# Correlation between Plasma Vitamin Levels and Core Symptoms in Children with Autism Spectrum Disorders

YUE WANG, LEI WANG AND LILI ZHANG*

Department of Child Health, The Affiliated Wuxi People's Hospital, Wuxi Children's Hospital of Nanjing Medical University, Suzhou, Wuxi 241023, China

**Wang *et al.*: To Investigate the Plasma Levels of Vitamins in Autism Spectrum Disorder Children**

**The aim of the study is to investigate the plasma levels of vitamins in children diagnosed with autism spectrum disorder and explore their relationship with developmental milestones and core symptoms. High-performance liquid chromatography/tandem mass spectrometry was utilized to measure the levels of lipid-soluble and water-soluble vitamins in plasma. The development and behavioral assessment scale was employed to evaluate the developmental progress of children aged 0-6, with data quality values representing their abilities in five domains; gross motor skills, fine motor skills, adaptability, language and social communication. The clinical core symptoms and behavioral traits of children in the autism spectrum disorder group were assessed using the childhood autism rating scale and childhood autism behavior scale. In the autism spectrum disorder group, higher plasma vitamin A levels were found to be positively associated with fine motor skills, adaptability, and social interaction scores (correlation coefficients r=0.517, 0.615 and 0.648; p=0.048, 0.015 and 0.009). Conversely, plasma nicotinamide levels exhibited a negative correlation with fine motor skills and social interaction scores (correlation coefficients r=-0.662, -0.535 and -0.535; p<0.05). Additionally, there was a positive correlation observed between plasma folate levels and overall developmental scores (correlation coefficient r=0.545 and p=0.036). No significant correlations were found between plasma vitamin levels and childhood autism rating scale and childhood autism behavior scale scores in the autism spectrum disorder group (p>0.05). In summary, the plasma levels of vitamin E, vitamin B1, niacinamide, and pyridoxamine hydrochloride were significantly elevated in children with autism spectrum disorder compared to the control group, although all values remained within the normal range.**

**Key words: Autism spectrum disorder, lipophilic vitamins, water-soluble vitamins, endocrine diseases**

The incidence of Autism Spectrum Disorder (ASD) is increasing every year. According to a 2023 study by the centers for disease control and prevention, the frequency of loneliness among 8 y old children in the United States of America is 1 in 36, with a male to female ratio of 3.8:1[1]. The current occurrence rate among children in China is 0.7 %[2]. It is commonly accepted that the origins of ASD are intricate and arise from a mix of genetic elements and environmental influences[3]. Being a significant environmental element, vitamins play a role in controlling the development of the brain during the initial phases of life. Insufficient vitamin intake has an impact on brain development and is a common cause of neurodevelopment-related diseases in children. Children with ASD are at increased risk of inadequate or excessive micronutrient intake due to particular dietary patterns. Based on the understanding of the importance of vitamins, some scholars have carried out studies on routine supplementation or therapeutic intervention with vitamins. According to a Canadian survey, 75 % of children with autism take dietary supplements[4]. In a separate investigation, the administration of vitamin supplements to children diagnosed with autism did not rectify their nutritional deficits. This is because a significant number of children with autism were deficient in just a couple of vitamins, and the excessive intake of vitamins or doses beyond the recommended limits often posed health risks to the body[5]. Studies have shown that plasma vitamin levels in children with ASD are different from those in normal children, but most studies only focus on the observation of a single or several vitamin levels[6,7], and fail to analyze vitamin

***Address for correspondence**
E-mail: zlljxf@126.com

levels in children with ASD through targeted metabolomics methods. In this research, metabolomics techniques were employed to analyze the levels of lipid-soluble and water-soluble vitamins in the blood plasma of children with ASD. The objective was to investigate the patterns of vitamin concentrations in the blood of children with ASD and their relationship with developmental milestones and core symptoms. The study aimed to offer insights into the potential benefits of preventive or therapeutic vitamin supplementation for children with ASD. A total of 15 autistic children (ASD group), with an average age of (3.59±0.78) y old, and 15 children with normal development (Typically Developing (TD) group), with an average age of (4.19±1.08) y old, were enrolled in the Children's Health Care Department of Wuxi People's Hospital Affiliated to Nanjing Medical University from January 2022 to December 2022. Inclusion criteria for ASD group include the boys, aged 2 y-6 y; someone who meets the Diagnostic and Statistical manual of Mental disorders, 5th edition, (DSM-5) diagnostic criteria for ASD. In exclusion criteria, someone who has a history of major organ diseases, endocrine diseases and genetic metabolic diseases; someone who has history of acute respiratory or digestive tract infection within 1 w and someone who supplements or adds vitamin preparations within a week except vitamin D supplement 400 μ/d within the age were excluded from this study. Inclusion criteria of TD group include the growth quotient ≥85, age similar to ASD group and no diagnosis of ASD or family history of ASD. In exclusion criteria, the genetic or neurological disease with a known cause and someone who has history of language impairment. Early morning fasting blood samples of children in ASD group and TD group were collected with EDTA anticoagulant tube. In sampling requirements; children fast for 6 h-8 h, avoid strenuous exercise, and do not take medications or dietary supplements for 72 h. The samples were centrifuged within 1 h (4°, 1000 Revolutions Per Minute (RPM), 10 min) and the plasma was separated and stored in a -80 refrigerator for subsequent metabolic analysis. The levels of lipid-soluble and water-soluble vitamins in children's plasma were determined by High Performance Liquid Chromatography (HPLC)-tandem Mass Spectrometry (MS). Detection instruments including the liquid chromatography waters acquity (Ultra-Performance LC (UPLC)), MS AB SCIEX 5500 Qtrap-(MS). UPLC-QQ-MS include the chromatographic separation conditions; column temperature 40°, flow rate 0.350 ml/min. Mobile phase composition A-water (0.1 % formic acid), B-acetonitrile (0.1 % formic acid). Running time 8 min, sample size 5 μl. MultiQuant software is used for integration and standard curve is used for content calculation. Children's development levels between 0 y and 6 y old were assessed using the developmental and behavioral assessment scale, where the Data Quality (DQ) values stand for developmental quotient and cover five skill categories; gross motor, fine motor, adaptability, language and social communication. The Childhood Autism Rating Scale (CARS) and Autism Behavior Scale (ABC) are employed to evaluate the primary clinical symptoms and behavioral traits of children in the ASD category. The measurement data were tested by Shapiro and Wilk (S-W test) for normal distribution, and the non-normal distribution data were represented by median and interquartile distance (M (P25, P75)). The measurement data between the two groups were compared by Mann-Whitney U test for rank sum. Spearman correlation analysis was used to analyze the correlation between vitamin level and DQ, five energy area scores, CARS and ABC scale scores in ASD group. $p<0.05$ was considered to be statistically significant. Comparison of plasma vitamin levels of children in the two groups vitamin E, vitamin B1, niacinamide and pyridoxamine hydrochloride in the ASD group were significantly higher than those in the TD group ($p<0.05$). However, there were no significant differences in vitamin A, 25-hydroxyvitamin D2, vitamin B2, pantothenic acid, pyridoxal hydrochloride, vitamin B7 and folic acid between the two groups (Table 1). The levels of 25-hydroxyvitamin D3, niacin and vitamin K1 did not reach the lower limit of detection. Spearman correlation analysis showed that plasma vitamin A level in ASD group was positively correlated with fine motor, adaptive ability and social interaction score ($p<0.05$), while plasma nicotinamide level was negatively correlated with fine motor and social interaction score ($p<0.05$). Plasma pyridoxine hydrochloride level was negatively correlated with DQ value, fine motor score and social interaction score ($p<0.05$), plasma folate level was positively correlated with gross motor score ($p<0.05$), while other vitamin levels were not significantly correlated with DQ value and scores of each dimension ($p>0.05$), as shown in Table 2. Spearman correlation analysis showed that there was no significant

correlation between plasma vitamin level and CARS and ABC scale scores in ASD group ($p>0.05$), as shown in Table 3. Studies have indicated that various vitamins play crucial roles in regulating early brain development, influencing neurotransmitter metabolism, and maintaining the function of the nervous system. Given that ASD is a neurodevelopmental condition, the role of vitamins in its onset and progression is significant. This research aimed to compare the vitamin levels in children with autism and TD children, as well as investigate the impact of varying vitamin levels on the developmental progress of children with autism. To achieve this, high-performance liquid chromatography tandem mass spectrometry was utilized to analyze the lipid-soluble and water-soluble vitamin levels in the blood plasma of children in the ASD group and TD group. The study sought to examine the relationship between vitamin levels and developmental milestones, as well as core symptoms, in children with ASD. It was observed that the plasma vitamin E concentrations of children in the ASD group were notably elevated in comparison to those in the TD group. Past investigations have indicated that children diagnosed with ASD intake higher amounts of vitamin E through their dietary habits in contrast to children with typical development[6,7]. Other studies have found that the plasma vitamin E level of children with ASD is not significantly different from that of the control group[8] or significantly lower than that of the control group[9], which is different from the results of this study. Considering that the targeted metabolomics method used in this study is different from the detection method used in the above studies, the results are different. There is insufficient evidence to suggest that vitamin E levels in children with ASD differ from those in TD children. The concentrations of plasma vitamin B1 (thiamine) were found to be notably elevated in children belonging to the ASD group when compared to those in the TD group. Anwar *et al*.[10] also found that plasma and urine thiamine levels were normal in children with ASD, but plasma Thiamine Pyrophosphate (TPP) concentration was reduced. TPP is produced from thiamine *via* TPP kinase, serving as the primary coenzyme form of vitamin B1. Studies have suggested that the reduction in TPP levels in the blood of children diagnosed with ASD could be associated with potential irregularities in the breakdown and uptake of TPP by the microorganisms residing in the gastrointestinal system[10]. The elevated thiamine concentrations observed in children with ASD in this research could be linked to various factors such as the microorganisms in the gastrointestinal tract and metabolic irregularities. Further investigations are required to explore these potential connections in more detail. It was observed that the plasma nicotinamide concentrations among children with ASD were notably elevated compared to those in the TD group. Furthermore, a negative association was identified between the plasma nicotinamide levels in children with ASD and their scores in fine motor skills and social interaction, suggesting an adverse effect of nicotinamide on the developmental progress of children with ASD. The role of niacinamide in nervous system development is complex. It can prolong the neurotoxic effects of methamphetamine in mice[11]; nicotinamide with appropriate concentration has a protective effect on animal cerebral ischemia[12]. High levels of niacinamide can alter cell methylation metabolism and affect Deoxyribonucleic Acid (DNA) and protein methylation[13], adversely affecting the body. In this study, the plasma nicotinamide level in children with ASD was significantly higher than that in the control group but within the reference value range, and the level of nicotinamide in children with ASD was negatively correlated with fine motor scores and social interaction scores, suggesting that the concentration of nicotinamide may have a threshold, and if it is higher than the threshold, it will have a negative effect on children's development. Further research is needed to confirm this theory. The analysis identified the forms of vitamin B6, specifically pyridoxal hydrochloride and pyridoxamine hydrochloride. The findings indicated a marked increase in the plasma concentrations of pyridoxamine hydrochloride in children with ASD compared to those in the TD group. Most previous studies have shown that children with ASD have vitamin B6 deficiency[14-16]. However, the results of one study were consistent with this study. The plasma vitamin B6 level in children with ASD was also found to be significantly higher than that in the control group, but the activity of pyridoxal kinase and the level of Pyridoxal Phosphate (PLP) were also found to be reduced, which the researchers assumed was due to the low conversion of pyridoxamine and pyridoxine to PLP[16]. PLP, together with cysteine beta-synthase, acts as a cofactor for the condensation of serine and homocysteine to form cysteine and further conversion

to cysteine[17]. Lower concentrations of PLP will block the formation of cysteine and lead to the accumulation of toxic homocysteine, which will have an adverse effect on the body. Since the pyridoxal kinase activity and PLP level were not detected in this study, although we found that the level of pyridoxamine hydrochloride in children with ASD was negatively correlated with DQ, fine motor, and social interaction scores, it was not clear that the negative effect was caused by PLP metabolism disorder. This study found that vitamin A level in children with ASD group was positively correlated with fine motor, adaptive ability and social interaction. Similar to this result, Guo *et al*.[18] found that there was no difference between the vitamin A level of children in the ASD group and the control group, and the vitamin A level of children in the ASD group was negatively correlated with CARS score. The results of a multicenter study in China showed that vitamin A levels in male patients with ASD were positively correlated with the total value of their developmental quotient[19], language, motor, and social interaction dimensions. In addition, other studies have shown that vitamin A increases Oxytocin (OXT) levels in patients with ASD through the CD38-OXT signaling pathway, thereby increasing brain activity and social skills in patients with ASD[20,21]. Vitamin A deficiency is not necessarily present in children with ASD, but vitamin A levels in children with ASD may be related to their developmental level or the severity of symptoms. In addition, this study did not find a correlation between vitamin levels and ABC and CARS scores in children with ASD. Due to the heterogeneity of children with ASD and the coordination of physiological interactions of multiple vitamins, it is necessary to carry out studies on vitamin metabolic pathways in ASD population. To further investigate the characteristics of vitamin level in children with ASD and its role in the pathogenesis of ASD. In summary, this study obtained vitaminomic profile of children with ASD through targeted metabolomics methods, which provided a basis for the study of nutritional level of children with ASD. However, there are some limitations in this study. First, this study was a cross-sectional survey conducted in a small sample, so a larger controlled study is warranted to assess plasma vitamin levels in patients with ASD. In addition, since most vitamins require exogenous intake, further analysis is needed in conjunction with dietary surveys.

**TABLE 1: COMPARISON OF PLASMA VITAMIN LEVELS BETWEEN ASD GROUP AND TD GROUP (M (P25, P75), ng/ml)**

| Vitamin | ASD group (n=15) | TD group (n=15) | Z | p |
|---|---|---|---|---|
| Vitamin A | 681.49 (573.81, 811.17) | 589.38 (483.85, 759.30) | -0.933 | 0.878 |
| 25-hydroxyvitamin D2 | 4.51 (3.45, 6.53) | 4.67 (4.25, 5.38) | -0.394 | 0.694 |
| Vitamin E | 4863.67 (2665.18, 6265.29) | 1552.2 (1240.29, 1963.93) | -4.667 | 0.001 |
| Thiamine | 96.61 (59.54, 177.21) | 37.59 (26.22, 71.42) | -3.132 | 0.002 |
| Vitamin B2 | 5.67 (4.50, 8.08) | 6.33 (4.66, 8.10) | -0.311 | 0.756 |
| Niacinamide | 2.47 (2.27, 2.78) | 2.15 (1.76, 2.31) | -2.201 | 0.028 |
| Pantothenic acid | 40.70 (31.61, 50.54) | 42.56 (37.55, 51.84) | -0.29 | 0.772 |
| Pyridoxal hydrochloride | 0.65 (0.35, 0.93) | 0.41 (0.23, 0.71) | -1.328 | 0.184 |
| Pyridoxamine hydrochloride | 69.04 (63.75, 75.33) | 59.72 (54.68, 65.01) | -3.227 | 0.001 |
| Vitamin B7 | 14.14 (9.42, 24.52) | 12.18 (10.13, 47.11) | -0.311 | 0.756 |
| Folic acid | 0.64 (0.11, 0.98) | 0.86 (0.36, 1.06) | -0.871 | 0.384 |

**TABLE 2: CORRELATION BETWEEN VITAMIN LEVEL AND DQ SCORE OF CHILDREN IN ASD GROUP**

| Vitamin | | DQ value | Gross motor | Fine motor | Adaptation | Language | Social interaction |
|---|---|---|---|---|---|---|---|
| Vitamin A | r | 0.455 | -0.034 | 0.517 | 0.615 | 0.400 | 0.648 |
| | p | 0.088 | 0.904 | 0.048 | 0.015 | 0.140 | 0.009 |
| 25-hydroxyvitamin D2 | r | -0.244 | -0.095 | -0.14 | -0.169 | 0.389 | 0.050 |
| | p | 0.381 | 0.737 | 0.620 | 0.548 | 0.152 | 0.859 |
| Vitamin E | r | 0.054 | 0.306 | -0.358 | -0.108 | 0.204 | -0.065 |
| | p | 0.849 | 0.268 | 0.190 | 0.703 | 0.467 | 0.819 |
| Thiamine | r | -0.113 | 0.216 | -0.310 | -0.077 | -0.188 | -0.333 |
| | p | 0.668 | 0.438 | 0.261 | 0.784 | 0.503 | 0.225 |
| Vitamin B2 | r | 0.082 | -0.043 | 0.098 | 0.179 | -0.229 | 0.004 |
| | p | 0.770 | 0.879 | 0.727 | 0.552 | 0.413 | 0.990 |
| Niacinamide | r | -0.418 | -0.050 | -0.662 | -0.325 | -0.292 | -0.535 |
| | p | 0.121 | 0.859 | 0.007 | 0.237 | 0.292 | 0.04 |
| Pantothenic acid | r | 0.158 | 0.243 | 0.147 | 0.493 | -0.221 | 0.158 |
| | p | 0.575 | 0.383 | 0.602 | 0.062 | 0.428 | 0.574 |
| Pyridoxal hydrochloride | r | 0.152 | 0.297 | -0.285 | -0.043 | -0.227 | -0.162 |
| | p | 0.588 | 0.282 | 0.304 | 0.879 | 0.416 | 0.563 |
| Pyridoxamine hydrochloride | r | -0.549 | -0.383 | -0.557 | -0.449 | -0.282 | -0.616 |
| | p | 0.034 | 0.159 | 0.024 | 0.058 | 0.308 | 0.015 |
| Vitamin B7 | r | 0.301 | -0.257 | 0.435 | 0.339 | 0.307 | 0.226 |
| | p | 0.276 | 0.354 | 0.105 | 0.216 | 0.265 | 0.418 |
| Folic acid | r | 0.190 | 0.545 | 0.054 | 0.197 | 0.079 | 0.204 |
| | p | 0.498 | 0.036 | 0.849 | 0.481 | 0.781 | 0.465 |

**TABLE 3: CORRELATION BETWEEN VITAMIN LEVEL AND CARS AND ABC SCALE SCORES OF CHILDREN IN ASD GROUP**

| Vitamin | CARS scale score | | ABC scale score | |
|---|---|---|---|---|
| | r | p | r | p |
| Vitamin A | -0.011 | 0.970 | 0.220 | 0.431 |
| 25-hydroxyvitamin D2 | -0.036 | 0.899 | 0.309 | 0.262 |
| Vitamin E | 0.040 | 0.889 | 0.089 | 0.751 |
| Thiamine | 0.095 | 0.735 | 0.095 | 0.737 |
| Vitamin B2 | 0.248 | 0.372 | 0.352 | 0.198 |
| Niacinamide | 0.243 | 0.382 | -0.124 | 0.659 |
| Pantothenic acid | 0.076 | 0.789 | 0.284 | 0.305 |
| Pyridoxamine hydrochloride | -0.216 | 0.440 | 0.189 | 0.500 |
| Pyridoxal hydrochloride | 0.090 | 0.750 | -0.146 | 0.604 |
| Vitamin B7 | -0.263 | 0.344 | 0.089 | 0.751 |
| Folic acid | -0.165 | 0.556 | -0.009 | 0.975 |

## Conflict of interests:

The authors declared no conflict of interests.

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