ACTA ABP BIOCHIMICA POLONICA

Vol. 70, No 3/2023
609–614
https://doi.org/10.18388/abp.2020_6676

Regular paper

Bionics investigation of blood 25-hydroxyvitamin D in the interpretable biomechanics diagnosis of childhood anemia

Lechi Zhang[1#], Aijie Huang[2#], Jingye Cai[3], Jiangting Hou[3], Hongyan Deng[3] and Chenxiao Liu[2]✉

[1]Department of Rehabilitation Medicine, The Affiliated Su-zhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Gusu School, Nanjing Medical University, 286 Guangji Road, Jiangsu 215008, People's Republic of China; [2]Department of Endocrinology, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou Municipal Hospital, Gusu School, Nanjing Medical University, 242 Guangji Road, Jiangsu 215008, People's Republic of China; [3]Department of Child Health Care, Zhang Jiagang Maternity and Child Health Care Hospital, Zhang jiagang, People's Republic of China

Vitamin D deficiency (VDD) causes a wide range of health problems, including anemia in infants. If not treated promptly, it may create serious issues for infants with long-term impacts. Therefore, a satisfactory solution to this problem is required. This investigation was to explore the correlation between the blood 25-hydroxyvitamin D (25(OH)D) levels and childhood anemia. In this investigation, a cross-sectional examination was performed on 2,942 babies ranging in age from 2 to 36 months and classified into three cohorts: VDD (Vitamin D deficiency), VDI (Vitamin D insufficiency), and VDS (Vitamin D sufficiency). Multiple-variables and multinomially-related logistic regressions for examining the anemia status-vitamin D (Vit-D) relationship of the baseline as the interpretable visual quality models were examined. The median serum 25(OH)D level in 2,942 infants was 24.72±4.26 ng/l, with 661 cases (22.5%) of VDD and 1710 cases of deficiency (58.1%), and a noticeable seasonal variation (p<0.05). Anemia was present in 28.5% of the VDD group compared with 3.3% in vit-D sufficient infants (p<0.0001). Lower levels of 25(OH)D were found to be associated with an increased risk of anemia in a multiple-variable regression analysis. In healthy children, low 25(OH)D levels were associated with increased risk of anemia. Biologically inspired, primary care physicians should assess Vit-D levels and place a greater emphasis on adequate supplementation for deficiency prevention.

Keywords: Low 25(OH)D; childhood anemia; healthy children; vitamin D

Received: 17 February, 2023; revised: 18 April, 2023; accepted: 17 May, 2023; available on-line: 06 September, 2023

✉e-mail: chenxiaoliu2010@rediffmail.com
#Lechi Zhang an Aijie Huang contributed equally to this work.
Acknowledgements of Financial Support: This investigation was sponsored by the Zhang Jiagang Science and Technology Bureau's Science and Technology Support Project (ZKS1633) and the Suzhou City Introduces Clinical Medicine Expert Team Project (SZYJTD201808).
Abbreviations: Hb, hemoglobin; VDD, Vitamin D deficiency; VDI, Vitamin D insufficiency; 25(OH)D, blood 25-hydroxyvitamin D

INTRODUCTION

Anemia is a pervasive issue in pediatrics and is an important part of standard care for all pediatric patients. If not treated in time, it can cause significant problems for children, and the effects can be long lasting (Khan, 2018). The hemoglobin (Hb) level in these patients is too low to meet cellular oxygen demands (D'Souza, 2020). It is necessary to employ better tactics, like the targeted screening of high-risk children, such as iron insufficiency and VDD. VDD refers to a highly frequent nutrition-related deficiency globally, especially in young children (Laway *et al.*, 2014). The effect of 25(OH)D, a key circulation vitamin D (vit-D) state, on calcium absorption and bone metabolic processes is noteworthy (Uberti *et al.*, 2016; Alyasin *et al.*, 2011). It has been progressively found to that decreased vit-D extents display relationships to diabetes mellitus (Sharma *et al.*, 2015), hypertension, cancer and the maintenance of immune homeostasis (Atkinson *et al.*, 2014). Existing studies show that serum 25(OH)D, which has long been thought to be the greatest indication of overall vit-D status, has a relationship with Hb level (Alyasin *et al.*, 2011), but the studies either used small sample sizes or focused primarily on women, the elderly, or adults in a healthcare setting (Shin & Shim, 2013; Yoo & Cho, 2015). In addition, lower 25 (OH)D extents have displayed an individual associating process to anemia in adults exhibiting chronic diseases (e.g., heart failing state, end-stage renal disease and diabetes) (Ernst *et al.*, 2015; Holick, 2007), even among healthy adults (Yazici *et al.*, 2018). However, this association has not been explored in infants with health conditions. It is generally considered that vit-D impacts proliferating and differentiating processes of stem cells in marrow of bone and may impact red cell proliferating process (Uberti *et al.*, 2016). For this reason, VDD may have an influence on Hb metabolism and induce anemia (Balasubramanian, 2011). Nevertheless, the likely relating characteristic of the VDD and anemia is still unclear. Thus, our intention was to assess the degree of vit-D in Zhang Jiagang's common children. Furthermore, the current investigation revealed the components that might be impacting VDD, the associations between feeding category, Hb extents, anemia status, and VDD, and the connection between the blood 25-hydroxyvitamin D level and childhood anemia.

MATERIALS AND METHODS

This experiment was performed at the Zhang Jiagang Maternity and Child Health Care Hospital in China from 2011 to 2016. This experiment was approved by the ethical committee of the Zhang Jiagang Maternity and Child Health Care Hospital, China. (Reg. No. 38563/2010/VIT-D/11.07.2010), and written informed consent was obtained from each participant. Totally, 2,942 infants

were chosen from the Child Health Care outpatient department at Zhang Jiagang Maternity and Child Health Care in China.

Inclusion criteria

1. Infants age limit from 2 months to 3 years. 2. Experimental duration August 2011 to November 2016.

Exclusion criteria

1. Systemically-related illness, 2. covering celiac disease, 3. liver, kidney disorders, rickets, joint pain, malnutrition, hypothyroidism, 4. hematologically-related disorders (e.g., anemia, G-6PD deficiency and thalassemia) besides iron insufficiency anemia received the exclusion. Feeding figures received the grouping process to 3 types specific to formula, cow's milk, and breast milk applications.

Anemia

Anemia is brought on by disruptions in the Hb production process. Anemia received the definition of Hb less than 110 mg/dL for males and females. Mild anemia = Hb 100 to 109 mg/dL; Moderate anemia = Hb 70 to 99 mg/dL; Severe anemia = Hb <70 mg/dL (Molloy *et al*., 2017).

Further children were grouped as VDD was classified as serum 25(OH) D extents<20 ng/mL, VDI as 25(OH) D extents between 20 and 30 ng/mL, VDS as >30 ng/mL, VDS intoxication as >150 ng/mL (Balasubramanian, 2011; Holick *et al*., 2015).

ELISA analysis

Serum 25(OH)D levels were determined by ELISA (Immunodiagnostic Systems Ltd., Beijing Bohui Innovation Technology) according to the manufacturer's instructions. In the first analytical phase, calibrators and specimens of blood are diluted with biotin-labeled 25(OH)D and added to microplate wells coated with monoclonal anti-25(OH)D antibodies. During the incubation, an unknown quantity of 25(OH)D in the blood sample competes with a known amount of biotin-labeled 25(OH)D for antibody binding sites in the microplate wells. Washing removes unbound 25(OH)D. A second incubation with peroxidase-labelled streptavidin is used to identify bound biotin-labelled 25(OH)D. The attached peroxidase induces a colour response in a third incubation with the peroxidase substrate tetramethylbenzidine (TMB). To halt the process, an acidic stopping solution is introduced. The intensity of the colour is related to the concentration of 25(OH)D. The intraassay CVs were 4.9% at a 25(OH)D mean concentration of 27.0 nmol/L, 6.9% at a 25(OH)D mean concentration of 61.5 nmol/L, and 3.2% at a 25(OH)D mean concentration of 160.3 nmol/L.

Haemoglobin (Hb) determination

The Hb was determined using the spectrophotometric cyanmethaemoglobin (HiCN) method by the XK-2 analysing tool. This approach is to convert haemoglobin to cyanmethemoglobin by adding potassium cyanide and ferricyanide, and then measure the absorbance at 540 nm in a photoelectric calorimeter against a reference solution. The test was carried out exactly as described by Bhaskaram and others (Bhaskaram *et al*., 2003).

Statistical Analysis

Overall, statistically-related processes were carried out by SPSS,12.0 Ver software. The information had the expression "proportions" or "mean ± standard deviation. The key features were evaluated within the groups using *t*-testing methods. The chi-square and Fisher's test methods were utilised to effectively assess the categorical data. The Pearson correlation assay was undertaken to assess the connection among 25(OH)D and Hb levels. Multiple logistic regression analysis techniques were employed to assess the baseline anemia conditions of the different vit-D groups. The regulations for the mothers' years of schooling, family income, sex, season, and child age based on the models were mentioned. The multinomial logistic regression studies for measuring the relationship between VDD and various anemia (moderate and mild) in contrast to non-anemia at baseline were

Table 1. Subject population characteristics

Characteristics	6 Mo~	12 Mo~	24 Mo~	36 Mo~
Total no	594 (20.2)	318 (10.8)	1,094 (37.2)	936 (31.8)
Male	300 (50.5)	186 (58.5)	585 (53.5)	470 (50.2)
Female	294 (49.5)	132 (41.5)	509 (46.5)	466 (49.8)
Feeding pattern				
Breast milk only	567 (95.5)	226 (71.1)	477 (43.6)	15 (1.6)
Breast milk and formula	27 (4.5)	65 (20.4)	502 (45.9)	715 (76.4)
Formula only	–	27 (8.5)	115 (10.5)	206 (22.0)
25(OH)D deficiency (levels<20 ng/mL)	164 (27.6)	125 (39.3)	298 (27.2)	74 (7.91)
25(OH)D insufficiency (levels20-30ng/mL)	426 (71.7)	187 (58.8)	640 (58.5)	457 (48.8)
25(OH)D sufficiency (levels>30ng/mL)	4 (0.7)	6 (1.8)	156 (14.3)	405 (43.3)
25(OH)D intoxication (levels>150 ng/mL)	0 (0)	0 (0)	0 (0)	0(0)
Anemia (Hb<110mg/dL)	393 (66.2)	210 (66.0)	593 (54.2)	307 (32.8)

Values are presented as-number (%). Month: Mo; 25-hydroxyvitamin D: 25 (OH)D.

Table 2. Comparison of haematological and biochemical profiles according to vitamin D status (in total 2,942 patients)

Parameters	25 (OHD) <20 ng/mL (VDD)	25(OHD) 20~30 ng/mL (VDI)	25(OHD) >30 ng/mL (VDS)	*p* value
Total no. (%)	661 (22.5)	1,710 (58.1)	571 (19.4)	<0.05*
Mean age (MO)	11.58±7.98	19.40±9.64	27.77±8.55	<0.001*
Male (%)	334 (50.5)	838 (49)	385 (67.4)	0.088
Female (%)	327 (49.5)	872 (51)	186 (32.6)	0.081
Breast feeding (%)	258 (39.0)	373 (21.8)	372 (65.1)	<0.05*
Mean 25(OH)D (ng/mL)	18.68±0.36	25.05±2.84	30.81±0.72	<0.001*
Mean Hb (mg/dL)	112.54±10.67	118.63±8.52	122.77±8.21	<0.001*

Values are presented as Mean ± S.D. or %. MO, month.25 (OH)D, 25-hydroxyvitamin D. Hb, hemoglobin; VDD, vitamin D deficiency. VDI, vitamin D insufficiency. VDS, vitamin D sufficiency.

also employed. A *p* value of <0.05 exhibited statistics-related significance.

RESULTS

The characteristics of study population characteristics

The features of the investigated participant are demonstrated in Table 1. Among the total 2942 infants enrolled here. Among these, 1541 (52.4%) were men and 1401 (47.6%) were girls. In this, 594 (20.2%) were below 6 months of age, whereas 318 (10.8%), 1094 (37.2%) and 936 (31.8%) from 6–12, 12–24, and 24–36, respectively. In infants <6 month, 95.5% were breastfed, whereas 4.5% received Breast milk and formula. In 6-12 months, 71.1% were breastfed and 20.4% were received Breast milk and formula, 8.5% received only formula. In 12–24 months, 43.6% were breastfed only, 45.9% were received only breast milk and formula and 10.5% received only formula. In 24–36 months, 1.6% were only breastfed and 75.4% were received breast milk and formula, 22% received only formula. The 25(OH) D deficiency levels <20 ng/mL showed 27.6% in the below 6 month. The 25(OH)D deficiency levels of <0 ng/mL were noted 27.6% in the below six months babies, 39.3% in 6–12 months, 27.2% in 12 to 24 months and 7.9% in 24 to 36 months. The 71.7% of below 6 months babies were showed 25(OH)D insufficiency levels of 20–30 ng/mL, 58.8% showed by 6–12 months babies, 58.5% were showed by 12 to 24 months, 48.8% showed in 24 to 36 months. In the study, 71.7%, 58.8%, 58.5%, and 48.8% of babies as follows: below 6 months, 6–12 months, 12–24 months, and 24–36 months showed 25(OH)D insufficiency levels (20–30 ng/mL). In the study, 0.7%, 1.9%, 14.2%, and 43.3% of babies as follows: below 6 months, 6–12 months, 12–24 months, and 24–36 months showed 25(OH)D sufficiency (levels >30 ng/mL). No 25(OH)D intoxication levels >150 ng/mL were reported in this study. In the study, 66.2%, 66%, 54.2%, and 32.8% babies as follows: below 6 months, 6–12 months, 12–24 months, and 24–36 months showed Anemia (Hb <110 mg/dL).

The associating process of VDD presence and breastfeeding exhibited statistics-related significance

In the tested babies, 22.5% showed by VDD, 58.1% expressed by VDI and 19.4% showed by VDS (Table 2). Mean age of the children who in VDD cohort was 11.58±7.98 months, while it in VDI group reached 19.40±9.64 months and in the VDS group was 27.77±8.55 months. The male (%) in the VDD was 50.5%, 49.0% in VDI and in VDS 67.4%. the female (%) in the VDD was 49.5%, 51.0% in VDI and in VDS 32.6%. The rate of exclusive breast feeding in the three cohorts was 39.0%, 21.8% and 65.1%. For this reason, the associating process of VDD presence and breastfeeding exhibited statistics-related significance (p<0.05). The significant diversification between Hb extents is among 3 cohorts. Among children VDD, 28.5% of infants were with Hb <110 mg/dL and mean Hb as 112.54±10.67 mg/dL. However, 8.2% of overall in the VDI cohort exhibiting mean Hb as 118.63±8.52 mg/dL and 3.3% of overall in the VDS cohort exhibiting mean Hb as 122.77±8.21 mg/dL (p<0.001). Thus, in vit-D deficient cohort, cases number exhibiting low Hb extents (<110 mg/dL) was higher in comparison with vit-D sufficient cohort (Fig. 1).

Anemia condition of different patients

Table 3 lists the anemia condition for different levels of vit-D. About 95.2% of the recruited infants had Mild anemia, covering 73.4% Moderate anemia and 17.6% sever anemia. When confounders (the mothers' years of schooling, family income, sex, season, and infant age) were controlled for, no connections were noted between vit-D and anaemia condition; however, moderate anaemia had a higher risk in 25(OH)D 30 mg/mL infants than in 25(OH)D 30 mg/mL infants [relative risk (RR), 1.59; 95% confidence interval (CI), 1.09-2.31]. The frequency of mild anaemia showed a weak correlation with vit-D status (RR, 1.04; 95% CI, 0.97–1.29). In Fig. 2, the authors also analyzed case number with 25(OH)D deficiency, insufficiency and sufficiency and the extents of 25(OH)D in line with seasons. The number of cases with vit-D levels of <30 ng/mL in the autumn (September to November) was 536 (76.9%), 625 (74.4%) in the summer

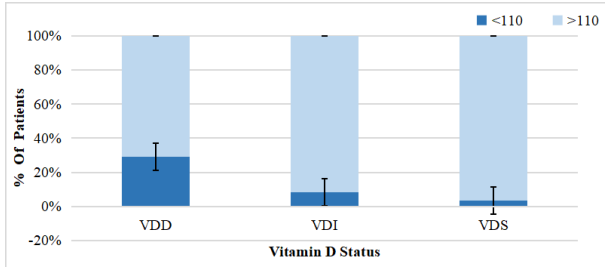

Figure 1. The Ratio of anaemia in VDD was significantly higher than that in VDI and VDS, p<0.05*.
VDD, vitamin D deficiency. VDI, vitamin D insufficiency. VDS, vitamin D sufficiency.

Table 3. Prevalence of anemia in different levels of 25 (OH)D children

Variable	25(OH)D<30 mg/mL (n=2371)	25(OH)D≥30 mg/mL (n=571)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)*
Mild anemia (Hb<110mg/dL)	1264 (53.3)	239 (41.9)	1.23 (0.45, 1.68)	1.23 (0.12, 1.35)
Subgroup				
			Unadjusted OR (95% CI)	Adjusted OR (95% CI)**
Anemia (Hb 70-99 mg/dL)	367 (15.5)	12 (2.1)	1.53 (1.11, 2.09)	1.59 (1.09, 2.31)
Moderate anemia (Hb 100-109 mg/dL)	897 (33.6)	227 (39.8)	1.06 (0.73, 1.55)	1.04 (0.97, 1.29)

*ORs were calculated by using logistic regression and adjusted for age of the child, season, sex, family income and mothers' years of schooling. **ORs were calculated by using multinomial logistic regression and adjusted age of the child, season, sex, family income and mothers' years of schooling.

Table 4. Factors associated with vitamin D deficiency

Variable	Odds ratio	95 % CI
Male	0.89	0.72-3.36
Female	0.81	0.66-3.27
Age (<12 Mo)	1.88*	0.65-6.06
Breast feeding	0.78	0.45-1.57
Serum mean Hb level	1.51*	1.02-2.84
Vitamin D tested in winter/spring	3.22*	1.13-4.57

*p value <0.001; VDD, vitamin D deficiency.

(June to August), 453 (87.3%) in the winter (December to February), and 757 (85.4%) in the spring (March to May). The average serum 25(OH)D level was significantly lower in spring/winter than in summer/autumn (25.28±4.31 *v/s* 23.78±4.34 ng/mL, p<0.01). The winter season exhibited the maximum VDD ratio (31.6%), and the spring season achieved the maximum VDI ratio (62.4%). The lone risk element for VDD determined by multiple-variable logistic regression was 25OHD in the winter or spring [odds ratio, 3.22; 95% CI, 1.13 to 4.57]. Several factors related to sex and breastfeeding that were seen in the univariate research had no independent relationship with VDD (Table 4). The risk element in terms

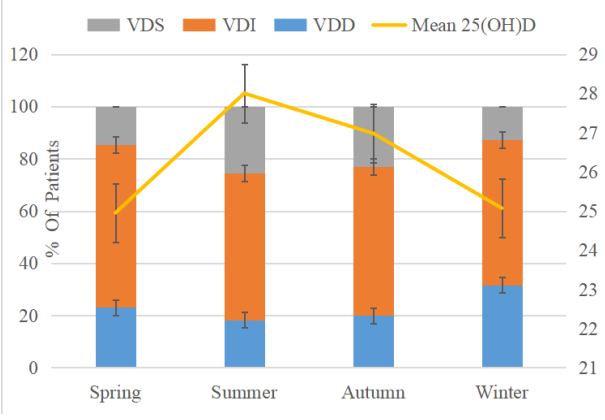

Figure 2. The levels of 25(OH)D status increased from spring to summer and then decreased in autumn and winter.
VDD, vitamin D deficiency. VDI, vitamin D insufficiency. VDS, vitamin D sufficiency.

of VDD developing process was evident in babies with anemia (p<0.001) and if 25(OH) D extents received the testing process in winter/spring (p<0.001).

DISCUSSION

The present work suggests that in a large, group-related Zhang Jiagang infants cohort with health condition, lower 25(OH)D extents displayed associations to elevated anemia risk. The identified associating process of vit-D condition and anemia was determined by other elements probably inducing anemia risk such as the mothers' years of schooling, family income, sex, season and age of infants. In the present work 28.5% and 8.2% of the infants achieved Hb <110 mg/dL in the vit-D deficient cohort (VDD and VDI), but merely 3.3% of the infants achieved Hb <110 mg/dL in the vit-D sufficient cohort. vit-D is vital nutrient required by an infant to grow and develop (Harinarayan & Joshi, 2009). The experts recommended that all only breastfeeding-based infants were required to undergo 400 IU per day of vit-D supplementing processes, which should be initiated several days after birth. No diversification received the identification in mean extents of vit-D in sex. However, the vit-D adequacy ratios of boy were slightly higher than in girls, which may be related to boys spending more time outdoors than girls. The authors further identified a noticeable correlating characteristic of <6 months age and VDD. Such correlating characteristic is likely to result from declined vit-D stores in such age cohort, breastfeeding almost exclusively and increased vit-D requirements and/or insufficient sun exposure. Balasubramanian and others (Balasubramanian *et al.*, 2011) suggest that a greater risk of VDD received the identification in infants having undergone exclusive breastfeeding process with no adequate vit-D supplementing process and appropriate exposure to sunlight. Furthermore, only a small number of breastfed infants get vit-D supplements since very few mothers follow their physician's suggestion for vit-D supplement because they think breast milk may provide critical nutrients (Yoon *et al.*, 2012). The majority of them prefer to buy health supplements like fish glycerides and DHA online. They consider that the vit-D presented by the hospital is easy to poison, and only part of the mother will take vit-D supplements during pregnancy.

The relating characteristic between VDD and anemia identified here complies with several recently conducted

researched on a range of groups (Alyasin *et al.*, 2011; Ernst *et al.*, 2015; Htet *et al.*, 2014). Several potential mechanisms could explain the associating characteristic of VDD and anemia in healthy infants. Major possibility is that VDD in infants is suggested displaying relationship to lifestyle and nutrition-related elements covering reduced milk intake and obesity as aforesaid. Inadequate 25(OH)D levels decrease local calcitriol synthesis in the bone marrow, resulting in decreased erythropoiesis (Sim *et al.*, 2010). Calcitriol could reduce cytokine producing process, thereby reducing inflammation-related milieu and anemia. The abundance of vitamin D receptors and vit-D in bone marrow seem to trigger erythroid precursors. High levels of 25(OH)D in hematopoietic tissues have been linked to erythroid progenitor cell paracrine activation. Moreover, there is considerable evidence that inflammatory cytokines affect erythropoiesis. Immunomodulation-related influences exerted by vit-D are likely to critically impact its role to prevent anemia by the modulating process related to systemically-related cytokine producing process, probably suppressing particular inflammation-related channels facilitating anemia progression. Furthermore, because of anemia people are commonly tired and less probably going externally for obtaining adequate sun exposing to generate vit-D. Anemia may also in turn contribute to VDD.

The season-related varying process acted as a single predicting element for VDD among infants subjected to anemia, abiding by existing works (Jin *et al.*, 2013). Given vit-D is primarily sourced by skin casually exposed to sunlight. 7-dehydrocholesterol (7-DHC) is isomerized to pre-vitamin D3 in the exposing process of the UVB (290 to 315 nm) part pertaining to sun photolyzes. When the relevant forming process is achieved, pre-vitamin D3 will undergo thermally isomerizing process for synthesize vit-D3. Solar UVB radiation amount accessing into the biosphere refers to one function pertaining to wavelength and ozone amount traveled by solar radiating process via the air, one function pertaining to the solar zenith angle, is determined by day time, season as well as latitude. Because the sun largely drives the vit-D synthesising process in the body, it was reported that VDD was more common; serum 25(OH)D extent was noticeably low in the spring/winter season than in the autumn/summer season. This is most likely due to summer/autumn sun exposure is adequate illumination than winter/spring and children outdoor time is longer. Human skin can be exposed to ultraviolet B radiation to produce more vit-D. Moreover, as suggested from recently conducted researches, genetically-related predisposition is likely to critically impact susceptible characteristic to VDI, and both vit-D uptake via diet and supplementing processes and skin-forming process when solar exposure is achieved may be determined by single genetically-related varying processes (Anastasiou *et al.*, 2017). The present multiple-variables-based logistic study suggested that season-based varying process in 25(OH)D acted as the merely noticeable single risk element in terms of VDD. For this reason, the present work demonstrates the season-based varying process is the primary risk component of VDD in Infants with anemia. Accordingly, the significance of sunshine and the requirement for vit-D supplement, especially throughout the winter and spring, are stressed. More research is required to explore the relationship among blood 25-hydroxyvitamin D levels and childhood anaemia.

CONCLUSION

To be specific, the present work on Zhang Jiagang infants suggested the correlative characteristics between anemia severity and VDD. For presenting the optimal nutritional condition for infants, breastfeeding continues to be worth recommending. However, educational efforts are required publicly and those breastfed in an exclusive manner (even under asymptomatic child) for increasing abidance by vit-D supplementing requirements.

Limitations

The present work showed several limitations. Subjects had varying ages; thus, their dietary intake and habits of feeding were likely to vary, probably causing a memory bias. It is better to compare plasma Hb and ferritin extents in the same patients, complying with the vital elements in Hb synthesis. It will be better to demonstrate the relationship to vit-D condition and categories of anemia and the strength of association differs among types of anemia. Considerable trials under randomization-based controlling should be conducted for determining if enhancing vit-D condition is capable of reducing anemia severity in normal groups.

Declarations

Acknowledgments. The authors like to acknowledge the employees at Suzhou Municipal Hospital and Zhang Jiagang Maternity and Child Health Care Hospital for their assistance with this study. Furthermore, the authors acknowledge and appreciate the interviewers' and respondents' time and effort put into this research.

Data and resources Transparency. The data obtained and compiled for this work are not publicly accessible; however, they may be obtained from the corresponding author if you make a request.

Consent for publication. Not applicable.

Conflict of interest. There are no conflicts of interest.

REFERENCES

Alyasin S, Momen T, Kashef S, Alipour A, Amin R (2011) The relationship between serum 25 hydroxy vitamin d levels and asthma in children. *Allergy Asthma Immunol Res* **3**: 251–255. https://doi.org/10.4168/aair.2011.3.4.251

Anastasiou A, Karras SN, Bais A, Grant WB, Kotsa K, Goulis DG (2017) Ultraviolet radiation and effects on humans: the paradigm of maternal vitamin D production during pregnancy. *Eur J Clin Nutr* **71**: 1268–1272. https://doi.org/10.1038/ejcn.2016.188

Atkinson MA, Melamed ML, Kumar J, Roy CN, Miller ER 3rd, Furth SL, Fadrowski JJ (2014) Vitamin D, race, and risk for anemia in children. *J Pediatr* **164**: 153–158. e151. https://doi.org/10.1016/j.jpeds.2013.08.060

Balasubramanian S (2011) Vitamin D deficiency in breastfed infants & the need for routine vitamin D supplementation. *Indian J Med Res* **33**: 250–252

Bhaskaram P, Balakrishna N, Radhakrishna KV, Krishnaswamy K (2003) Validation of hemoglobin estimation using Hemocue. *Indian J Pediatr* **70**: 25–28. https://doi.org/10.1007/BF02722739

D'Souza AM (2020) A general pediatrician's approach to anemia in childhood. *Pediatr Ann* **49**: e10–e16. https://doi.org/10.3928/19382359-20191212-01

Ernst JB, Becker T, Kuhn J, Gummert JF, Zittermann A (2015) Independent association of circulating vitamin D metabolites with anemia risk in patients scheduled for cardiac surger. *PLoS One* **10**: e0124751. https://doi.org/10.1371/journal.pone.0124751

Harinarayan CV, Joshi SR (2009) Vitamin D status in India – its implications and remedial measures. *J Assoc Physicians India* **57**: 40–48.

Holick MF (2007) Vitamin D deficiency. *N Engl J Med* **357**: 266–281. https://doi.org/10.1056/NEJMra070553

Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM; Endocrine Society (2011) Evaluation, treatment, and prevention of vitamin D deficien-

cy: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* **96**: 1911–1930. https://doi.org/10.1210/jc.2011-0385

Htet MK, Fahmida U, Dillon D, Akib A, Utomo B, Thurnham DI (2014) The influence of vitamin A status on iron-deficiency anaemia in anaemic adolescent schoolgirls in Myanmar. *Public Health Nutr* **17**: 2325–2332. https://doi.org/10.1017/S1368980013002723

Jin HJ, Lee JH, Kim MK (2013) The prevalence of vitamin D deficiency in iron-deficient and normal children under the age of 24 months. *Blood Res* **48**: 40–45. doi: 10.5045/br.2013.48.1.40.

Khan L (2018) Anemia in childhood. *Pediatr Ann* **47**: e42–e47. https://doi.org/10.3928/19382359-20180129-01

Laway BA, Kotwal SK, Shah ZA (2014) Pattern of 25 hydroxy vitamin D status in North Indian people with newly detected, type 2 diabetes: A prospective case control study. *Indian J Endocrinol Metab* **18**: 726–730. https://doi.org/10.4103/2230-8210.139242

Molloy J, Koplin JJ, Allen KJ, Tang MLK, Collier F, Carlin JB, Saffery R, Burgner D, Ranganathan S, Dwyer T, Ward AC, Moreno-Betancur M, Clarke M, Ponsonby AL, Vuillermin P; BIS Investigator Group (2017) Vitamin D insufficiency in the first 6 months of infancy and challenge-proven IgE-mediated food allergy at 1 year of age: a case-cohort study. *Allergy* **72**: 1222–1231. https://doi.org/10.1111/all.13122

Sharma S, Jain R, Dabla PK (2015) The role of 25-hydroxy vitamin D deficiency in iron deficient children of north India. *Indian J Clin Biochem* **30**: 313–317. https://doi.org/10.1007/s12291-014-0449-x

Shin JY, Shim JY (2013) Low vitamin D levels increase anemia risk in Korean women. *Clin Chim Acta* **421**: 177–180. https://doi.org/10.1016/j.cca.2013.02.025

Sim JJ, Lac PT, Liu IL, Meguerditchian SO, Kumar VA, Kujubu DA, Rasgon SA (2010) Vitamin D deficiency and anemia: a cross-sectional study. *Ann Hematol* **89**: 447–452. https://doi.org/10.1007/s00277-009-0850-3

Uberti F, Morsanuto V, Lattuada D, Colciaghi B, Cochis A, Bulfoni A, Colombo P, Bolis G, Molinari C (2016) Protective effects of vitamin D3 on fimbrial cells exposed to catalytic iron damage. *J Ovarian Res* **9**: 34. https://doi.org/10.1186/s13048-016-0243-x

Yazici KU, Percinel Yazici I, Ustundag B (2018) Vitamin D levels in children and adolescents with obsessive compulsive disorder. *Nord J Psychiatry* **72**: 173–178. https://doi.org/10.1080/08039488.2017.1406985

Yoo EH, Cho HJ (2015) Prevalence of 25-hydroxyvitamin D deficiency in Korean patients with anemia. *J Clin Lab Anal* **29**: 129–134. https://doi.org/10.1002/jcla.21740

Yoon JW, Kim SW, Yoo EG, Kim MK (2012) Prevalence and risk factors for vitamin D deficiency in children with iron deficiency anemia. *Korean J Pediatr* **55**: 206–211. https://doi.org/10.3345/kjp.2012.55.6.206

Zhou S, Glowacki J (2017) Chronic kidney disease and vitamin D metabolism in human bone marrow-derived MSCs. *Ann N Y Acad Sci* **1402**: 43–55. https://doi.org/10.1111/nyas.13464