



Association of Serum Vitamin A and D Status with Neuropsychological Development Outcomes in Children Aged 4 to 24 Months: A Retrospective Cross-Sectional Study

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(Received 19 Aug 2024; accepted 20 Nov 2024)

Abstract

Background: We aimed to assess the prevalence of serum Vitamin A and D status and their potential association with neuropsychological development outcomes in Southern China.

Methods: A hospital-based retrospective study was conducted in Guangzhou, China, with 4,206 children aged 4 to 24 months between 2018 and 2020. Data from the hospital's electronic database included serum levels of Vitamin A and D, along with neuropsychological outcomes. Linear regression model was used to assess the association between serum Vitamin A and D status and neuropsychological outcomes, while multiple binary logistic regression model was applied to determine the association of these Vitamins' levels with different neuropsychological developmental delays, adjusting for age, gender, and other potential confounders.

Results: Overall, 12.7% of children were found to be deficient in Vitamin A, while 2.5% were deficient in Vitamin D. Marginal Vitamin A deficiency (MVAD) was prevalent in 58.5% of the children, and 19.4% exhibited Vitamin D insufficiency (VDI). Neuropsychological developmental delays were observed in 7.7% to 16.8% of the children across various domains. However, there were no significant differences in neuropsychological outcomes among children with varying Vitamin A and D statuses (P -value>0.05). Even after adjusting for potential confounders, the association between Vitamin A and D levels and neuropsychological development outcomes remained statistically non-significant.

Conclusion: Our study reveals a higher prevalence of VAD and MVAD in children than VDD and VDI. However, neither Vitamin A nor Vitamin D status showed a significant association with neuropsychological development outcomes in early childhood.

Keywords: Early childhood; Serum vitamin A; Serum vitamin D; Cognitive development; Developmental delays

Introduction

Neuropsychological development includes various milestones that a child is expected to achieve

from infancy to childhood, categorized into gross motor, fine motor, language, adaptive, and per-



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DOI: <https://doi.org/10.18502/ijph.v54i5.18636>

sonal-social behaviors. These milestones are essential indicators of a child's neurodevelopmental progress, helping to identify potential impairments or delays. Early detection allows for timely interventions, which can improve overall developmental outcomes (1). Developmental delay is a common diagnosis when these outcomes are not achieved within the expected age-related time frame (2). Approximately 250 million children, constituting about 43%, fail to reach their full cognitive development, particularly in developing countries (3). China ranks second globally, with 45 million children experiencing cognitive delays (4). According to WHO, the 0-8 yr age is crucial for early childhood development, marked by rapid brain growth and the acquisition of core cognitive and motor skills, which are foundational for later life (5). Nutritional deficiency during this developmental period can significantly impact cognition, behavior, and productivity throughout school years and adulthood. Emphasizing the prevention of nutrients deficiencies in childhood holds the potential for profound and enduring benefits in adulthood and society at large (6).

Micronutrient deficiency among children is a critical concern, particularly in developing countries (7). Vitamins, essential micronutrients vital for proper organismal development, exert a significant influence on initial brain development and play ongoing roles as coenzymes in metabolism (8). Notably, among vitamins, A and D stand out as crucial fat-soluble vitamins essential for the optimal growth and development of children (9). Vitamin A contributes to the regulation of numerous biochemical and physiological processes critical for maintaining normal vision, growth, reproduction, immune competency (10), as well as cell regulation, tissue growth, and differentiation (11). Similarly, vitamin D plays an indispensable role in normal growth and development (12). Functioning as a neuro-steroid, vitamin D dynamically fulfills neuroprotective roles during brain development and is involved in cell multiplication, differentiation, immune modulation, and the regulation of neurotransmission and steroidogenesis (13). Given the multifaceted roles of vitamin A and D, their deficiency has the po-

tential to hinder neuropsychological development in childhood, underscoring the need for comprehensive studies in this domain.

Vitamin A deficiency (VAD) is a significant public health challenge, affecting approximately 250 million children and 19.1 million pregnant women, primarily in developing nations (14). VAD contributes to mortality and morbidity in children under five, compromising resistance to infection and increasing mortality risk (14, 15), while severe cases can lead to permanent blindness, wasting and stunting (16). Positive correlations have been observed between cord blood retinol levels and motor skill development by age two in healthy Chinese mothers and their newborns (17, 18). Similarly, vitamin D deficiency (VDD) is a pervasive micronutrient deficiency and often undiagnosed medical condition globally, with high prevalence among children and adults (19). In South-eastern China, both VDD and vitamin D insufficiency (VDI) have been documented in various age groups (20). Cord blood vitamin D status at birth has been associated with cognitive, language, and behavioral development throughout childhood (21). Adequate vitamin D status during pregnancy has been linked with improved infants' neurodevelopmental outcomes (22), while low maternal vitamin D status in early pregnancy has been associated with neurocognitive delays in early childhood (23).

Vitamin A and D are integral to children's neuropsychological development. However, there is a paucity of studies elucidating the impact of these micronutrients on neuropsychological outcomes in early childhood, particularly in Southern China. Our objective was to assess the prevalence and association of serum Vitamin A and D status with neuropsychological development outcomes, focusing on developmental delays, in children aged 4-24 months in Southern China. The aim was to provide scientific evidence to healthcare professionals for the timely supplementation of Vitamin A and D in the event of deficiencies during early childhood. This study is innovative in its focus on a comprehensive evaluation of both serum Vitamin A and D levels in relation to neuropsychological outcomes within a specific pediat-

ric population, providing valuable insights that would guide public health policy-makers not only in Southern China but also in similar contexts globally.

Materials and Methods

Study design and population

This retrospective study ($n=4206$) was conducted on children aged 4-24 months in the Department of Children's Health Care at Guangdong Women and Children Hospital, Guangzhou, China, between 2018 and 2020.

Data were retrieved from the hospital electronic database, and the study received ethical approval from the Medical Research Ethics Board of Guangdong Women and Children Hospital (No.201801057).

Ethical Consideration

This study was approved by the Medical Research Ethics Board of Guangdong Women and Children Hospital (No.201801057).

Inclusion and exclusion criteria

Children with serum Vitamin A and D levels and neuropsychological development outcomes were included, while children with mental or physical disabilities and unreliable neuropsychological development assessments were excluded.

Assessment of serum Vitamin A and D levels

Serum retinol (vitamin A) levels were determined using an electro-chemical method with LK3000V vitamins' detector, following manufacturer's guidelines (24). Vitamin A status was classified as deficiency ($VA < 0.35 \mu\text{mol/L}$), marginal vitamin A deficiency ($VA 0.35 - 0.7 \mu\text{mol/L}$), and sufficiency ($VA > 0.7 \mu\text{mol/L}$) based on established cut-off values (25, 26). Concurrently, serum Vitamin D [25(OH)D] levels were analyzed through electro-chemiluminescence immuno-assay, employing equipment from Abbot Laboratories, Lake Bluff, IL, USA. Comprehensive details of the laboratory procedures are elaborated in our prior study (27). Similarly, serum Vitamin D lev-

els were categorized as sufficiency ($25(\text{OH})\text{D} > 75 \text{ nmol/L}$), insufficiency ($25(\text{OH})\text{D} 50 - 75 \text{ nmol/L}$), and deficiency ($25(\text{OH})\text{D} < 50 \text{ nmol/L}$) (28).

Assessment of children's neuropsychological development outcomes

Children's neuropsychological development outcomes (NSDO) were assessed using the Children Neuropsychological and Behavior Scale - Revision 2016 (CNBS-R2016), developed by the Capital Institute of Paediatric in China. It encompasses on a general or developmental quotient and five different sub-scales, including Gross Motor, Fine Motor, Language, Adaptive, and Personal-social behaviors. A developmental or sub-scales quotients less than eighty (< 80) points denoting mild delay (< 70 points indicating significant delay), and greater than or equal to eighty (≥ 80) points means no delay (29). The children were stratified into three age groups: (i) 4 to 6 months, (ii) 7 to 12 months, and (iii) 13 to 24 months (30).

Statistical analysis

The data were analyzed using SPSS 25.0 (IBM, Armonk, NY, USA). Continuous variables were presented as mean and standard deviation, while categorical variables were expressed as frequencies and percentages. Chi-square tests were employed to assess differences between categorical variables. Linear regression analysis for continuous variables was conducted to evaluate the association between serum Vitamins A, D levels, and neuropsychological development outcomes. Additionally, to assess the association of Vitamin A and D status with different neuropsychological developmental delays, a binary logistic regression model was employed, presenting adjusted odds ratios with a 95% CI. The independent variables were the serum levels of Vitamin A and D, while the dependent variables were included various neuropsychological development outcome scores. All statistical tests were two-tailed and statistical significance was considered at P -value < 0.05 for all tests.

Results

General descriptions of the study participants

Overall, 4,206 children aged 4-24 months (mean age 9.59 ± 5.001 months; 57.1% boys, 42.9% girls) were participated. Mean serum levels for Vitamin A and D were 0.59 ± 0.22 $\mu\text{mol/L}$ and

94.93 ± 25.62 nmol/L , respectively. Overall, 12.7% and 2.5% of the children were deficient in Vitamin A and D, respectively. The proportion of children delayed in different neuropsychological development domains ranged from 7.7% to 16.8% (Table 1).

Table 1: General characteristics and descriptive statistics ($n = 4,206$)

Variables		<i>n</i>	%	Mean	SD
Age (Months)				9.59	5.001
Age groups	4-6 Months	1,642	39.0		
	7-12 Months	1,620	38.5		
	13-24 Months	944	22.4		
Gender	Boys	2,400	57.1		
	Girls	1,806	42.9		
Serum Vitamin A levels ($\mu\text{mol/L}$)				0.59	0.22
Vitamin A status	Deficiency	535	12.7		
	MVAD	2,462	58.5		
	Sufficiency	1,209	28.7		
Serum Vitamin D levels (nmol/L)				94.93	25.62
Vitamin D status	Deficiency	107	2.5		
	Insufficiency	817	19.4		
	Sufficiency	3,282	78.0		
CNBS-R2016 scores in different neuropsychological domains					
Developmental quotient				92.13	8.69
No delay		3,884	92.3		
Delay		322	7.7		
Gross motor				91.19	14.03
No delay		3,500	83.2		
Delay		706	16.8		
Fine motor				90.87	11.58
No delay		3,579	85.1		
Delay		627	14.9		
Adaptive behavior				93.06	10.84
No delay		3,796	90.3		
Delay		410	9.7		
Language				91.76	12.09
No delay		3,635	86.4		
Delay		571	13.6		
Personal-social behavior				93.79	10.95
No delay		3,817	90.8		
Delay		389	9.2		

Note: MVAD = marginal vitamin A deficiency.

The study found no significant differences (P -value >0.05) in the serum levels of Vitamin A and D in relation to neuropsychological devel-

opment domains (Tables 2, 3). After adjusting for confounding variables, the association between serum Vitamins A, D status, and neuropsychological

logical developmental delays across different CNBS-R2016 domains remained statistically non-significant (P -value >0.05) (Tables 4, 5).

Table 2: Comparison of serum Vitamin A status across different CNBS-R2016 domains

Variables	Vitamin A status ($\mu\text{mol/L}$)						χ^2	P -value
	Deficiency (VA < 0.35 $\mu\text{mol/L}$)		MVAD (VA 0.35–0.7 $\mu\text{mol/L}$)		Sufficiency (VA > 0.7 $\mu\text{mol/L}$)			
	n	%	n	%	n	%		
Developmental quotient								
No delay	493	12.7	2,272	58.5	1,119	28.8	0.118	0.942
Delay	42	13.0	190	59.0	90	28.0		
Gross motor								
No delay	446	12.7	2,035	58.1	1,019	29.1	1.549	0.461
Delay	89	12.6	427	60.5	190	26.9		
Fine motor								
No delay	451	12.6	2,092	58.5	1,036	28.9	0.635	0.728
Delay	84	13.4	370	59.0	173	27.6		
Adaptive behavior								
No delay	483	12.7	2,217	58.4	1,096	28.9	0.338	0.845
Delay	52	12.7	245	59.8	113	27.6		
Language								
No delay	460	12.7	2,119	58.3	1,056	29.1	1.229	0.541
Delay	75	13.1	343	60.1	153	26.8		
Personal-social behavior								
No delay	478	12.5	2,241	58.7	1,098	28.8	1.483	0.476
Delay	57	14.7	221	56.8	111	28.5		

Note: Data are expressed as frequency (n) and percentages (%), MVAD = marginal vitamin A deficiency.

Table 3: Comparison of serum Vitamin D status across different CNBS-R2016 domains

Variables	Vitamin D status (nmol/L)						χ^2	P -value
	Deficiency (25(OH)D < 50 nmol/L)		Insufficiency (25(OH)D 50-75 nmol/L)		Sufficiency (25(OH)D > 75 nmol/L)			
	n	%	n	%	n	%		
Developmental quotient								
No delay	97	2.5	754	19.4	3,033	78.1	0.458	0.795
Delay	10	3.1	63	19.6	249	77.3		
Gross motor								
No delay	85	2.4	691	19.7	2,724	77.8	2.289	0.318
Delay	22	3.1	126	17.8	558	79.0		
Fine motor								
No delay	86	2.4	694	19.4	2,799	78.2	1.986	0.370
Delay	21	3.3	123	19.6	483	77.0		
Adaptive behavior								
No delay	96	2.5	743	19.6	2,957	77.9	0.566	0.753
Delay	11	2.7	74	18.0	325	79.3		
Language								
No delay	92	2.5	697	19.2	2,846	78.3	1.116	0.572
Delay	15	2.6	120	21.0	436	76.4		
Personal-social behavior								
No delay	99	2.6	743	19.5	2,975	77.9	0.479	0.787
Delay	8	2.1	74	19.0	307	78.9		

Note: Data are expressed as frequency (n) and percentages (%).

Table 4: Association between serum Vitamin A status and neuropsychological developmental delays

Vitamin A status ($\mu\text{mol/L}$)							
Variables	Sufficiency	MVAD			Deficiency		
	1.00 (reference)	aOR	95% CI	<i>P</i> value	aOR	95% CI	<i>P</i> -value
DQ	Ref.	1.05	0.81, 1.36	0.716	1.06	0.72, 1.56	0.754
GM	Ref.	1.13	0.93, 1.36	0.224	1.05	0.79, 1.39	0.746
FM	Ref.	1.07	0.88, 1.30	0.508	1.12	0.84, 1.49	0.429
AB	Ref.	1.09	0.86, 1.37	0.490	1.05	0.74, 1.49	0.778
LG	Ref.	1.16	0.94, 1.44	0.162	1.17	0.86, 1.59	0.327
PSB	Ref.	0.99	0.78, 1.27	0.981	1.21	0.86, 1.70	0.276

Note: aOR = adjusted odds ratio; adjusted for age, and gender; AB = adaptive behavior; CI = confidence interval; DQ=developmental quotient; FM=fine motor; GM=gross motor; LG=language; MVAD= marginal vitamin A deficiency; PSB=personal-social behavior.

Table 5: Association between serum Vitamin D status and neuropsychological developmental delays

Vitamin D status (nmol/L)							
Variables	Sufficiency	Insufficiency			Deficiency		
	1.00 (reference)	aOR	95% CI	<i>P</i> value	aOR	95% CI	<i>P</i> -value
DQ	Ref.	1.02	0.76, 1.36	0.883	1.29	0.66, 2.52	0.451
GM	Ref.	0.92	0.74, 1.14	0.429	1.41	0.86, 2.31	0.177
FM	Ref.	1.02	0.82, 1.27	0.837	1.41	0.86, 2.30	0.169
AB	Ref.	0.91	0.69, 1.19	0.491	1.09	0.57, 2.07	0.795
LG	Ref.	1.11	0.88, 1.39	0.380	1.02	0.57, 1.82	0.947
PSB	Ref.	0.93	0.71, 1.23	0.624	0.74	0.35, 1.55	0.427

Note: aOR = adjusted odds ratio; adjusted for age, gender, and seasons of test; AB = adaptive behavior; CI = confidence interval; DQ = developmental quotient; FM = fine motor; GM = gross motor; LG = language; PSB = personal-social behavior

Table 6 shows a non-significant association ($P>0.05$) between neuropsychological develop-

ment outcome scores and serum Vitamin A and D levels.

Table 6: Linear association between different CNBS-R2016 domains and Vitamin A and D levels

Neuropsychological development outcome scores	Vitamin A ($\mu\text{mol/L}$)		
	β	95% CI	<i>P</i> -value
Developmental quotient	0.014	-0.663, 1.745	0.378
Gross motor	0.014	-1.047, 2.843	0.365
Fine motor	0.013	-0.894, 2.317	0.385
Adaptive behavior	0.008	-1.118, 1.887	0.616
Language	0.005	-1.416, 1.936	0.761
Personal-social behavior	0.009	-1.078, 1.957	0.570
	Vitamin D (nmol/L)		
Developmental quotient	0.000	-0.010, 0.010	0.980
Gross motor	-0.014	-0.024, 0.009	0.350
Fine motor	0.006	-0.011, 0.016	0.693
Adaptive behavior	-0.009	-.017, 0.009	0.570
Language	0.011	-0.009, 0.019	0.480
Personal-social behavior	0.010	-0.008, 0.017	0.502

Note: CI = confidence interval.

Discussion

Our findings revealed a prevalence of 12.7% for VAD, and 2.5% for VDD, with MVAD and VDI reported at 58.5% and 19.4%, respectively. Interestingly, we observed a non-significant correlation between the levels of these vitamins and the outcomes of neuropsychological development in this specific population. Despite the absence of statistically significant association, the elevated prevalence of MVAD, emphasizes the imperative for more extensive research efforts in this crucial area.

Micronutrient deficiency particularly Vitamin A and D, remains a critical concern in developing countries (7), which are vital for the healthy growth and development of children (9). We observed a relatively higher prevalence of VAD and MVAD at 12.7% and 58.5%, respectively, aligning with previous findings (15.1%, 14.9% and 64.7%) (25, 26). Conversely, VDD and VDI reported lower prevalence in our study (2.5% and 19.4%), consistent with a previous study reporting 12.1% VDD, and 35.1% VDI among healthy infants and neonates (26, 31). Potential contributors to MVAD may include lower maternal Vitamin A status, inadequate diets during pregnancy, and genetic factors. Conversely, the lower prevalence of VDD and VDI in the sun-exposed Southern region could be attributed to ample sunlight exposure and Vitamin D-rich diets during pregnancy. Additionally, all children are taking 400 IU of Vitamin D daily during their first two years of life, as recommended by the Chinese Medical Association. Despite these findings, further research is warranted to fully understand and address micronutrients deficiencies in this context.

Our study found a non-significant association between serum Vitamin A and D status and developmental delay levels across different neuropsychological outcomes, a departure from some earlier research literature (17, 18, 22). Notably, our results align with a study in India, emphasizing the absence of a significant association be-

tween Vitamin D status and early childhood cognitive development adds an intriguing layer to the discussion (32). Moreover, we observed moderately elevated, yet statistically non-significant, adaptive and personal-social behaviors scores in the insufficient and sufficient Vitamin D groups, findings that align with prior research (33). This nuanced exploration necessitates further investigations to unravel the intricate interplay between vitamins' statuses and developmental outcomes in early childhood. Nevertheless, these outcomes align with a study demonstrating that maternal Vitamin A supplementation, in a Vitamin A-deficient context, positively impacted vision and mortality but did not show long-term effects on cognitive or motor skill development of pre-pubescent offspring (34). Furthermore, our results are consistent with earlier research, indicating that Vitamin D status during early childhood did not correlate with gross motor function in school-aged children (35).

Various investigations examining the correlation between neurodevelopment and cord blood Vitamin D concentrations have yielded mixed results, particularly in early and middle stages of childhood (36). The association between Vitamin D deficiency during pregnancy and subsequent neurodevelopment in early and middle childhood has shown varied outcomes in existing research (37). This nuanced exploration prompts continued scrutiny into the intricate relationship between cord blood Vitamin D concentrations and the unfolding landscape of neurodevelopment in children.

Our study has several notable strengths, including its focus on a critical period of early childhood, a large sample size, and the use of robust statistical methods to explore the association between serum vitamin A and D status and neuropsychological development outcomes. However, we acknowledge that our study had some limitations such as single-center study and lacking data on complementary feeding, micronutrients supplementation, particularly Vitamin A and D and other socio-demographic factors that may bias the results.

Conclusion

We observed a higher prevalence of VAD than VDD in the study population. Moreover, the association between Vitamin A and D status and different neuropsychological developmental levels remained statistically non-significant. Given the relatively higher prevalence Vitamin A deficiency, recommendations for Vitamin A supplementation in early childhood are encouraged to prevent clinical complications. Further studies are suggested to replicate our findings.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

We would like to acknowledge the Guangdong Women and Children Hospital for providing the data.

Financial Support

This work was supported in part by the Guangdong Provincial Key Laboratory of Tropical Disease Research (2017B030314035).

Conflict of interest

The authors declare that there is no conflict of interests.

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