ISSN (electrónico): 1699-5198 - ISSN (papel): 0212-1611 - CODEN NUHOEQ S.V.R. 318

ARÁN

Nutrición Hospitalaria

Trabajo Original

Pediatría

Clinical value of vitamin K testing in children aged 1-2 years with vitamin D deficiency rickets

El valor clínico de la determinación de la vitamina K en niños de 1 a 2 años de edad con raquitismo por déficit de vitamina D

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Abstract

Objective: to investigate the clinical value of vitamin K testing in children with vitamin D deficiency rickets.

Methods: fifty children with vitamin D deficiency rickets admitted to our hospital from March 2021 to March 2022 were recruited as the case group using convenience sampling; and an additional 50 children without bone health diseases during the same period of health checkup were selected as the control group. The relevant indexes between the two groups were compared.

Results: there were statistically significant differences between the two groups in the level of 25 hydroxyvitamin D3 [25-(OH)D3], the proportion of breastfeeding, and the proportion of preterm birth ($p < 0.001$). The levels of vitamin K1 and K2 were lower in the case group than in the control group, and the proportion of those with vitamin K1 deficiency and vitamin K2 deficiency were higher than the control group ($p < 0.001$) . Positive correlations were found between vitamins K1 and K2 and 25-(OH)D3, blood calcium, and blood phosphorus ($p < 0.05$); artificial feeding, preterm birth, vitamin K1 deficiency, and vitamin K2 deficiency were risk factors for the development of vitamin D deficiency rickets, and the highest AUC of the combination of each index in predicting the occurrence of vitamin D deficiency rickets was 0.951 (*95 % CI*: 0.910-0.991).

Conclusion: preterm birth, artificial feeding, and vitamin K1 and K2 deficiency are independent risk factors for bone metabolism in children with vitamin D-deficiency rickets. And these risk factors have predictive and diagnostic value in the diagnosis and management of vitamin D deficiency rickets.

Keywords:

Vitamin D deficiency rickets. Vitamin K. Bone mineral density. Carboxylated osteocalcin.

Resumen

Objetivo: investigar el valor clínico de la determinación de vitamina K en niños con raquitismo por deficiencia de vitamina D.

Métodos: cincuenta niños con raquitismo por deficiencia de vitamina D admitidos en nuestro hospital desde marzo de 2021 hasta marzo de 2022 se reclutaron como grupo de casos mediante muestreo de conveniencia y se seleccionaron adicionalmente 50 niños sin enfermedades óseas durante el mismo período de revisión de la salud como grupo de control. Se compararon los índices relevantes entre los dos grupos.

Resultados: existían diferencias estadísticamente significativas entre los dos grupos en los niveles de 25 hidroxivitamina D3 [25-(OH)D3], la proporción de lactancias maternas y la proporción de nacimientos prematuros ($p < 0,001$). Los niveles de vitaminas K1 y K2 eran inferiores en el grupo de casos que en el grupo de control, y la proporción de aquellos con deficiencia de vitamina K1 y vitamina K2 era mayor que en el grupo de control ($p < 0,001$). Se encontraron correlaciones positivas entre las vitaminas K1 y K2 y la 25-(OH)D3, el calcio sanguíneo y el fosforo sanguíneo ($p < 0,05$); la alimentación artificial, el nacimiento prematuro, la deficiencia de vitamina K1 y la deficiencia de vitamina K2 son factores de riesgo para el desarrollo de raquitismo por deficiencia de vitamina D, y el AUC más alto de la combinación de cada índice en la predicción del raquitismo por deficiencia de vitamina D fue de 0,951 (IC 95 %: 0,910-0,991).

Conclusión: el nacimiento prematuro, la alimentación artificial y la deficiencia de vitaminas K1 y K2 son factores de riesgo independientes para el metabolismo óseo en niños con raquitismo por deficiencia de vitamina D. Y estos factores de riesgo tienen valor predictivo y diagnóstico en el diagnóstico y manejo del raquitismo por deficiencia de vitamina D.

Palabras clave:

Raquitismo por deficiencia de vitamina D. Vitamina K. Densidad mineral ósea. Osteocalcina carboxilada.

Received: 02/04/2024 • Accepted: 23/07/2024

Fund: study on the clinical value of vitamin K detection in 1-2 year old children with vitamin D deficiency rickets, No.A2024015.

Conflict of interest: the authors declare no conflict of interest.

Artificial intelligence: the authors declare not to have used artificial intelligence (AI) or any AI-assisted technologies in the elaboration of the article.

Jie L, Niu L, Lu T, Sun Q. Clinical value of vitamin K testing in children aged 1-2 years with vitamin D deficiency rickets. Nutr Hosp 2024;41(6):1172-1179
DOI: http://dx.doi.org/10.20960/nh.05251

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INTRODUCTION

Nutritional vitamin D deficiency rickets is a systemic chronic nutritional disease featuring bone lesions resulting from disorders of calcium and phosphorus metabolism due to vitamin D deficiency in children. Epidemiologic surveys (1) show that the incidence of nutritional vitamin D deficiency rickets decreases yearly in response to the improvement of socio-economic and cultural levels. However, preterm and twin-born infants are prone to nutritional vitamin D deficiency rickets as a result of increased vitamin D requirements due to rapid growth and development, which is complemented by insufficient vitamin D stored in the body.

Vitamin K, also known as clotting vitamins, is vital to human health. Vitamin K can be obtained from food and can also be synthesized and synthesized by intestinal bacteria. However, newborns are at risk of vitamin K deficiency because their immature livers do not use vitamin K effectively. In addition, due to the low content of vitamin K in breast milk, and vitamin K is difficult to transfer through the placental barrier, intestinal asepsis, their vitamin K reserves tend to be low (1,2). Vitamin K has been shown to be associated with bone metabolism in addition to coagulation and neonatal bleeding disorders. It affects the bone health of infants and children to a great extent (3,4).

Bone R-hydroxy glutamic acid protein (GLa), short for osteocalcin, falls into the category of vitamin K-dependent calcium-binding proteins. It is a sensitive and specific indicator reflecting osteoblast activity and bone metabolism status, and serves as a new biochemical marker with a broader application prospect.

Vitamin K is necessary for the activation of coagulation factors VII (proconvertin), IX (antihemophilic B), X (stuart factor) and prothrombin. By differentiating osteogenic, osteoclastogenic and stimulating cells in bone tissue, it upregulates the expression of bone marker genes by promoting bone mineralization in the extracellular matrix and inhibits the functional expression of osteoclasts. In other words, it exerts beneficial effects on both osteoclastogenesis and bone quality enhancement. Furthermore, vitamin K increases the rate of bone mineralization and inhibits bone matrix dissolution and bone calcium loss by promoting GLa protein γ-glutamate carboxylation and calcium salt deposition to achieve net bone calcium accumulation (5). In this sense, vitamin K can be inferred to be related to bone mineral density (BMD). A study in The Netherlands showed that infants and children have six times higher concentrations of uncarboxylated osteocalcin (ucOCN) compared to adults; this, combined with their low vitamin K status, increases the potential risk of osteoporosis (5). Data from a domestic study related to the nutritional status of pediatric skeletal vitamin K revealed a correlation between pediatric skeletal vitamin K deficiency and age; the more severe degree of skeletal vitamin K deficiency was predominantly in the age group of more than 3 months to 1 year, which is also the stage where the supply of vitamin K to the skeleton is also the most deficient. This is consistent with the finding that children in this age group are clinically prone to abnormal bone metabolism and susceptible to rickets (6,7).

Therefore, vitamin K is crucial in preventing fractures in children, apart from the relevance of vitamin D for children's bone health (8). Late vitamin K deficiency usually coexists with vitamin D deficiency rickets (9). A favorable vitamin K status has a close bearing on a significant increase in bone mass in children during puberty. It has been found that concomitant vitamin K and vitamin D supplementation in children with osteoporosis caused by long-term glucocorticoid use is significantly more effective in improving lumbar spine bone mineral density and blood osteocalcin concentration in children (10). Vitamin D levels are broadly accepted as one of the biochemical indexes for evaluating healthy bone growth. A favorable vitamin K status has a close bearing on a significant increase in bone mass in children during puberty. As for the possibility of vitamin K level as one of the biochemically sensitive indexes for the evaluation of bone metabolism and as one of the diagnostic indexes of vitamin D deficiency rickets, more in-depth investigations need to be carried out. In this study, children with vitamin D deficiency rickets were recruited to analyze the serum vitamin K levels in children with vitamin D deficiency rickets and to investigate the clinical diagnostic and therapeutic value of vitamin K in vitamin D deficiency rickets. By doing so, we aimed to provide some clinical guidance for the diagnosis and treatment of vitamin D deficiency rickets.

STUDY SUBJECTS AND METHODS

STUDY SUBJECTS

Fifty children with vitamin D deficiency rickets admitted to our hospital from March 2021 to March 2022 were recruited as the case group using convenience sampling; and an additional 50 children without bone health diseases during the same period of health checkup were selected as the control group.

The inclusion criteria were as follows:

1. Children diagnosed with vitamin D deficiency rickets according to the Guidelines for the Diagnosis and Treatment of Osteochondrosis and Rickets (9) developed by the Osteoporosis and Bone Mineral Salt Diseases Branch of Chinese Medical Association;
2. Inclusion criteria of abnormal bone metabolism: normal BMD ($Z > -1.0$), mild BMD deficiency ($-1.5 < Z \leq -1.0$), moderate BMD deficiency ($-2.0 < Z \leq -1.5$), severe BMD deficiency ($Z \leq -2.0$); this index was formulated and implemented with reference to the Z-value scoring criterion of Ultrasonic velocity in *Study on the Correlation Between Vitamin K Deficiency and Abnormal Bone Metabolism in Children* (11) by Wanyan Zewei; for the convenience of the study, those with mild, moderate, and severe BMD deficiencies were included in the abnormal BMD group;
3. Those aged 3 months-2 years of either gender;
4. Those with normal weight by reference to the *Reference Values for Body Weight, Body Length and Head Circumference for Chinese Infants aged 0 to 13 Weeks* (12);
5. Those with complete and valid medical records; and

6. Those in the control group who did not suffer from vitamin K deficiency, vitamin D deficiency rickets, bone pain, low bone density, bone morphology and other abnormal changes in bone diseases.

The exclusion criteria were as follows:

1. Children < 1 or > 2 years old;
2. Those with normal bone metabolism;
3. Those with combination of other underlying diseases, such as cold, cough, and runny nose;
4. Those with combination of severe respiratory, cardiovascular, cerebrovascular, urinary, immune, hepatic, biliary, endocrine system and other critical diseases;
5. Those with allergic diseases (e.g., skin needling, drug allergy) and history of taking vitamin D, K, etc. and history of taking drugs for other diseases within 3 months prior to enrollment;
6. Children with genetic diseases;
7. Those with an overweight;
8. Those with an incomplete grade of information; and
9. Those whose guardians did not sign the informed consent form or who were unwilling to participate in the experiment, whose clinical data were missing, whose compliance was poor and who did not follow the prescribed treatment, or who withdrew from the experiment in the middle of the study, resulting in the inability to obtain the real medical records, or whose data were mutilated, damaged, or had unclear handwriting.

The study was approved by the Ethics Committee of the hospital. The guardians of the subjects signed an informed consent form.

STUDY METHODS

Measurement of vitamin D

Sample collection

In the early morning fasting state, 2 mL of blood from the external elbow vein was collected and stored in a biochemical tube (labeled) (normal room temperature: 23 °C, storage time: 1 h), and centrifuged (centrifugation rate: 4000 rev/min, centrifugation time: 10 min); then the supernatant (≥ 200 μL) was placed in clean EP tubes and stored in a light-proof environment (light-proof environment: 4 °C) for subsequent testing.

Testing method

HPLC – MS / MS: 10 μL of internal standard working solution was placed in a centrifuge tube (1.5 mL), followed by the addition of 100 μL of blood sample to be tested, and then ethyl acetate (1.2 mL) was pipetted into the above centrifuge tube with a pipette gun, and mixed well (time: 5 min), then centrifuged again (centrifugation rate: 15,000 rev/min, centrifugation time: 10 min), and 1 mL of the supernatant was taken and blown dry; next, 100 μL of methanol was added for mixing (time: 1 min), and 100 μL of the supernatant was fed in five injections of 20 μL/dose; finally, the final results were obtained.

Measurement of vitamin K

Sample collection

The same procedure as that for vitamin D.

Testing method

HPLC – MS / MS: 10 μL of internal standard working solution was placed in a centrifuge tube (1.5 mL), followed by the addition of 200 μL of blood sample to be tested, and then ethyl acetate (1000 μL) was pipetted into the above centrifuge tube with a pipette gun, and mixed well (time: 5 min), then centrifuged again (centrifugation rate: 15000 rev/min, centrifugation time: 10 min), and 900 μL of the supernatant was taken and blown dry; next, 150 μL of methanol was added for mixing (time: 1 min), and 100 μL of the supernatant was fed in ten injections of 10 μL/dose; finally, the final results were obtained.

Measurement of BMD, bone alkaline phosphatase (BALP) and trace elements: BMD was measured by ultrasonic bone strength and electronic spondrometer, BALP was measured by automated enzyme marker, and trace elements (calcium, phosphorus, etc.) were measured by atomic absorption spectrophotometry.

Diagnostic criteria for decreased BMD and osteoporosis

Normal bone mass ($Z > -1$), decreased BMD ($-2.5 \leq Z < -1$), osteoporosis ($Z < -2.5$), and abnormal BMD defined as $Z \leq -1$, which was formulated according to the *Guidelines for the Diagnosis and Treatment of Primary Osteoporosis (2017 Edition)* (13).

Classification criteria of vitamin D

Deficiency (< 10 ng/mL), insufficiency (10-20 ng/mL), normal (20-100 ng/mL) and overdose (> 100 ng/mL) in four dimensions according to the *Recommendations for the Prevention and Control of Micronutrient Deficiencies in Children (2010 Edition)* (14).

Classification criteria of vitamin K

Vitamin K1 and K2 are divided into three dimensions: deficiency (K1 < 0.1 ng/mL, K2 < 0.1 ng/mL), normal ($0.1 \leq K1 \leq 2.2$ ng/mL, $0.1 \leq K2 \leq 0.86$ ng/mL), and overdose (K1 > 2.2 ng/mL, K2 > 0.86 ng/mL) according to relevant standards issued by the Mayo Clinic of the U.S. and the MDI Biological Laboratory and in Germany (15).

DATA COLLECTION

Routine clinical data such as age, gender, height, weight, blood calcium, blood phosphorus, bone alkaline phosphatase (BALP), and 25 hydroxyvitamin D3 [25-(OH)D3] were collected from both groups.

STATISTICAL ANALYSIS

All data in this study were statistically processed using SPSS 26.0 statistical software. The K-S method was used for normality test; normally distributed measurement data were expressed as (x ± s), and the *t* test was used for comparison of means between groups. Count data were expressed as frequency (*n*) or rate (%), and were tested by the χ^2 test. Pearson's correlation coefficient was used to calculate the correlation coefficient *r*. Furthermore, the risk factors for the development of vitamin D deficiency rickets were explored using logistic regression analysis; the predictive value of each index for the development of vitamin D deficiency rickets was investigated using the receiver operating characteristic curve (ROC). All tests were two-sided, with the test level set at $\alpha = 0.05$.

RESULTS

GENERAL DATA

In the case group, there were 50 cases, 29 males and 21 females, with a mean age of 1.29 ± 0.56 years; in the control group, there were 50 cases, 31 males and 19 females, with a mean age of 1.23 ± 0.34 years. No statistically significant difference was observed between the two groups in terms of gender, age, height, weight, blood calcium, blood phosphorus, and BALP ($p > 0.05$). However, there were statistically significant differences between the two groups in the level of 25-(OH)D3, the proportion of breastfeeding, and the proportion of preterm birth, as shown in table I.

Comparison of vitamin K levels and deficiency between the two groups

There were statistically significant differences in the levels of vitamin K1, the proportion of those with vitamin K1 deficiency, the levels of vitamin K2, and the proportion of those with vitamin K2 deficiency between the two groups; the levels of vitamin K1 and K2 in the case group were lower than those in the control group, and the proportion of those with deficiency was higher than that in the control group, as shown in table II.

Pearson correlation analysis of vitamin K and vitamin D deficiency rickets

Positive correlations were found between vitamin K1 and 25-(OH)D3, blood calcium, and blood phosphorus; likewise, positive correlations were also found between vitamin K2 and 25-(OH)D3, blood calcium, and blood phosphorus, as shown in table III.

Table I. Comparison of general data

Index	Control group (*n* = 50)	Case group (*n* = 50)	t/χ^2 value	*p*-value
Age (yrs, x ± s)	1.23 ± 0.34	1.29 ± 0.56	0.156	0.870
Gender (male/female)	31/19	29/21	0.167	0.683
Height (cm, x ± s)	73.65 ± 5.87	73.45 ± 5.69	0.805	0.423
Weight (kg, x ± s)	9.43 ± 1.56	8.88 ± 1.72	1.563	0.087
Blood calcium (mmol/L, x ± s)	2.30 ± 0.13	2.28 ± 0.14	0.452	0.653
Blood phosphorus (mmol/L, x ± s)	1.43 ± 0.31	1.41 ± 0.26	0.599	0.551
BALP (U/L, x ± s)	181.11 ± 25.57	180.45 ± 26.54	0.144	0.886
25(OH)D3 (ng/L, x ± s)	57.06 ± 13.57	13.88 ± 2.09	17.398	< 0.001
Feeding mode (cases)			43.717	< 0.001
Breastfeeding	40	7		
Artificial feeding	10	43		
Preterm birth (cases)	7	23	12.190	< 0.001

BALP: bone alkaline phosphatase; 25(OH)D3: 25-hydroxyvitamin D3.

MULTIFACTORIAL ANALYSIS OF VITAMIN D DEFICIENCY RICKETS

A logistic regression model was constructed with the occurrence of vitamin D deficiency rickets as the dependent variable (occurrence = 1, no occurrence = 0) and the statistically significant factors in the univariate analysis as the independent variables (25-(OH) D3 is an indicator of the body's vitamin D reserve. Low levels of 25-(OH) D3 indicate vitamin D deficiency, and the causal association between 25-(OH) D3 and vitamin D deficiency rickets has been established, so it was not included in the analysis). The grouping and coding of the variables are shown in table IV. Regression analysis showed that artificial feeding, preterm birth, vitamin K1 deficiency, and vitamin K2 deficiency were risk factors for the development of vitamin D deficiency rickets, as shown in table V.

PREDICTIVE VALUE OF INDEXES ON THE OCCURRENCE OF VITAMIN D DEFICIENCY RICKETS

Artificial feeding, preterm birth, vitamin K1, and vitamin K2 were all of predictive value for the development of vitamin D deficiency rickets. The area under the curve (AUC) of artificial feeding for predicting vitamin D deficiency rickets was 0.843 (95 % CI: 0.771-0.916); the AUC of preterm birth for predicting vitamin D deficiency rickets was 0.856 (95 % CI: 0.745-0.924); the AUC of vitamin K1 deficiency in predicting vitamin D deficiency rickets was 0.867 (95 % CI: 0.784-0.948); and the AUC of vitamin K2 deficiency in predicting vitamin D deficiency rickets was 0.912 (95 % CI: 0.897-0.988). Among them, the highest AUC of the combination of each index in predicting the occurrence of vitamin D deficiency rickets was 0.951 (95 % CI: 0.910-0.991), as shown in table VI.

Table II. Comparison of vitamin K levels and deficiency between the two groups

Index		Control group (n = 50)	Case group (n = 50)	t/χ^2 value	p-value
Vitamin K1	Level (ng/L, x ± s)	1.432 ± 0.654	0.076 ± 0.021	9.393	< 0.001
	Number of deficiencies	2	15	11.977	< 0.001
Vitamin K2	Level (ng/L, x ± s)	0.719 ± 0.213	0.078 ± 0.021	8.593	< 0.001
	Number of deficiencies	5	38	44.431	< 0.001

Table III. Pearson's correlation analysis of vitamin K and vitamin D deficiency rickets

Item		Correlation coefficient r	p-value
Vitamin K1	25-(OH)D3	0.359	0.011
	Blood calcium	0.322	0.021
	Blood phosphorus	0.326	0.018
Vitamin K2	25(OH)D3	0.512	< 0.001
	Blood calcium	0.489	< 0.001
	Blood phosphorus	0.368	0.011

Table IV. Variable grouping and coding

Variable	Grouping	Coding
Artificial feeding	No	1
	Yes	2
Preterm birth	No	1
	Yes	2
Vitamin K1 level	Input at original value	
Vitamin K2 level	Input at original value	

Table V. Logistic regression analysis of risk factors for vitamin D deficiency rickets

Influencing factors	S.E.	Wald χ^2	p-value	OR	OR (95 % CI)
Artificial feeding	0.345	8.234	0.001	2.796	1.575-7.123
Preterm birth	2.256	6.208	0.001	3.456	2.757-5.112
Vitamin K1 deficiency	0.978	6.763	0.001	6.371	5.731-21.633
Vitamin K2 deficiency	1.123	7.423	0.001	9.563	7.931-32.263

Table VI. Predictive value of indexes on the occurrence of vitamin D deficiency rickets

Item	AUC	95 % CI	Cut-off value	Sensitivity (%)	Specificity (%)
Artificial feeding	0.843	0.771-0.916	-	81.31	84.54
Preterm birth	0.856	0.745-0.924	-	83.61	81.70
Vitamin K1 deficiency	0.867	0.784-0.948	0.216 ng/mL	90.44	89.48
Vitamin K2 deficiency	0.912	0.897-0.988	0.184 ng/mL	93.01	95.82
Combined prediction	0.951	0.910-0.991	-	95.24	97.73

DISCUSSION

Vitamin D deficiency rickets is associated with a deficiency of vitamin D during bone growth. It was previously believed that adequate vitamin D supplementation was sufficient for patients with vitamin D deficiency rickets. As modern medicine develops and advances, vitamin K intake is also found to be of necessity. Vitamin K is involved in human bone metabolism (16). Osteocalcin, also known as vitamin K-dependent bone γ-hydroxyglutamic acid protein, is abundant to the extent that its concentration and degree of carboxylation reflect the status of BGP in the femur, which accounts for 1-2 % of bone protein. BGP levels in children with vitamin D deficiency rickets were significantly higher than in normal children before treatment, which decreased significantly after treatment (17). Under the action of vitamin K-dependent carboxylase, all three glutamate residues at the BGP site (amino acids 17, 21, and 24) are evolved into carboxylated osteocalcin (cOC) by carboxylation. Once Ca^{2+} and carboxyapatite are deposited in combination with cOC, they participate in the bone mineralization process. Uncarboxylated osteocalcin (ucOC), as a specific expression in the structure of BGP (< 3 carboxylated residues), can be used as a sensitive indicator of the body's vitamin K status due to its extreme ease of release into the bloodstream (18). The BALP levels in the vitamin K2 group (< 0.1 ng/mL) were lower than those in the vitamin K2 group (≥ 0.1 ng/mL), suggesting a correlation between vitamin D deficiency and BALP. In the case of vitamin D deficiency, the absorption of calcium and phosphorus in the intestines is reduced, resulting in impaired bone formation and compensatory osteoblastic activity. BALP is a direct response to osteoblast activity. The degree of increased BALP activity has a significant grade correlation with children, susceptibility to fright, sleep disturbance, hyperhidrosis, cranial softening and pregnancy factors (such as dietary monotony, no vitamin D preparations).

The possible mechanisms linking vitamin K2 and vitamin D deficiency rickets can be categorized as follows: First, vitamin K2 is involved in the transcription of bone-specific genes. Vitamin K2 has been shown to induce the production of osteoblast markers, indirectly activating steroids and human steroid xenobiotics. It is not only beneficial for increasing ALP and insulin-like growth factor expression, but also broadens bone formation capacity and growth space (supported by osteocalcin, collagen, etc.), thereby contributing to the structure of calcium salt deposition. Secondly, vitamin K2 is a coenzyme of γ-glutamyl-carboxylase, which not only maintains a special affinity and activity for carboxylation into osteocalcin (OC) and Ca^{2+} structure, but also facilitates calcium salt deposition and bone mineralization rate enhancement. Adequate vitamin K2, a source of bone deposition and mineralization, is beneficial in improving Ca^{2+} expression in the blood of children with vitamin D deficiency rickets (19). Third, vitamin K2 inhibits calcium loss and the expression of osteoclast differentiation factors such as IL-1 and IL-6 in the blood of children with vitamin D deficiency rickets. All these confirm the correlation of bone metabolism between vitamin K2 deficiency and children with vitamin D deficiency rickets.

It was also shown in the present study that there is an association between vitamin K and vitamin D deficiency rickets and that vitamin K deficiency is a risk factor for the development of vitamin D deficiency rickets in children, which is consistent with the above findings. This suggests that vitamin K2 deficiency may lead to reduced bone mass and undermineralization in children with vitamin D deficiency rickets, thereby participating in the onset, progression, and prognosis of the disease (20,21). Regarding the improvement of BMD and BGP, the clinical effects of vitamin K2 supplementation are superior to those of vitamin D supplementation as evidenced by numerous previous studies (22,23).

We also found that preterm delivery and artificial feeding are risk factors for the development of vitamin D deficiency rickets. Seventy-five percent of fetal calcium and phosphorus are stored during the last three months of gestation. Therefore, the calcium and phosphorus levels in infants and children born prematurely are significantly lower than those in normal term infants, depending on the date of preterm birth and individual circumstances. It has been reported in the relevant literature (24) that preterm infants may have relatively weak calcium retention capacity due to early separation from their mothers, and they are at high risk of rickets because of their fast growth rate, presence of growth catch-up growth period, and high requirement of vitamin D and calcium compared with normal term infants.

Recent years have witnessed the rapid development of modern society, with scientists and nutritionists sparing no effort

to improve dairy products. This has led to the widespread use of artificial feeding, which is gradually replacing breastfeeding. Chinese infant formula food industry standard "Food Safety National Standard Infant Formula Food" (GB10765-2010) (25) and "Food Safety National Standard Infant and early Childhood Formula Food" (GB10767-2010) (26) clearly specify the nutritional requirements of infant artificial milk, including the content of vitamins, protein, fatty acids, etc., in order to ensure that the nutritional needs of infants are met. However, despite being as close as possible to breast milk in terms of nutritional value, artificial milk products have never been able to replace breast milk. This is because the nutritional composition of breast milk is very complex and perfect, with hundreds of ingredients, many of which are bioactive and contain many unique growth factors, which are very important for the development and growth of neonatal brain nerve tissue. Although formula can imitate and add certain nutrients, formula lacks many ingredients in breast milk, including many kinds of living cells, cholesterol, polyamines, free amino acids, enzymes and many other bioactive ingredients. Moreover, the disinfection process used to produce formula slightly changes the structure of milk protein, thus missing the anti-infective protection of cross-species (27). There is a significant correlation between the feeding mode and the prevalence of rickets in infants within 6 months of age, and the prevalence of rickets is higher in artificially fed infants. Breast milk contains all the nutrients needed by infants. While preventing diarrhea and respiratory infections, it reduces nutrient loss and consumption, and plays a vital preventive role in the prevention and treatment of rickets (28). In this regard, breastfeeding should be strongly advocated, and complementary foods should be added in a timely manner, so as to develop good eating habits, avoid partiality and picky eating, and rationalize the diet to ensure balanced nutrition.

Nevertheless, there are some limitations in this study. First, the relatively small sample included in this study may lead to a bias between the results of the study and the actual clinical situation. Second, the inclusion of only those admitted to our hospital may have biased the extrapolation of the findings. Finally, time constraints in the study process may have resulted in some shortcomings in the observation time.

CONCLUSION

In conclusion, children suffering from vitamin D deficiency rickets present significant vitamin K deficiency, being more pronounced in the case of vitamin K2 deficiency. Vitamins K1 and K2 are known to influence bone metabolism in children with vitamin D deficiency rickets; they show a positive correlation with vitamin D deficiency rickets. Preterm birth, artificial feeding, and vitamin K1 and K2 deficiency are independent risk factors for the development of bone metabolism in children with vitamin D-deficiency rickets. Preterm birth, artificial feeding, and vitamins K1 and K2 are of predictive and diagnostic value in the diagnosis and management of vitamin D deficiency rickets.

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