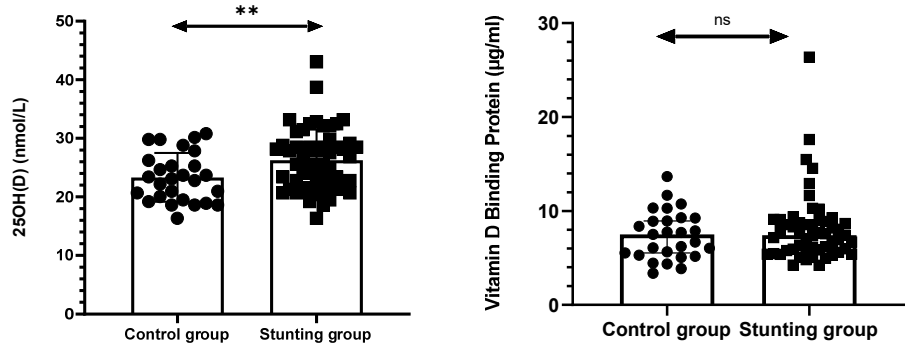


Figure 2. 25OH(D) and DBP between the stunting and control groups

consuming foods containing vitamin D, and supplements. But the difference was not seen in the stunting group.

Discussion

Serum retinol is the most often used biomarker of vitamin A status. Approximately 95% of serum vitamin A is retinol bound to Retinol Binding Protein (RBP) under normal conditions. About 5% is in the unbound form and in the form of retinyl esters (23). The average retinol levels in stunted children, while lower than those in control children in this study, did not exhibit a significant difference.

Likewise, RBP levels did not exhibit a significant difference between the two groups. Previous research on Ugandan children aged 6–59 months stated that micronutrient deficiencies (ferritin, RBP) were not linked to stunting, underweight, or wasting categories (24). A cross-sectional study of growth-stunted toddlers aged 12 to 59 months in eastern Uganda, RBP was detected in 54% of stunted toddlers with levels exceeding 0.7 µmol/L (25). Changes in plasma RBP4 levels in Bangladeshi children aged 12 to 18 months after nutritional intervention (eggs, milk, and micronutrients comprising 12.5 mg iron, 5 mg

Table 3. 25OH(D) serum based on characteristic of subjects

Variable	Stunting group		Control group	
	25OH(D), Mean (SD)	p value	25OH(D), Mean (SD)	P value
Gender				
Boy	26.04 (4.84)	0.850 ^a	22.7 (4.03)	0.269 ^b
Girl	26.61 (5.92)		24.62 (4.38)	
Currently breastfeeding				
Yes	27.83 (5.19)	0.116 ^a	18.74 (2.32)	0.040 ^{b*}
No	25.59 (5.26)		23.91 (4.01)	
Children consume foods such as milk, cereal, margarine, cheese, eggs, liver, and sardines				
Never	21.74 (1.5)	0.134 ^c	23.72 (-)	0.032 ^{c*}
1 to 3 times per month	22.13 (2.95)		30.31 (0.71)	
Once each week	28.2 (5.6)		18.9 (-)	
Once per day	26.5 (4.04)		22.1 (3.85)	
More than once per day	26.5 (6.7)		25.28 (3.08)	
Children consume supplements now.				
No	25.56 (5.44)	0.423 ^d	21.97 (3.52)	0.013 ^{b*}
Vitamin D	29.86 (11.44)		-	
Multivitamin	26.79 (3.8)		26.08 (4.19)	
Children are exposed to sunlight from 09.00-15.00				
Never or less than one hour per month	27.13 (3.9)	0.496 ^c	26.5 (6.09)	0.430 ^c
1 to 3 hours monthly	-		29.8 (-)	
One hour weekly	21.38 (0.76)		-	
2 to 4 hours each week	25.32(5.3)		-	
5 to 6 hours each week	-		22.8 (-)	
0.5 to 1 hour weekly	26.83 (6)		22.9 (4.13)	
More than two hours each week	25.27 (5.3)		22.33 (3.25)	
Children are given/applied sunscreen/sunblock.				
Never	27.22 (5.53)	0.457 ^d	21.83 (3.87)	0.057 ^c
Occasionally	24.2 (4.78)		27.03 (3.23)	
Often	25.69 (2.37)		24.1 (4.23)	
Always	25.38 (9.57)		24.66 (-)	
Child skin color				
Pale white skin	-	0.554 ^d	24.66 (-)	0.179 ^d
White skin	25.67 (4.08)		25.7 (4.52)	
Light brown skin	25.71 (5.42)		20.87 (4.15)	
Brown skin	27.29 (6.01)		23.99 (2.74)	
Dark brown skin	-		25.29 (-)	
Mothers taking vitamin D while pregnant/breastfeeding.				
Yes	25.19 (3.18)	0.511 ^a	23.41 (4.12)	0.960 ^b
No	26.49 (5.63)		23.32 (4.29)	

^a Analyzed using mann-whitney test, ^b Analyzed using independent t-test, ^c Analyzed using one-way ANOVA, LSD Post hoc analysis for stunting group: children consume foods such as milk, cereal, margarine, cheese, eggs, liver, sardines 1-3 times/month vs 1 times/week (p value = 0.020), 1-3 times/month vs 1 times/day (p value = 0.070), 1-3 times/month vs >1 times/day (p value = 0.091); LSD Post hoc analysis for control group: children consume foods such as milk, cereal, margarine, cheese, eggs, liver, sardines 1-3 times/month vs 1 times/day (p value = 0.009), 1-3 times/month vs >1 times/day (p value = 0.072), 1 times/day vs >1 times/day (p value = 0.081); children are given/applied sunscreen/sunblock: never vs often (p value=0.008); ^dAnalyzed using Kruskal-wallis, mann-whitney analysis for control group: child skin color light brown skin vs brown skin (p value=0.046), white skin vs light brown skin (p value=0.042), *Statistically significant at p≤0.05, ***Statistically significant at p≤0.001

zinc, 300 mg vitamin A, 150 mg folic acid, 50 mg vitamin C) did not affect anthropometric changes (26). Similarly, no correlation was found between serum retinol and indicators of anthropometric or socioeconomic status (27). Research on Middle Eastern children under three years of age failed to establish a correlation between serum vitamin A levels and stunting (28), a finding similarly reported in research involving preschool children in Sri Lanka (29).

Previous research indicated varying results, revealing that infants of normal height (aged 6-59 months) exhibited significantly elevated retinol concentrations (1.54 ± 0.55 µmol/L) in contrast to those with stunting (1.37 ± 0.47 µmol/L) and severe stunting (1.32 ± 0.39 µmol/L) (30). However, in this study, higher retinol concentrations were found compared to that research, both in the control child

group (1.89 ± 0.64 µmol/L) and in the stunting group (1.79 ± 0.59 µmol/L). The results of the retinol serum survey in toddlers in Indonesia in 2023 showed that 86.6% fell into the normal concentration category (20-40 µg/dL), 5.2% had mild deficiency (10-20 µg/dL), and 8.1% were above normal (>40 µg/dL) in the age group of 24-35 months (3). The effective vitamin A supplementation program may yield knowledge regarding these outcomes. Serum retinol levels in children under five years of age are heightened in those who have undergone consistent supplementation compared to those who have not (31). The administration of vitamin A supplements is significantly inversely correlated with stunted growth and underweight status, after adjusting for additional risk factors. The probability of experiencing stunting is 50% greater, and the incidence of

underweight is 75% greater among children who do not receive vitamin A supplements compared to those who do (32).

The study's findings indicate that retinol levels in the control group who were still consuming breast milk were markedly lower than those in children who had ceased breast milk consumption. The group of children who were stunted showed a similar tendency, however, without substantial differences. The American Academy of Paediatrics (AAP) advocates for exclusive breastfeeding for about six months postnatally. The AAP advocates for sustained nursing and the introduction of suitable supplementary foods at approximately 6 months, provided it is wanted by both the mother and child for a duration of 2 years or more. This advice aligns with the guidance provided by the World Health Organisation (WHO) (33). In this study, certain children, from both the stunting group and the control group, aged over 2 years, continued to receive breast milk. Prior studies indicate that the likelihood of vitamin A deficiency in breastfed infants over six months of age is significant, with 89.5% failing to ingest vitamin A-rich foods thrice weekly. This occurs because breastfed infants may not consume foods rich in vitamin A, and while exclusive breastfeeding is advised, most breast milk is deficient in retinol, hence heightening the risk of vitamin A insufficiency (34). Long-term breastfeeding alone may not guarantee protection for children from vitamin A deficiency (VAD) in areas with mild subclinical vitamin A deficiency (SVAD) (35). The high-risk demographic for low vitamin A in China comprises young children, individuals residing in rural regions, those with parents possessing little educational attainment, and individuals who do not consistently intake vitamin A supplements (36).

Vitamin D is composed of two molecules: cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2) (37). Cholecalciferol and ergocalciferol are physiologically inactive and require liver and kidney hydroxylation processes to activate. Both types of vitamin D are transformed into 25(OH)D in the liver and stored in body fat (38). 25(OH)D has a lengthy half-life of two to three weeks, which is consistent with the primary circulating form of vitamin D. Its concentration indicates the organism's vitamin D level (39). 25(OH)D bound to its DBP enters circulation and is transported to the kidneys, where the second hydroxylation occurs. Vitamin D is converted into its biologically active form (1,25(OH)2D) within the kidneys by the enzyme 1- α hydroxylase as needed, under normal parathyroid gland function. The vitamin D and active D hormone metabolite 1,25(OH)2D have particular effects (38).

In this study, significantly higher levels of 25(OH)D were found in the stunted children group (26.67 ± 5.29 nmol/L) compared to the control group (23.34 ± 4.17 nmol/L), while the DBP level results have the same pattern but are not statistically significant. The intricate link between DBP and total and free 25(OH)D is described by earlier research. Under certain conditions, a decrease or increase of DBP can be accompanied by low or normal free 25(OH)D (40). Inflammation redistributes carrier proteins

such as albumin, RBP, and DBP to the extravascular space, lowering micronutrient concentrations (41). DBP levels in human serum are typically in the micromolar range (~ 6 μ mol/L or 300 ml/L), with lab levels ranging from 200 to 600 mg/L (42). DBP, which is in the mid-micromolar range, exists in serum at elevated amounts compared to other vitamin D metabolites combined, particularly 25OHD, which is normally below 100 nmol/L. Fewer than 5% of DBP is holoprotein; the rest is apoprotein. By binding all vitamin D metabolites and having a high affinity for 25OH(D) and 1,25(OH)2D, DBP ensures a large circulating pool to prevent rapid depletion. Feedback does not impact human or animal free 25OH(D). Vitamin D consumption or sun exposure enhances total and free 25OH(D) concentrations, while vitamin D deficiency does not impact DBP; therefore, they fall nearly equally. 25OH(D) activates genes and cells in many target cells without fully understanding how its low free concentration or bioavailability does so. With DBP, different cells have limited access to and effectiveness of vitamin D metabolites. DBP does not directly alter inflammation. DBP transports unsaturated fatty acids, which compete with vitamin D metabolites and diminish their affinity for 25OH(D) and 1,25(OH)2D. DBP increases C5a-activated neutrophil chemotactic activity by binding to immune cell membrane proteoglycans. This role in inflammation is uncertain. No correlation exists between 25(OH)D3 concentration and DBP, proving feedback does not regulate free 25OHD3 (42).

Prior studies indicated that the vitamin D levels in children aged 2.0 to 2.9 years were 54.0 ± 2.3 nmol/L (43). Interestingly, the results of the study on Indonesian children aged 6–59 months showed that serum 25OH(D) was higher in the severely stunted group (56.3 ± 9.8 nmol/L) compared to the stunted group (51.9 ± 13.4 nmol/L) and the control group (54.1 ± 14.7 nmol/L) (30). Findings from a narrative review of eight articles show that there is a link between vitamin D status and stunting in two articles, not related to each other, in five articles, and related to linear growth in children with slow growth in one article. Because there are many things that can cause stunting in children, vitamin D levels are not always the main cause of stunting in children (44). Prior studies indicated an inability to establish a correlation between stunting, underweight, and wasting with vitamin D insufficiency in children aged 6–12 months in Africa (24). Vitamin D insufficiency is not linked to physical growth (45). Serum concentrations of 25(OH)D3 do not exhibit a significant correlation with stunted growth in the research cohort aged 10–36 months (28). The vitamin D status is not correlated with linear development in Indian children aged 6 to 9 (46). The HAZ scores of Indonesian school-age children are not correlated with their vitamin D intake. Deficiencies in macro and micronutrients that restrict the growth of other growth factors, such as calcium, zinc, and vitamin B12, may be the primary causes of children's growth (47).

In the stunted children group, elevated levels of 25OH(D) were observed in those who ingested vitamin D supplements; however, the results were not statistically significant. According to other research, children who

consume less vitamin D than the recommended amount are more likely to suffer from stunting. These findings support a prior study undertaken in low- and middle-income nations (48). Meanwhile, several clinical trials reported different results (49, 50). The administration of vitamin D supplementation to infants was 2000 IU/day (50 mg/day) in Finland, observed in a cohort study, and it was found that neither the frequency nor the dosage of vitamin D supplementation was associated with short stature at any age studied (50). Four investigations demonstrated that vitamin D supplementation had no impact on the length of newborns, which serves as a measure of stunting. Seven articles demonstrated that vitamin D supplementation administered to pregnant women influenced several anthropometric parameters, including neonatal length (51). Children aged 12-15 with normal growth and very low serum vitamin D levels can temporarily improve height by taking 800 IU of vitamin D3 daily for six months. A shorter study of 300 IU of fortified milk every day for 7 weeks had no impact (48). Serum 25 (OH)D3 concentration is positively associated with stunted growth in children who take nutritional supplements but not those who do not (28).

The vitamin D levels in the control group of this study, who continued breastfeeding, were lower than those in the group that had ceased breastfeeding. Breastfed infants are especially vulnerable to vitamin D insufficiency due to inadequate vitamin D levels in breast milk, restricted sun exposure, heightened pollution, and scarce natural food sources of vitamin D. Breast milk generally has low concentrations of vitamin D (about $<25-50$ IU/L), with its concentration affected by the vitamin D status of the lactating mother and seasonal variations (52). Certain studies indicate that the protective benefits of breast milk are most pronounced during the initial 6–12 months of life, but others suggest that the substantial decrease in infection risk persists until the age of 2 years or beyond (53). In contrast to the stunting group, children who continued nursing exhibited elevated vitamin D levels. Prior studies indicated that sociodemographic factors do not influence the vitamin D level of preschool children in Nepal; however, extended breastfeeding correlates with improved vitamin D status (54). This indicates the potential for distinct processes of vitamin D metabolism between the stunted children group and the control group.

The vitamin D levels of the children evaluated in this study have not reached the recommended ideal levels. A meta-analysis of 6 months to 19-year-olds found an average blood vitamin D level of 22.74 ng/mL, with a predicted range of 15.96 to 29.52. Indonesia has a 33% prevalence of hypovitaminosis D, with more women than men affected. Serum vitamin D levels depend on food, supplementation, BMI, physical activity, genetics, and disease (55). Vitamin D deficiency among healthy children living in Southeast Asia is common. The prevalence of individuals with levels below 50 nmol/L varies from 0.9% to 96.4%, exceeding 50% in newborns, while the prevalence of those with levels below 30 nmol/L ranges from 0% to 55.8% (44). Severe vitamin D deficiency, characterized by a 25(OH)D concentration below 30 nmol/L (or 12 ng/ml), is associated with heightened risks of excess mortality, infections, and

various illnesses, necessitating mitigation efforts (56). A contributing factor to the elevated incidence of hypovitaminosis D among Indonesian children and adolescents is malnutrition and the presence of darker skin types (55). Vitamin D deficiency impacts all age demographics, particularly babies, owing to restricted sun exposure, insufficient vitamin D concentrations in breast milk, and cultural unawareness regarding regular supplementation. The efficacy of vitamin D supplementation is contingent upon timing and demographic factors. Infants, toddlers, and adults may suffer from vitamin D inadequacy due to limited solar exposure, breastfeeding complications, and cultural misconceptions (57).

Previous meta-analysis studies found that subjects with Vitamin D levels below the standard had a 1.86 times higher risk of experiencing stunting and a 2.76 times higher risk of being overweight than those with normal Vitamin D levels. However, all these results were not statistically significant (58). In Ecuadorian children, those who were underweight faced a twofold risk of having blood 25(OH)D levels below 42.5 nmol/l when compared to their normal weight counterparts, and a lower vitamin D status was linked to stunted growth (OR=2.8; 95% CI 1.6–4.7) (49). A longitudinal study in Bogota, Colombia, linked vitamin D insufficiency (<50 nmol/l) to growth abnormalities in girls but not boys (59). The definition and limits of low vitamin D status have been contested, especially in children. There is insufficient data to establish child thresholds. Definitions from the Institute of Medicine specify serum levels necessary to prevent rickets and ensure sufficient bone mineral density (60). Meanwhile, in this study, it was found that a 25OH(D) level > 23.875 nmol/L (obtained from ROC analysis) is at a 2.59 times higher risk of causing stunting with a 95% confidence interval (0.996–6.736) compared to children with a 25OH(D) level < 23.875 nmol/L, and it is statistically significant ($P = 0.048$). Thus, our findings on the relationship between vitamin D and stunting may be unexpected. Science is fascinated by vitamin D since its mystery remains unsolved and its functions in human health are continuously being uncovered (57).

The cross-sectional study design is subject to certain limitations because it cannot analyze the definite causal relationship between variables. The limited number of samples makes these results less generalizable to other populations. The absence of food intake data is also another limitation in this study. Therefore, recommendations for further research include conducting longitudinal research with a larger number of samples and more complete nutritional data. The advantages of this study are the complete data for vitamin A and D status, both metabolites and vitamin-carrying proteins. As far as the researcher knows, this topic is the first to be written on a specific age group, namely 2-3 years old, which is the age group with the most stunting prevalence in Indonesia.

Conclusion

The findings of this study indicate high vitamin D status in stunted children compared with the control group, while the vitamin A status is identical in both groups. The cross-

sectional design of this study reveals a correlation between high vitamin D status and currently breastfeeding and consumption of vitamin D supplements in stunted children. Conversely, the levels of vitamins A and D were diminished in the control group of 2-3 year-old children who continued to receive breast milk. However, we cannot confirm the causality between parameters with this research design. Therefore, our findings must be confirmed by prospective research. Further research is required to elucidate the mechanisms of vitamin D metabolism in stunted children.

Authors' Contributions

MS: Research study conception and design, review and editing; RF: study design, material preparation, acquisition of data, writing original draft, editing; HW: statistical analysis, interpretation of the results, review and editing; DRG: organization, review and editing.

Ethical Considerations

This research protocol adheres to the Declaration of Helsinki and has been approved by the Health Research Ethics Committee - Faculty of Medicine Universitas Indonesia and Dr. Cipto Mangunkusumo National Hospital (HREC FMUI-CMH) with Registration Number KET-363/UN2.F1/ETIK/PPM.00.02/2024. Each participant gave written consent for this study.

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Conflict of Interests

The authors declare that they have no competing interests.

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