Current Science
CS 6(4), 6533-6543 (2026)
Received 14 July 2026 | **Accepted** 31 July 2026 | **Online Available** 10 August 2026
https://doi.org/10.52845/CS/2026-6-4-10

Current Science

Current Science

OPEN ACCESS

ISSN(O) 2795-8639

Original Article

Correlation Study of Serum Vitamin D with Attention Deficit Hyperactivity Disorder in Children in China

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Abstract:
Background: To investigate serum vitamin D levels in children with attention deficit hyperactivity disorder (ADHD) , and to evaluate the associations of serum vitamin D levels with symptom severity, clinical subtypes and comorbid tic disorder (TD) of ADHD. **Methods**: Serum vitamin D (VD) levels and trace element contents were measured in 264 children with ADHD and 234 healthy children undergoing physical examination. The associations of VD levels with symptom severity, clinical subtypes and comorbid TD were analyzed. All statistical analyses were performed using SPSS 27.0 software. **Results**: 1. Serum VD level and contents of zinc, iron, calcium and magnesium among trace elements in the case group were all lower than those in the control group, and the differences were statistically significant (all $P<0.05$) . Serum VD level of ADHD children with onset in autumn and winter was lower than that of those with onset in spring and summer ($P=0.008$). 2. Serum VD levels in children with moderate and severe ADHD were significantly lower than those in the mild ADHD group and the control group (all $P<0.01$) . 3. Among all clinical subtypes, children with combined type (CT) ADHD had the lowest serum VD level, which was significantly lower than that of the control group ($P<0.001$) . 4. Serum VD level in the ADHD comorbid TD group was significantly lower than that in the pure ADHD group ($P<0.001$) . 5. Serum VD level in ADHD children was negatively correlated with symptom severity ($r=-0.516$; $P<0.001$) , clinical subtype ($r=-0.431$; $P<0.01$) , comorbid TD ($r=-0.373$; $P<0.001$) , inattention score on the SNAP-IV scale ($r=-0.229$; $P=0.008$) , oppositional defiant score ($r=-0.189$; $P=0.03$) and total SNAP-IV score ($r=-0.218$; $P=0.012$) , and was positively correlated with serum calcium level ($r=0.502$; $P<0.001$) . **Conclusion**: Children with ADHD have serum VD deficiency, as well as significantly reduced levels of zinc, iron, calcium and magnesium. Serum VD level is negatively correlated with symptom severity, clinical subtype, comorbid TD, inattention score, oppositional defiant score and total score on the SNAP-IV scale, and positively correlated with serum calcium level in children with ADHD.
Keywords: Attention Deficit Hyperactivity Disorder,Vitamin D,Children

Introduction

Attention deficit hyperactivity disorder (ADHD) , commonly known as hyperactivity disorder, is characterized by core symptoms including inattention, hyperactivity and impulsive behavior. It mostly onsets in preschool age with a relatively high incidence, and is one of the most common chronic neurodevelopmental disorders in childhood. Its prevalence among Chinese children is approximately 6.4%[1]. Clinically, ADHD is classified into three subtypes: Predominantly inattentive type (PIT) , Predominantly hyperactive-impulsive type (HIT) , and Combined type (CT) . Research indicates that ADHD symptoms often persist into adolescence and even adulthood, exerting long-term effects on individuals' cognition and behavior. It adversely affects academic achievement, social function and mental health, and leads to inferiority, anxiety, behavioral problems and other issues, seriously impairing the health and long-term development of children in China[2-5].

At present, clinical intervention for ADHD is mainly based on pharmacological therapy, combined with behavioral therapy and psychological support. Although certain therapeutic effects can be achieved, limitations remain such as restricted applicable population, unstable therapeutic efficacy, and intolerance or obvious adverse reactions in some affected children[6]. Therefore, identifying safe and easily implementable nutrition-related intervention targets has important clinical value.

Children with ADHD generally have serum vitamin D (Vitamin D, VD) deficiency, and insufficient serum VD may be one of the risk factors for the onset of ADHD[7-8]. Based on this background, this study takes the assessment of symptom severity, clinical subtypes and comorbid tic disorders (TD) in children with ADHD as an entry point to investigate the association between serum VD levels and the clinical characteristics of ADHD, so as to provide a basis for nutritional intervention.

Methods

1 Research Objects

A total of 264 children with attention-deficit hyperactivity disorder (ADHD) who first visited the Department of Pediatrics of Bishan hospital of Chongqing medical university，Bishan Hospital of Chongqing between January 1, 2024 and January 1, 2026 were enrolled as the case group. The case group comprised 212 males (80.3%) and 52 females (19.7%) , with an age of (104.4±23.9) months. The control group consisted of 234 healthy children who underwent physical examination at the pediatric outpatient department during the same period, including 192 males (82.1%) and 41 females (17.9%) , with an age of (101.4±32.1) months. Inclusion criteria: (1) aged 3 to 18 years; (2) meeting the diagnostic criteria for ADHD specified in the 5th edition of the "Diagnostic and Statistical Manual of Mental Disorders" (DSM-V)[9]; (3) given that the incidence of ADHD is significantly higher in males than in females, with the number of male pediatric patients more than doubling that of female pediatric patients in childhood[2], subjects were included with a male-to-female ratio close to 4:1; (4) no vitamin D (VD) supplements have been taken in the past three months; (5) the guardians of the subjects were informed of the study protocol, provided consent and signed the written informed consent form. Exclusion criteria: (1) unclear diagnosis; (2) comorbid genetic metabolic diseases or other neuropsychiatric disorders; (3) severe hepatorenal dysfunction, etc.

2 Research Methods

1.1 Assessment Tools: (1) Swanson, Nolan and Pelham Rating Scale Version IV (SNAP-IV): the scale contains 26 items divided into three dimensions: inattention, hyperactivity-impulsivity, and oppositional defiant disorder, with each item scored on a 4-point 0-3 Likert scale. (2) Vanderbilt ADHD Diagnostic Parent Rating Scale (VADPRS) : the scale consists of two sections: behavior and performance. The behavior section includes 47 items covering five factors: inattention, hyperactivity/impulsivity, oppositional behavior, conduct disorder, and anxiety/depression, with each item scored on a 4-point 0-3 Likert scale. The performance section consists of 8 items, with each item scored on a 5-point 1-5 Likert scale. All scales were independently completed by the guardians of children included in the case group.

1.2 Grouping Method: For children with first-diagnosed ADHD, diagnosis and grouping were completed by pediatric neurologists. Basic information of children was collected through outpatient consultation. The severity of symptoms was assessed according to the ADHD diagnostic criteria in DSM-V: children with mild ADHD were assigned to the mild ADHD group (n=112) , children with moderate ADHD to the moderate ADHD group (n=130) , and children with severe ADHD to the severe ADHD group (n=22) , based on clinical symptoms and VADPRS results, children with predominant inattention type (PIT) , hyperactive-impulsive type (HIT) and combined type (CT) of ADHD were assigned to the PIT group (n=132) , HIT group (n=52) and CT group (n=80) respectively; according to the diagnostic criteria for ADHD comorbid tic disorder (TD) in DSM-V, subjects were divided into the ADHD comorbid TD group (n=80) and the pure ADHD group (n=184) . The relationships between VD level and ADHD clinical symptom severity, clinical subtype, and comorbid TD were statistically analyzed.

1.3 Laboratory Tests: Serum VD level and trace elements (zinc, iron, calcium, magnesium, copper) were detected for ADHD children in the case group and healthy children in the control group. (1) VD level detection: 5 mL of fasting peripheral venous blood was collected, stood for 30 minutes, and centrifuged at 3000 rpm at 4℃ for 10 minutes. After separation, serum was stored in a -80 °Crefrigerator until detection. Electrochemiluminescence was used to rapidly determine the level of serum VD (25(OH)D) . Diagnostic criteria: according to the "Expert Consensus on Assessment and Improvement of Vitamin D Nutritional Status", serum 25(OH)D ≤12 μg/L (30 nmol/L) is defined as VD deficiency, 12-20 μg/L (30-50 nmol/L) as VD insufficiency, and >20 μg/L (50 nmol/L) as normal VD[10]. (2) Trace element detection: 2-5 mL of peripheral venous blood was drawn from children in both groups, and atomic absorption spectrometry was used to analyze the contents of iron, zinc, copper, calcium and magnesium.

1.4 Quality Control: (1) Diagnosis was completed by attending pediatric neurologists; (2) All scales including SNAP-IV and VADPRS were independently completed by the children's guardians; (3) After grouping and investigation, data was preliminarily sorted, double-checked by two investigators, and 10% of questionnaires were randomly selected for telephone recheck to ensure the accuracy of the data.

3 Statistical Analysis

SPSS 27.0 software was used for statistical analysis of the data. Measurement data conforming to normal or approximately normal distribution are expressed as mean ± standard deviation ($\bar{x}\pm s$) . Independent samples t-test was used for comparison of means between two groups, and one-way analysis of variance (ANOVA) was used for comparison of means among multiple groups; count data are expressed as percentage (%) , and chi-square (χ^2) test was used for comparisons between two groups and among multiple groups. Spearman correlation analysis was used to analyze the correlation between two variables. $P<0.05$ was considered as statistically significant difference.

4 Ethics Statement

This study was reviewed and approved by the Ethics Committee of Bishan District People's Hospital of Chongqing (approval No. Cqbyky-20260326-22). Informed consent was submitted by all subjects when they were enrolled. There were no statistically significant differences between the case group and the control group in terms of age, gender, body mass index (BMI), enrollment season, family structure, and family history of mental illness ($P>0.05$) .

Results

1 Serum vitamin D levels and trace element analysis in children of the two groups

1.1 Serum vitamin D levels were lower in children with ADHD compared to those in the control group [(25.87±6.27) μg/L vs. (30.11±10.55) μg/L, P=0.018]. The proportion of children with insufficient or deficient serum vitamin D (15.15%, 40/264) was also higher than that in the control group (7.69%, 18/234) , and the difference was statistically significant (t=-2.97, P=0.006) ,as shown in Table 1.

1.2 Comparison of trace elements between ADHD children and controls: Zinc levels were lower in ADHD children compared to controls [(75.21±12.17) μmol/L vs. (78.82±13.52) μmol/L, P=0.002]; iron levels were lower in ADHD children [(7.08±2.50) mmol/L vs. (8.29±0.69) mmol/L, P<0.001]; calcium levels were lower in ADHD children [(1.78±0.65) mmol/L vs. (1.63±0.15) mmol/L, P<0.001]; magnesium levels were lower in ADHD children [(1.53±0.15) mmol/L vs. (1.56±0.13) mmol/L, P=0.006]; however, there was no statistically significant difference in copper levels between ADHD children and controls (P=0.059) ,as shown in Table 1.

1.3 Correlation analysis between vitamin D levels and trace elements in ADHD children: Spearman correlation analysis was performed between serum vitamin D levels and trace elements in the case group. The results showed no significant correlation between vitamin D levels and zinc, iron, magnesium, or copper in ADHD children (P>0.05) , however, a positive correlation was observed between vitamin D levels and calcium (r=0.502; P<0.001) ,as shown in Figure 1.

Table 1 Comparison of serum vitamin D levels and trace elements between two groups of children ($\bar{x}$±s)

Item	Case Group (n=264)	Control Group (n=234)	t value	p value
VD（μg/L）	25.87±6.27	30.1±**10.55**	-5.51	<0.001
Trace Elements				
Zinc (μmol/L)	75.21±12.17	78.82±13.52	-3.121	0.002
Iron (mmol/L)	7.08±2.50	8.29±0.69	-7.193	<0.001
Calcium (mmol/L)	1.78±0.65	1.63±0.15	3.433	<0.001
Magnesium (mmol/L)	1.53±0.15	1.56±0.13	-2.776	0.006
Copper (μmol/L)	15.22±3.82	15.87±3.75	-1.894	0.059

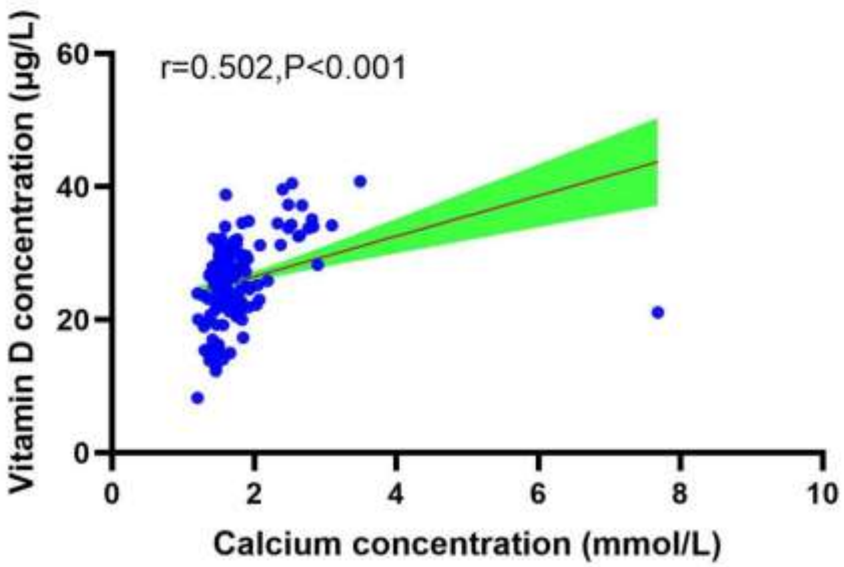

Figure 1.Correlation between vitamin D and calcium levels in children with ADHD (n=264)

1.4 Spring-summer and autumn-winter seasons were divided as March-September and October-February of the following year, respectively. The vitamin D level of ADHD children in the autumn-winter onset group was lower than that in the spring-summer onset group [(26.80±6.51) μg/L vs (24.73±5.79) μg/L, P=0.008], while there was no statistically significant difference in vitamin D level between ADHD children enrolled in autumn-winter and those enrolled in spring-summer (P=0.55) .

2 Analysis of Serum Vitamin D Levels in Children with ADHD of Different Symptom Severities

2.1 Children in the case group were divided into mild, moderate and severe ADHD groups according to the severity of clinical symptoms.

The difference in VD levels among different groups was statistically significant (P<0.01). The VD levels of children with moderate and severe ADHD were significantly lower than those of children with mild ADHD and children in the control group (P<0.01) , while the difference between the mild ADHD group and the control group was not statistically significant (P>0.05) ,as shown in Table 2.

2.2 Correlation analysis between vitamin D level and symptom severity in children with ADHD: Spearman correlation analysis was performed on serum vitamin D level and symptom severity of children in the case group. The results showed that vitamin D level in children with ADHD was negatively correlated with the severity of clinical symptoms (r=-0.516; P<0.001) ,as shown in Figure 2.

Table 2 Comparison of serum vitamin D levels among four groups (`x±s，μg/L)

Group	n	VD	F value	p value
Mild ADHD group	112	28.78±6.08	55.657	<0.001
Moderate ADHD group	130	24.97±4.66		
Severe ADHD group	22	16.4±4.41		
Control group	234	30.11±10.55		

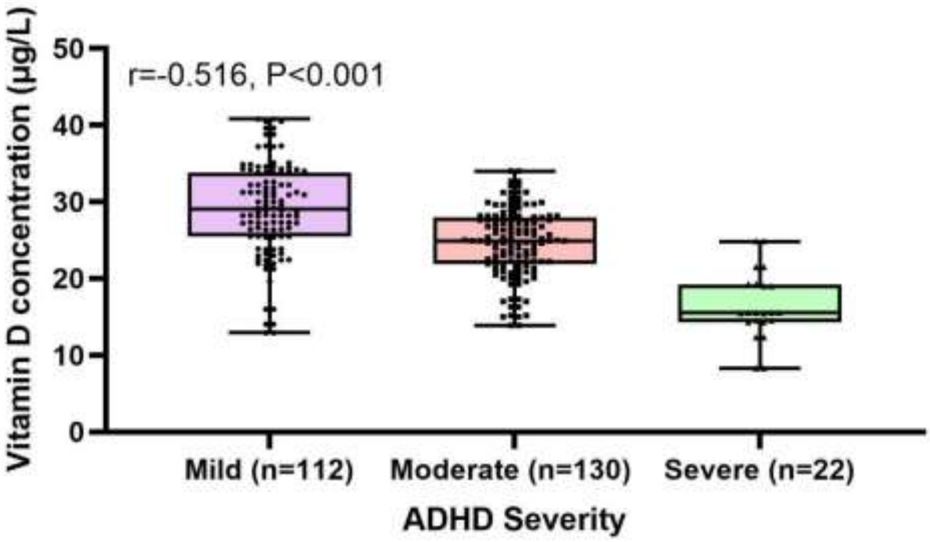

Figure 2.Correlation between vitamin D level and ADHD severity

3 Analysis of Serum Vitamin D Levels in Children with ADHD of Different Clinical Subtypes

3.1 Children in the case group were divided into three subgroups by clinical subtype: the Predominantly Inattentive Type (PIT) group (n=132) , the Hyperactive-Impulsive Type (HIT) group (n=52) , and the Combined Type (CT) group (n=80) . The difference in serum VD levels among children with different ADHD subtypes and the control group was statistically significant

(P<0.001) . Specifically, the CT group had the lowest serum VD level, which was significantly lower than that of the control group (P<0.001) , no statistically significant difference was observed between the PIT group, the HIT group and the control group (P>0.05) , as shown in Table 3. The proportion of children with insufficient or deficient average serum VD in the HIT group and CT group was significantly higher than that in the PIT group and the control group, and the difference was statistically significant (F=7.784; P<0.001) .

3.2 Correlation analysis between VD level and clinical subtypes in children with ADHD: Spearman correlation analysis was performed between serum VD level of children in the case group and different clinical subtypes. The results showed that serum vitamin D level was negatively correlated with clinical subtypes in children with ADHD (r=-0.431; P<0.01) , as shown in Figure 3.

Table 3 Comparison of serum vitamin D levels among four groups($\bar{x}$±s，μg/L)

Group	n	VD	F value	p value
PIT group	132	28.39±5.86	30.795	<0.001
HIT group	52	25.05±5.53		
CT group	80	22.25±5.46		
Control group	234	30.11±10.55		

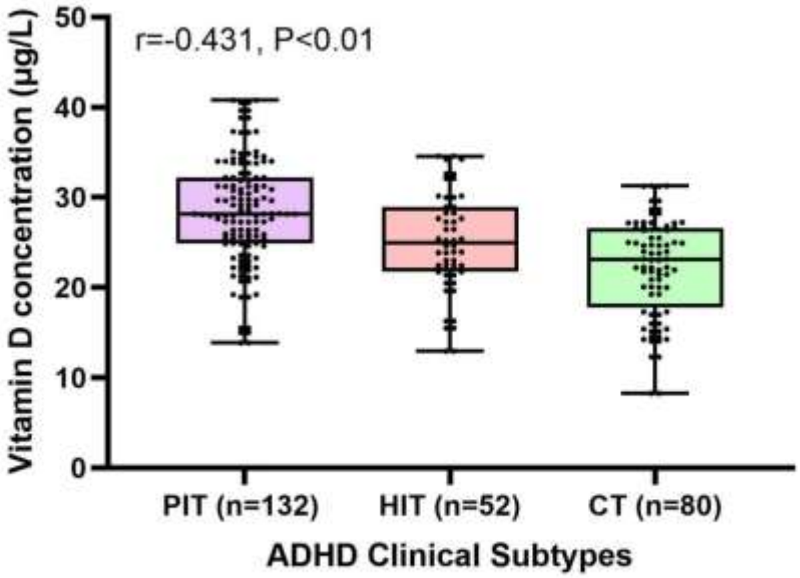

Figure 3.Correlation between vitamin D level and ADHD clinical subtypes

4 Analysis of serum vitamin D levels in children with ADHD comorbid with TD

4.1 Children in the case group were divided into the ADHD comorbid TD group and the pure ADHD group based on the presence or absence of comorbid TD. The serum VD level of the ADHD comorbid TD group was lower than that of the pure ADHD group [(22.33±5.99) μg/L vs (27.41±5.76) μg/L], and the difference was statistically significant (t=-6.508; P<0.001) .

4.2 Correlation analysis between serum VD level and comorbid TD status in children with ADHD: Spearman correlation analysis was performed between serum VD content and comorbid TD status, and the results showed that vitamin D level was negatively correlated with comorbid TD status in children with ADHD (r=-0.373; P<0.001) ,as shown in Figure 4.

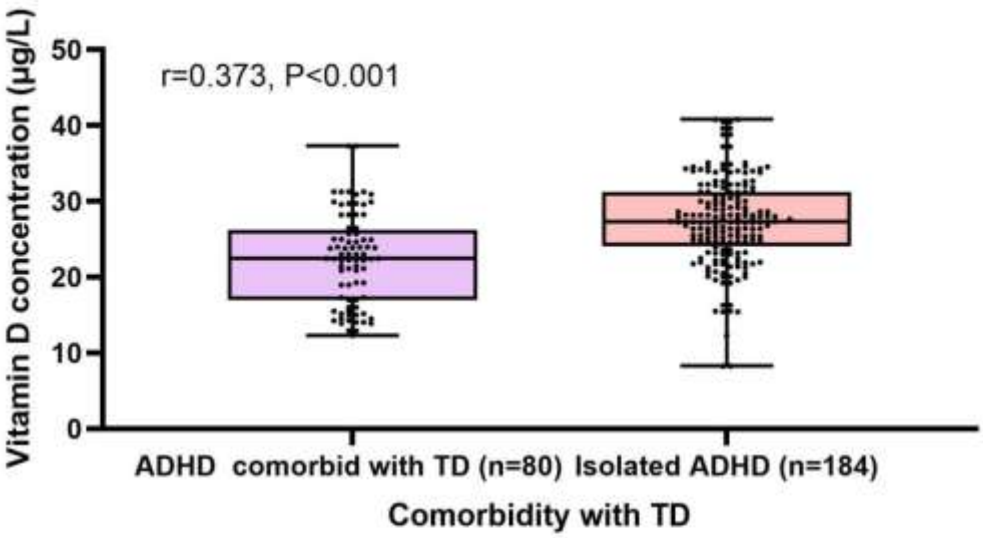

Figure 4.Correlation between vitamin D level and TD comorbidity in ADHD children

5 Correlation analysis between serum vitamin D (VD) level and scores of various assessment scales in children with ADHD:

5.1 Correlation analysis between VD level and SNAP-IV scores in children with ADHD: Spearman correlation analysis was performed on serum VD content and SNAP-IV scores of children in the case group. The results showed that vitamin D level in children with ADHD was negatively correlated with inattention scores (r=-0.229; P=0.008) , showed no significant correlation with hyperactivity-impulsivity scores (P>0.05), was negatively correlated with oppositional defiant scores (r=-0.189; P=0.03) , and was negatively correlated with total scores (r=-0.218; P=0.012) .

5.2 Correlation analysis between VD level and hyperactivity index of CONNERS scale in children with ADHD: Spearman correlation analysis was performed on serum VD content and hyperactivity index of CONNERS scale of children in the case group. The results showed that there was no significant correlation between vitamin D level and hyperactivity index of CONNERS scale in children with ADHD (P>0.05) .

Discussion

The etiology of attention-deficit/hyperactivity disorder (ADHD) is complex and associated with multiple factors, including genetic factors, neurotransmitters, immunity, environment and nutrition. Abnormal neurodevelopment and impaired behavior regulation in children with ADHD result from the combined effect of multiple factors. During the onset and progression of ADHD, nutritional imbalance, as a modifiable factor, plays a critical role. Previous studies have identified that abnormalities in neurotransmitter-related genes such as dopamine, norepinephrine and 5-hydroxytryptamine, fragile X syndrome, 22q11 microdeletion syndrome, tuberous sclerosis and Williams-Beuren syndrome are important genetic basis of ADHD, which shows significant familial aggregation.

The neurobiological mechanism of ADHD is centered on dopamine system dysfunction, and increased oxidative stress and disordered catecholamine metabolism (including dopamine metabolism) are key pathogenic mechanisms in the pathological process of ADHD. Drugs targeting dopamine transporters and dopamine receptor functions effectively alleviate ADHD symptoms by increasing dopamine (DA) concentration, which further verifies the critical role of the DA system in ADHD. In addition, structural and functional abnormalities in cognition-related brain regions, such as reduced prefrontal cortex volume and abnormal basal ganglia function, may also be causally linked to the pathophysiological process of ADHD, leading to cognitive and behavioral regulation disorders in affected children. In terms of pathogenesis, environmental factors including maternal bad habits during pregnancy (e.g., smoking and alcohol consumption) , preterm birth, low birth weight, asphyxia and hypoxia, high psychological stress, and adverse family rearing environment may all increase the risk of ADHD and exacerbate clinical symptoms. Abnormalities in nutritional metabolism may exist in children with ADHD, which further affects the release and balance of neurotransmitters and thereby aggravates the clinical manifestations of ADHD. As a modifiable environmental factor, nutritional status plays an important role in ADHD progression. Specifically, vitamin D, as a key nutrient, the association between its deficiency and ADHD has been validated by a growing number of studies [11].

This study demonstrates that serum vitamin D (VD) levels in children with ADHD are significantly lower than those in healthy children. VD levels in the moderate and severe ADHD groups are also significantly lower than those in the mild ADHD group and the control group, indicating that VD levels decrease as symptoms worsen. This finding is consistent with the results reported by Han et al. [12]. The potential mechanisms underlying this association may be as follows: Low 25-hydroxyvitamin D (25(OH)D) levels tend to attenuate its antioxidant capacity, making the body unable to effectively resist oxidative stress. Since 25(OH)D receptors and active enzymes are widely distributed in the hypothalamus and substantia nigra, 25(OH)D deficiency reduces the expression levels of proteins critical to dopaminergic neurons, leading to dopaminergic neuronal damage and promoting the progression of ADHD. VD levels are significantly reduced in children with comorbid tic disorder (CT) , and show a negative correlation with different clinical types of ADHD. Studies by Li et al. [12-13] found that VD levels in all ADHD subtypes differ significantly from those in the control group, and there are correlations between SNAP-IV scores (including total score, inattention score and hyperactivity-impulsivity score) and VD levels in the hyperactive-impulsive subtype and combined subtype. Regarding comorbidity with TD, serum VD levels in the ADHD with comorbid TD group are lower than those in the pure ADHD group, and VD levels are negatively correlated with comorbid TD. This finding is consistent with the results reported by Wang et al. [14], indicating that lower VD levels correlate with higher risk of comorbid TD in ADHD patients. However, some studies have shown that serum VD levels in TD patients are not associated with comorbid ADHD [15].

Serum VD levels in ADHD children with onset in autumn and winter are lower than those in children with onset in spring and summer, which is mainly attributed to the difference in sun exposure associated with seasons. In Chongqing, shorter daylight duration and reduced ultraviolet intensity in autumn and winter lead to decreased cutaneous vitamin D synthesis in children; meanwhile, reduced outdoor activities and increased clothing coverage further lower vitamin D levels. However, Wang et al. [16] found that 25(OH)D levels in ADHD children in Huizhou area differ significantly across seasons, with the highest level in autumn, followed by summer, winter and spring in descending order. Among trace elements, serum levels of zinc, iron, calcium and magnesium in ADHD children are all lower than those in control children, while no statistically significant difference in copper levels is observed between the two groups, and VD level is positively correlated with calcium level. Deficiencies of vitamin D and trace elements may be involved in the occurrence and development of ADHD by affecting neurodevelopment and neurotransmitter function. Therefore, the balance of vitamin D and trace elements including iron, zinc and magnesium plays a crucial role in the prevention and improvement of ADHD symptoms [17]. In this study, VD levels in ADHD children are negatively correlated with inattention scores, oppositional defiant scores and total scores of the Swanson, Nolan, and Pelham IV Scale (SNAP-IV) , while no significant correlation is observed between VD levels and hyperactivity-impulsivity scores. Li et al. [18]

also found that serum 25(OH)D concentration is negatively correlated with the total SNAP-IV score.

Vitamin D, which is mainly synthesized via cutaneous photochemical reaction, is a class of fat-soluble vitamins and an important neuroactive steroid associated with nervous system development. It not only plays an irreplaceable role in calcium and phosphorus metabolism, bone development and hormone synthesis, but also participates in neuronal differentiation, axonal growth, neurotransmitter release and calcium signal transduction. VD deficiency may exert adverse effects on key developmental processes such as neuronal differentiation and synapse formation in the fetal brain, and is significantly correlated with neurodevelopmental disorders [19-20].

VD has antioxidant effects. VD deficiency tends to disrupt the maturation of dopaminergic neurons and impair the homeostasis of brain development, which may further induce or exacerbate ADHD-related symptoms, whereas vitamin D protects dopaminergic neurons from oxidative stress damage. VD can also regulate nutritional stress factors, help reduce damage to hippocampal neurons, and improve cognitive function. Specifically, VD promotes the expression of Dickkopf-related protein 1 and reduces neurological damage, thereby achieving the effect of improving cognitive function [21].

In conclusion, there may be an association between VD levels and the occurrence and development of ADHD, but the specific mechanism requires further investigation. This study has certain limitations: First, as a cross-sectional study, it can only demonstrate the correlation between VD levels and ADHD symptom severity, clinical types and comorbid tic disorder, and cannot confirm the causal relationship between these variables; second, this study did not comprehensively exclude the influence of confounding factors including children's diet, outdoor activities and sleep. Future studies can conduct large-sample prospective cohort studies to clarify the causal relationship between VD deficiency and the occurrence and development of ADHD; meanwhile, randomized controlled intervention trials should be carried out to investigate the improvement effect of VD supplementation on clinical symptoms of ADHD children, and determine the optimal supplementation dose and treatment course, so as to provide more reliable and solid evidence for the prevention, nutritional intervention and targeted therapy of ADHD.

Funding: None

Acknowledgments: The authors thank Mr. Wang Bo, Department of Pediatrics, Bishan hospital of Chongqing medical university， Bishan Hospital of Chongqing for his technical assistance for this study.

Disclosure: The authors have no potential conflicts of interest to disclose. Other authors have no potential conflicts of interest to disclose.

Author Contributions: (Use CRediT terms)

Conceptualization: Tang TT, Wang B, Han Zhb,Yang HQ. Data curation: Tang TT. Formal analysis: Tang TT. Funding acquisition: Wang B. Investigation: Tang TT. Methodology: Tang TT, Han Zhb. Project administration: Tang TT. Resources: Tang TT. Software: Tang TT. Supervision: Tang TT. Validation: Tang TT. Visualization: Tang TT. Writing – original draft:Tang TT. Writing – review & editing: Tang TT

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