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ORIGINAL ARTICLE

Association between Maternal Serum 25(OH)-Vitamin D_3 Levels in Late Pregnancy and Profiles of Newborn Amino Acid Concentrations

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SUMMARY

Background: **Vitamin D deficiency is common during pregnancy. 25(OH)-Vitamin D_3 is the major vitamin D circulating form in human organism. However, the effects of 25(OH)-vitamin D_3 deficiency in late pregnancy on the infant's amino acid metabolism has still not been studied. The aim of this study was to evaluate the relationship between maternal serum 25(OH)-vitamin D_3 levels in late pregnancy and profiles of newborn amino acid concentrations.**
Methods: **A total of 539 women in late pregnancy and their newborns enrolled in this study. The concentrations of 25(OH)-vitamin D_3 in maternal serum were measured by ABI 4500 high performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS). For newborns, their amino acids levels were measured by ABI 3200 LC/MS/MS. *T*-test and Spearman's correlation analyses were used in the study as statistical analysis methods.**
Results: **The concentrations of arginine (Arg) and glycine (Gly) in newborn blood spots were significantly different in each maternal serum 25(OH)-vitamin D_3 status group. There was a significant correlation between maternal serum 25(OH)-vitamin D_3 status and Arg concentration in their offspring ($p = 0.03$).**
Conclusions: **Maternal serum 25(OH)-vitamin D_3 concentration in late pregnancy may affect their newborn's amino acid metabolism, but the precise mechanisms underlying the relationship need further investigation.**
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KEY WORDS

late pregnancy, 25(OH)-vitamin D_3, newborn, amino acid

INTRODUCTION

Vitamin D deficiency is a worldwide health issue, especially in pregnant women. According to a recent report, 54% of pregnant women had suboptimal vitamin D levels, showing vitamin D status as a critical health problem in pregnant women [1,2]. Maternal vitamin D level is essential for optimal fetal development. The relationship between vitamin D deficiency and various adverse pregnancy outcomes have been widely reported in the past years [3]. Ingvild M. reported that there was a trend toward a higher risk of type 1 diabetes in infants with lower levels of maternal vitamin D during pregnancy

[4]. M K Javaid reported that reduced concentration of 25(OH)-vitamin D_3 in mothers during late pregnancy was associated with reduced whole body and lumbar spine bone mineral content in children at age 9 years [5]. Amino acids, which are a source of protein synthesis and supply to the tissues, play a pivotal role in maintaining organ and body protein homeostasis [6]. Amino acids are also the most important and direct factors in assessing neonatal nutrition and metabolism [7]. However, the effects of vitamin D deficiency in late pregnancy on the infant's amino acid metabolism still have not been studied. The aim of the study focuses on the relationship between maternal serum 25(OH)-vitamin D_3 levels in late pregnancy (> 28 weeks) and profiles of newborn amino acid concentrations and investigates whether lower maternal serum 25(OH)-vitamin D_3 concentrations in late pregnancy influenced the newborn's amino acids metabolism. It will provide some proof for the investigation of amino acid metabolism disorder and nutritional status of newborns.

MATERIALS AND METHODS

Study subjects

The study was carried out at the Fourth Hospital of Shijiazhuang (Shijiazhuang Gynaecology and Obstetrics Hospital) in China. The study consisted of 539 women in late pregnancy and their newborns. Women aged 20 - 42 years were divided into four groups, 25(OH)-vitamin D_3 severe deficiency group (< 10 ng/mL), deficiency group (10 - 20 ng/mL), insufficiency group (20 - 30 ng/mL), and sufficiency group (> 30 ng/mL). The groups were divided according to Olmos OA et al. [1]. Their newborns were also divided into four groups according to their mother's 25(OH)-vitamin D_3 status. The characteristics of these participants are presented in Table 1. We excluded pregnant women with multiple pregnancy, artificial assisted pregnancy, gestational diabetes, kidney disease, liver disease, and thyroid disease. Exclusion criteria for newborns include refusal of parental consent, evidence of a major congenital anomaly, and complicated maternal pregnancy.

Measurement of maternal serum 25(OH)-vitamin D_3

Blood of women in late pregnancy was collected after overnight fasting. Serum samples were centrifuged at 3,500 r/minute for 15 minutes. Before analyzing the serum samples, the analytical performance of HPLC-MS/MS (AB Sciex 4500) was verified. Intra-assay and inter-assay coefficients of variation were 3.8% and 4.3%, respectively. Concentrations of 25(OH)-vitamin D_3 (precursor ions 401.3, product ion 365.5) were measured by using HPLC-MS/MS and analyzed in the multiple reaction monitoring (MRM) modes by the directions for the use of tandem mass spectrometry kit for 25(OH)-vitamin D_3 (YingSheng, China). Analyst 1.6.3 software (AB Sciex, USA) was used for integrating the peak areas and fitting with calibration curves.

Measurement of newborns' amino acids

Dried blood spots (DBS) specimens were obtained from the newborns on 72 hours of life. Whole blood was drawn by heel prick and spotted on filter paper, then dried at room temperature for analysis. For sample treatment methods, please refer to Liu Q et al. [8]. Concentrations of the 9 amino acids, including alanine (Ala), glycine (Gly), valine (Val), methionine (Met), phenylalanine (Phe), tyrosine (Tyr), citrulline (Cit), ornithine (Orn), arginine (Arg) from DBS specimens were analyzed by liquid chromatography LC-20AD (Shimadzu, Japan) coupled with API 3200 triple-quadrupole tandem mass spectrometer (AB Sciex, USA). Amino acids were analyzed in MRM modes according to the directions for the use of amino acid kit (YingSheng, China).

Statistical analysis

Statistical analysis was performed with SPSS 20.0 software. Measurement data were expressed by mean ± SD. A *t*-test was used to determine statistically significant differences between the four groups. $p < 0.05$ was considered as statistically significant. The correlation between maternal serum 25(OH)-vitamin D_3 level and each amino acid concentration in newborn DBS was analyzed by Spearman's correlation analysis.

RESULTS

Concentrations of maternal serum 25(OH)-vitamin D_3 and amino acids in newborn DBS

The concentrations of maternal serum 25(OH)-vitamin D_3 and amino acids in newborn DBS are shown in Table 2. Data were expressed as mean ± SD. p-values between different groups (P1: 25(OH)-vitamin D_3 severe deficiency group vs. sufficiency group; P2: 25(OH)-vitamin D_3 deficiency group vs. sufficiency group; p3: 25(OH)-vitamin D_3 insufficiency group vs. sufficiency group) were calculated. $p < 0.05$ was required for statistical significance. Compared with maternal serum 25(OH)-vitamin D_3 sufficiency group, Arg in newborn DBS was significantly lower in maternal serum 25(OH)-vitamin D_3 severe deficiency group and deficiency group ($p < 0.05$). The concentrations of Gly in newborn DBS was significantly higher in maternal serum 25(OH)-vitamin D_3 severe deficiency group and deficiency group than those in sufficiency group ($p < 0.05$). The levels of the other 7 amino acids had no obvious difference between children born to mothers with insufficient concentrations of 25(OH)-vitamin D_3 group and sufficiency group

Correlations between maternal serum 25(OH)-vitamin D_3 status and newborns' amino acids levels

The correlation between maternal serum levels of 25(OH)-vitamin D_3 in late pregnancy and each amino acid concentration of their offspring was analyzed by Pearson's correlation analysis. The relationship was presented in Figure 1. Maternal serum 25(OH)-vitamin

Table 1. The baseline characteristics of maternal-infant participants in this study.

Variables	Severe deficiency (n = 25)	Deficiency (n = 252)	Insufficiency (n = 158)	Sufficiency (n = 104)
Mother's age (years)	28.00 ± 4.25	29.11 ± 3.91	29.16 ± 3.94	29.79 ± 4.04
Mother's gestational age (weeks)	32.80 ± 2.00	32.54 ± 2.15	32.18 ± 1.95	32.15 ± 1.78
Maternal 25(OH)-vitamin D_3 concentration (ng/mL)	8.75 ± 0.93	15.00 ± 2.87	24.61 ± 2.78	39.07 ± 7.43
Gender of newborns, female [n (%)]	11/25 (44.00%)	104/252 (41.27%)	78/158 (49.37%)	48/104 (46.15%)
Weight of newborns (g)	3,313.20 ± 280.56	3,309.88 ± 453.74	3,333.45 ± 458.10	3,323.46 ± 375.13

Quantitative and qualitative data were expressed as mean ± SD and proportions, respectively.

D_3 status was significantly positively associated with Arg concentration in their offspring ($p = 0.003$, $r = 0.130$). But the 25(OH)-vitamin D_3 levels were not correlated with Ala, Gly, Val, Met, Phe, Tyr, Cit, or Orn concentrations of the newborns ($p > 0.05$).

DISCUSSION

Vitamin D, which is mainly derived from endogenous synthesis after exposure of the skin to solar ultraviolet radiation, has been proven to have more functions in the body than the classical effects on calcium metabolism [9]. Previous studies reported that vitamin D_3 is widely expressed in human pancreatic β-cells as well as renal cell and numerous cell types of the immune system [10, 11]. The kidney is an important metabolic location for amino acids, which is the direct nutritional and metabolic profile for newborns [12]. However, there were hardly any related reports for the relationship between vitamin D and amino acids. We reported the first study about maternal serum concentrations of 25(OH)-vitamin D_3 in late pregnancy which might affect amino acid metabolism of their newborns. In this report, 25(OH)-vitamin D_3 and 9 amino acids were measured by using HPLC-MS/MS. It showed that Arg levels of the newborns were significantly lower in maternal serum 25(OH)-vitamin D_3 severe deficiency group and deficiency group than those in 25(OH)-vitamin D_3 sufficiency group ($p < 0.05$). Maternal serum 25(OH)-vitamin D_3 status was significantly positively associated with Arg concentration in their offspring ($p < 0.05$). Arginine, a conditionally essential amino acid, is the substrate for the synthesis of nitric oxide (NO), which is a potent vasodilator in systemic, gastrointestinal, and pulmonary circulation [13]. Newborns with necrotizing enterocolitis and persistent pulmonary hypertension have decreased plasma Arg concentrations, and Arg availability may be an important factor in limiting NO formation in this population [14]. Toshie reported that vitamin D receptor ligands have a carboxyl group as an anchor to Arg-274 in the ligand-binding domain. In particular, the 1-hydroxyl group in vitamin D could make hydrogen bonds with Arg-274 [15]. Vitamin D supplementation in hypertensive patients with 25(OH)-vitamin $D_3 < 20$ ng/mL had a significant increase in their Arg level [16]. Claudio showed that Arg and vitamin D_3 combined were able to induce a higher nitric oxide production. The effects on vasodilation induced by cooperative stimulation have been confirmed in an *in vivo* model as well [17]. Arginine and vitamin D might have the potential to enable shorter duration of treatment, reduced infectivity, and improved response in drug-resistant TB [18].

The study also found that the concentrations of Gly in newborn DBS was significantly higher in maternal serum 25(OH)-vitamin D_3 severe deficiency group and deficiency group than those in the sufficiency group ($p < 0.05$). Gly, a nonessential amino acid, exerts broad spectrum of anti-inflammatory, cytoprotective, and immunomodulatory properties [19]. However, there are few articles that focus on the relationship between vitamin D and glycine, and their interaction mechanism is not clear. Chen et al. successfully demonstrated that glycine supplement may restore the reduced serum vitamin D caused by incomplete bile duct ligation of guinea pigs [20]. In this study, Gly concentration in newborn DBS was significantly higher in maternal serum 25(OH)-vitamin D_3 severe deficiency group and deficiency group than that in sufficiency group. Maybe it is a stress response.

CONCLUSION

In conclusion, we observed that the concentration of Arg in newborn DBS was significantly lower and Gly was significantly higher in maternal serum 25(OH)-vitamin D_3 severe deficiency group and deficiency group ($p < 0.05$). The levels of another 7 amino acids showed no obvious differences between children born to mothers in the four groups. Maternal serum 25(OH)-vita-

Table 2. Concentrations of maternal serum 25(OH)-vitamin D_3 and neonates' amino acids in DBS.

Variables	Maternal (ng/mL)	Neonates (μmol /L)								
	Vitamin D	Ala	Gly	Val	Met	Phe	Tyr	Cit	Orn	Arg
Severe deficiency	8.75 ± 0.93	296.45 ± 62.93	497.19 ± 96.58	148.32 ± 19.68	22.47 ± 3.90	13.78 ± 5.46	97.55 ± 38.51	13.78 ± 5.46	128.71 ± 36.57	7.43 ± 4.57
Deficiency	15.00 ± 2.87	289.07 ± 76.28	479.26 ± 163.07	141.57 ± 34.16	22.17 ± 4.83	12.50 ± 3.66	100.74 ± 40.02	12.50 ± 3.66	128.82 + 42.66	9.43 ± 6.38
Insufficiency	24.61 ± 2.78	292.26 ± 74.89	453.97 ± 121.41	148.17 ± 35.20	22.84 ± 5.62	12.99 ± 3.85	99.25 ± 40.19	12.99 ± 3.85	136.94 ± 47.91	9.47 ± 7.40
Sufficiency	39.07 ± 7.43	291.93 ± 75.69	443.56 ± 121.63	143.82 ± 33.66	22.85 ± 5.10	13.03 ± 4.30	98.85 ± 34.10	13.03 ± 4.30	129.28 ± 46.50	11.10 ± 8.25
P1		0.782	0.042	0.522	0.733	0.460	0.868	0.460	0.955	0.035
P2		0.747	0.044	0.571	0.235	0.243	0.672	0.243	0.929	0.042
P3		0972	0.498	0.321	0.993	0.938	0.932	0.938	0.201	0.097

P1 - Severe deficiency group vs. sufficiency group, P2 - Deficiency group vs. sufficiency group, P3 - Insufficiency group vs. sufficiency group.

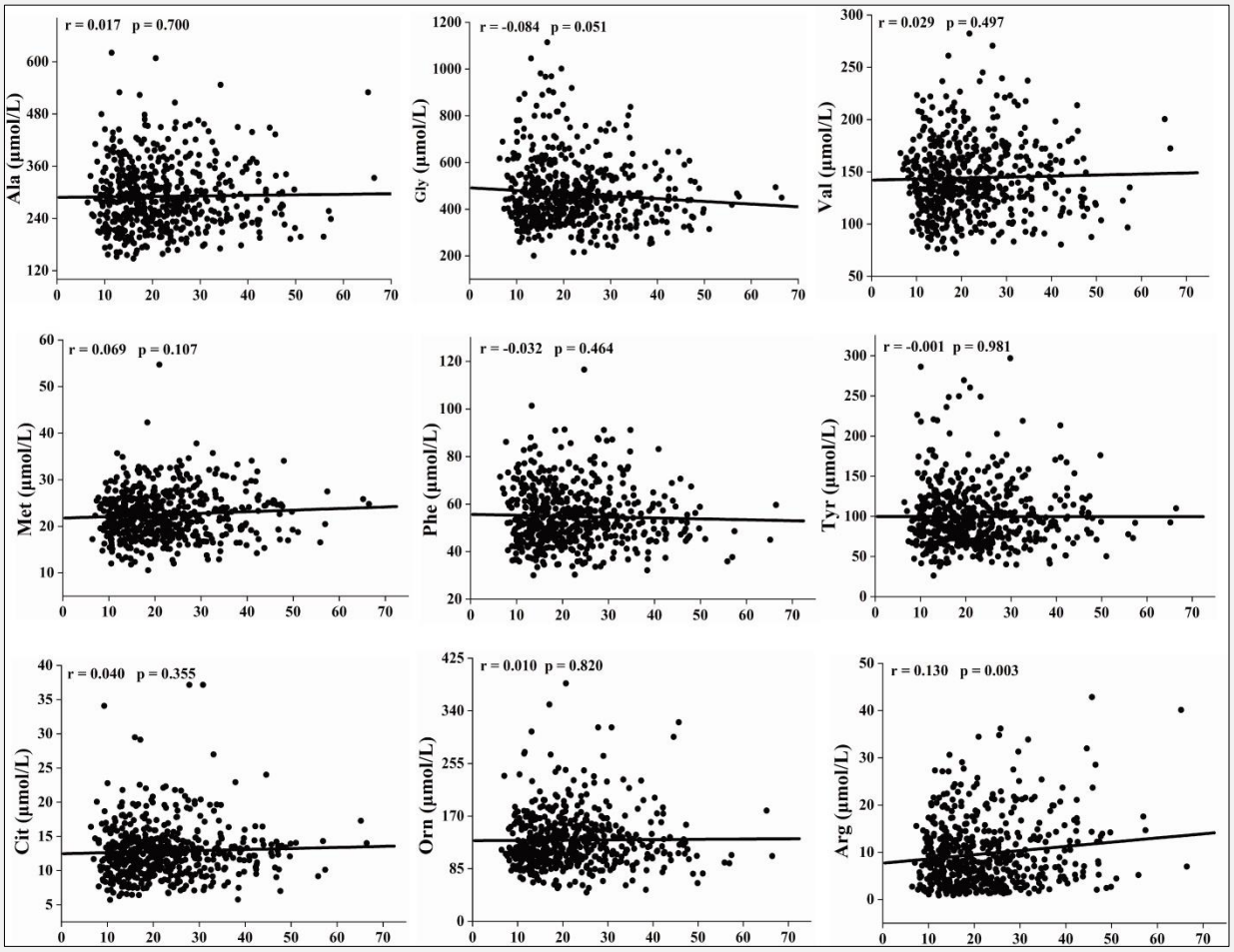

Figure 1. Correlations between maternal serum 25(OH)-vitamin D_3 concentration in late pregnancy and newborn amino acid levels. Linear regression lines are shown. r = Pearson's correlation coefficients.

min D_3 status was significantly, positively associated with Arg concentration in their offspring ($p < 0.05$), but not with Ala, Gly, Val, Met, Phe, Tyr, Cit, or Orn concentrations ($p > 0.05$). The precise mechanisms underlying the relationship need further investigation.

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Declaration of Interest:
The authors declared that they have no conflicts of interest. This study was approved by the ethics committee of the Fourth Hospital of Shijiazhuang.

References:

1. Olmos-Ortiz A, Avila E, Durand-Carbajal M, Diaz L. Regulation of calcitriol biosynthesis and activity: focus on gestational vitamin D deficiency and adverse pregnancy outcomes. Nutrients 2015;7:443-80 (PMID: 25584965).

2. Saraf R, Morton SM, Camargo CA, Grant CC. Global summary of maternal and newborn vitamin D status-a systematic review. Matern Child Nutr 2016;12:647-68 (PMID: 26373311).

3. Andraž D, Mujezinović F. The association of vitamin D levels with common pregnancy complications. Nutrients 2018;10:867 (PMID: 29976852).

4. Sørensen IM, Joner G, Jenum PA, Eskild A, Torjesen PA, Stene LC. Maternal serum levels of 25-Hydroxy-Vitamin D during pregnancy and risk of type 1 diabetes in the offspring, Diabetes 2012;61:175-8 (PMID: 22124461).

5. Javaid MK, Crozier SR, Harvey NC, et al. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. Lancet 2006;367:36-43 (PMID: 16399151).

6. van der Meij BS, Teleni L, Engelen MPKJ, Deutz NEP. Amino acid kinetics and the response to nutrition in patients with cancer. Int J Radiat Biol 2019;95:480-92 (PMID: 29667485).

7. Osborn DA, Schindler T, Jones LJ, Sinn JK, Bolisetty S. Higher versus lower amino acid intake in parenteral nutrition for newborn infants. Cochrane Database Syst Rev 2018;3:CD005949 (PMID: 29505664).

8. Liu Q, Wu J, Shen W, et al. Analysis of amino acids and acyl carnitine profiles in low birth weight, preterm, and small for gestational age neonates. J Matern Fetal Neonatal Med 2017;30:2697-704 (PMID: 27844490).

9. Wang Q, Ma A, Han X, et al. Is low serum 25-hydroxyvitamin D a possible link between pulmonary tuberculosis and type 2 diabetes? Asia Pac J Clin Nutr 2017;26:241-6 (PMID: 28244701).

10. Neelankal John A, Jiang FX. An overview of type 2 diabetes and importance of vitamin D3-vitamin D receptor interaction in pancreatic β-cells, J Diabetes Complications 2018;4:429-43 (PMID: 29422234).

11. Lajdova I, Spustova V, Oksa A, et al. The Impact of Vitamin D3 Supplementation on Mechanisms of Cell Calcium Signaling in Chronic Kidney Disease. BioMed Res Int 2015;2015:807673 (PMID: 26064953).

12. van de Poll MC, Soeters PB, Deutz NE, Fearon KC, Dejong CH. Renal metabolism of amino acids: its role in interorgan amino acid exchange. Am J Clin Nutr 2004;79:185-97 (PMID: 14749 222).

13. Engelen MP, Safar AM, Bartter T, Koeman F, Deutz NE. Reduced arginine availability and nitric oxide synthesis in cancer is related to impaired endogenous arginine synthesis, Clin Sci (Lond) 2016:130:1185-95 (PMID: 27129191).

14. Canpolat FE, Yurdakök M, Yiğit S, Korkmaz A, Tekinalp G. Blood L-Arginine Levels in Early Respiratory Distress Syndrome. Pediatr Pulmonol 2005;40:511-4 (PMID: 16229020).

15. Fujishima T, Tsuji G, Tanaka C, Harayama H. Novel vitamin D receptor ligands having a carboxyl group as an anchor to arginine 274 in the ligand-binding domain, J Steroid Biochem Mol Biol 2010;121:60-2 (PMID: 20435140).

16. Grübler M, Müllner C, Verheyen ND, et al. Effects of oral vitamin D3 on L-arginine to asymmetric dimethylarginine dimethylarginine ratio in arterial hypertension, J Hypertens 2017;35:e280. https://www.researchgate.net/publication/319425314_PP2210_EFFECTS_OF_ORAL_VITAMIN_D3_ON_L-ARGININE_TO_ASYMMETRIC_DIMETHYLARGININE_RATIO_IN_ARTERIAL_HYPERTENSION

17. Molinari C, Morsanuto V, Polli S, Uberti F. Cooperative effects of Q10, vitamin D3 , and L-arginine on cardiac and endothelial cells. J Vasc Res 2018;55:47-60 (PMID: 29301117).

18. Ralph AP, Kelly PM, Anstey NM, L-arginine and vitamin D: novel adjunctive immunotherapies in tuberculosis. Trends Microbiol 2008;16:336-44 (PMID: 18513971).

19. Pérez-Torres I, Zuniga-Munoz AM, Guarner-Lans V. Beneficial effects of the amino acid glycine. Mini Rev Med Chem 2017;17: 15-32 (PMID: 27292783).

20. Chen CY, Wang BT, Wu ZC, et al. Glycine ameliorates liver injury and vitamin D deficiency induced by bile duct ligation. Clin Chim Acta 2013;420:150-4 (PMID: 23103539).