

Press-Needle Therapy Combined with Vitamin D for Behavioral and Gastrointestinal Symptoms in Children with Autism Spectrum Disorder: A Clinical Study Postprint

Authors: Jianhui Gu, Jing Yuzhen, Lü Junfeng, Yang Lina, Wei Qinglin, Jia Yongnan, Jia Yongnan

Date: 2025-08-06T11:37:45+00:00

Abstract

Background: Special education and rehabilitation therapy is the primary method for improving core symptoms of autism spectrum disorder (ASD). However, most children with ASD have comorbidities of gastrointestinal dysfunction such as constipation, abdominal pain, and nausea, which seriously affect their physical and mental health. Press-needle therapy can improve gastrointestinal function, and vitamin D (VitD) participates in nervous system development and immune regulation, which are closely related to symptoms and function in children with ASD. The therapeutic efficacy of press-needle combined with VitD for behavioral and gastrointestinal symptoms in children with ASD remains to be studied.

Objective: To investigate the effects of press-needle combined with VitD on behavioral and gastrointestinal symptoms in children with ASD based on special education and rehabilitation therapy.

Methods: A total of 84 children with ASD who underwent rehabilitation training at Gansu Provincial Rehabilitation Center Hospital and the Affiliated Hospital of Gansu University of Chinese Medicine from April 2022 to June 2024 were selected as study subjects. They were randomly divided into two groups (42 cases each in the observation group and control group) using a random number table method, with 40 cases ultimately included in each group after applying exclusion criteria. The control group received special education and rehabilitation therapy, while the observation group received press-needle combined with VitD2 treatment in addition to special education and rehabilitation therapy. Both groups were treated for 3 months. The Autism Behavior Checklist (ABC), Childhood Autism Rating Scale (CARS), Social Responsiveness Scale (SRS),

Traditional Chinese Medicine gastrointestinal symptom scores, and serum 25-hydroxyvitamin D [25-(OH)D] levels were observed and compared before and after treatment to evaluate the efficacy of the two intervention methods.

Results: Compared with before treatment, ABC, CARS, SRS, and Traditional Chinese Medicine gastrointestinal symptom scores in both groups decreased after treatment ($P < 0.05$). After treatment, these scores in the observation group were lower than those in the control group ($P < 0.05$). Compared with before treatment, serum 25-(OH)D levels decreased in the control group but increased in the observation group after treatment ($P < 0.05$). After treatment, serum 25-(OH)D levels in the observation group were higher than those in the control group ($P < 0.05$). The observation group had 36 effective cases (90.0%), while the control group had 30 effective cases (75.0%), with the total effective rate being higher in the observation group than in the control group ($P < 0.05$).

Conclusion: Press-needle combined with VitD demonstrates significant therapeutic efficacy in treating children with ASD, effectively improving their social interaction and behavioral abilities, alleviating gastrointestinal discomfort symptoms, enhancing quality of life, and improving rehabilitation training outcomes.

Full Text

Clinical Study of Press-Needle Combined with Vitamin D on Behavioral and Gastrointestinal Symptoms in Children with Autism Spectrum Disorder

GU Jianhui¹, JING Yuzhen¹, LU Junfeng¹, YANG Lina², WEI Qinglin³, JIA Yongnan^{2*}

¹Department of Children's Rehabilitation, Gansu Province Hospital Rehabilitation Center, Lanzhou 730000, China

²Department of Acupuncture and Moxibustion, North Hospital, Affiliated Hospital of Gansu University of Chinese Medicine, Lanzhou 730000, China

³Acupuncture Center, Affiliated Hospital of Gansu University of Chinese Medicine, Lanzhou 730000, China

Corresponding author: JIA Yongnan, Associate chief physician; E-mail: 124270220@qq.com

Abstract

Background: Special education rehabilitation therapy is the main method for improving the core symptoms of autism spectrum disorder (ASD). However, most children with ASD have comorbid gastrointestinal dysfunction such as constipation, abdominal pain, and nausea, which severely affects their physical and mental health. Press-needle can improve gastrointestinal function, and vitamin

D (VitD) participates in neurological development and immune regulation that is closely related to symptoms and function in children with ASD. The efficacy of press-needle combined with VitD in treating behavioral and gastrointestinal symptoms in children with ASD needs to be studied.

Objective: To investigate the effects of press-needle combined with VitD on behaviors and gastrointestinal symptoms in children with ASD based on special education rehabilitation therapy.

Methods: A total of 84 children with ASD who received rehabilitation training at Gansu Province Hospital Rehabilitation Center and Affiliated Hospital of Gansu University of Chinese Medicine from April 2022 to June 2024 were selected as study subjects. They were randomly divided into two groups (42 cases in the observation group and 42 cases in the control group) using the random number table method. After applying exclusion criteria, 40 cases were included in each group. The control group received special education rehabilitation therapy, while the observation group received press-needle combined with Vitamin D₂ treatment on the basis of special education rehabilitation therapy. Both groups were treated for 3 months. The Autism Behavior Checklist (ABC), Childhood Autism Rating Scale (CARS), Social Responsiveness Scale (SRS), TCM gastrointestinal symptom scores, and serum 25-hydroxyvitamin D [25-(OH)D] levels were observed and compared before and after treatment to evaluate the efficacy of the intervention methods in both groups.

Results: Compared with before treatment, ABC, CARS, SRS, and TCM gastrointestinal symptom scores in both groups of ASD children decreased after treatment ($P < 0.05$). After treatment, these scores in the observation group were lower than those in the control group ($P < 0.05$). Compared with before treatment, serum 25-(OH)D levels in the control group decreased after treatment, while levels in the observation group increased ($P < 0.05$). After treatment, serum 25-(OH)D levels in the observation group were higher than those in the control group ($P < 0.05$). The total effective cases were 36 (90.0%) in the observation group and 30 (75.0%) in the control group. The total effective rate of the observation group was higher than that of the control group ($P < 0.05$).

Conclusion: Press-needle combined with VitD has significant therapeutic effects on children with ASD, effectively improving their social interaction and behavioral abilities, alleviating gastrointestinal discomfort, enhancing quality of life, and improving rehabilitation training outcomes.

Keywords: Autism spectrum disorder; Vitamin D; Press-needle; Child; Behavioral symptoms; Gastrointestinal tract

1. Subjects and Methods

1.1 Study Subjects

A total of 84 children with ASD who received rehabilitation training at Gansu Province Hospital Rehabilitation Center and Affiliated Hospital of Gansu University of Chinese Medicine from April 2022 to June 2024 were selected as study subjects. They were randomly divided into two groups (observation group and control group, 42 cases each) using the random number table method. In the observation group, 1 case was excluded for discontinuing intramuscular VitD₂ injections and 1 case for discontinuing press-needle treatment. In the control group, 2 cases were excluded for not undergoing venous blood collection for 25-(OH)D testing at the scheduled time. Therefore, the actual sample size was 40 cases in each group, totaling 80 cases. This study was approved by the Medical Ethics Committee of Gansu Province Hospital Rehabilitation Center.

Diagnostic criteria: (1) ASD was diagnosed according to the ASD diagnostic criteria in the *Diagnostic and Statistical Manual of Mental Disorders (5th Edition)* [7] (DSM-5). (2) Vitamin D nutritional status was determined according to the following criteria [8]: Vitamin D deficiency, serum 25-(OH)D ≤ 20 ng/mL; Vitamin D insufficiency, serum 25-(OH)D 21–29 ng/mL; Vitamin D sufficiency, serum 25-(OH)D ≥ 30 ng/mL.

Inclusion criteria: (1) Met diagnostic criteria; (2) Age 1–7 years; (3) Had gastrointestinal symptoms (stomach pain, nausea, poor appetite, constipation, insomnia, etc.); (4) Parents signed informed consent forms.

Exclusion criteria: (1) Bleeding tendency; (2) Severe organ damage; (3) Needle fainting; (4) Allergy to press-needle adhesive; (5) Participating in other clinical studies with medication.

Dropout, exclusion, and termination criteria: (1) Withdrew midway and did not complete the treatment protocol; (2) Incomplete data that prevented efficacy evaluation; (3) Developed severe adverse reactions during treatment that prevented continuation.

1.2 Sample Size Calculation

Sample size was estimated using the formula for comparing two independent sample rates from *Medical Statistics* [9]:

$$n_1 = n_2 = 2\pi_c(1 - \pi_c)\pi_1(1 - \pi_1) + \pi_2(1 - \pi_2)\pi_1 - \pi_2$$

where $\alpha=0.05$, $\beta=0.20$, and c is the correction coefficient. Literature review revealed that the effective rate of ASD rehabilitation therapy was approximately 72.73% [10]. Based on clinical observation, the expected effective rate of press-needle combined with VitD for ASD was 90%, with a maximum dropout rate of 20% considered, resulting in 42 cases per group and a total sample size of 84 cases.

1.3 Treatment Methods

1.3.1 Control Group The control group received special education rehabilitation therapy. For ASD children with significant training difficulties or behavioral problems, initial one-on-one personalized intervention or parent-child collaborative teaching models were recommended. After adaptation, children could gradually transition to a combined group and individual training system. Group interventions were conducted in units of 4-5 children through diversified training forms, including: (1) language ability training, (2) cognitive ability training, (3) motor ability training, (4) self-care ability training, and (5) social adaptation ability training—5 sessions/day, 30 min/session.

Language ability training: For nonverbal ASD children, the focus was on pronunciation and oral muscle training; for verbal ASD children, the focus was on language comprehension, guided language expression, and communication skills.

Cognitive ability training: Focused on external characteristics and attributes of objects through matching, classification, and sorting activities to strengthen understanding and differentiation of basic concepts, including color and shape discrimination and number conservation; specialized training for abstract cognitive abilities such as time and space perception, logical reasoning, and comparative analysis; integrated mathematical knowledge learning and reading ability cultivation to help children build a systematic and comprehensive cognitive system and improve comprehensive thinking ability.

Motor ability training: Included gross and fine motor training using equipment such as swings, slides, balance boards, and multivariate combinations to improve sensory integration function, limb coordination, and hand function.

Self-care ability training: Included training in drinking, eating, washing, dressing, toileting, and organizing to improve self-care abilities.

Social adaptation ability training: Included training in active consciousness, communication, and social integration abilities.

Although the above training content had different emphases, various 障碍 problems could not be completely separated, and stereotyped behaviors were continuously corrected during the training process. Parents were advised to conduct life-oriented training at home using daily routines as the main line, record ASD children's performance and abnormal behaviors, summarize weekly, and adjust training plans based on the summary. Training was conducted 5 days/week with 2 days of rest, for 3 months/course.

1.3.2 Observation Group Based on the control group's conventional treatment plan, the observation group additionally received combined press-needle and VitD₂ therapy.

(1) **Press-needle acupoint selection:** Zhongwan (CV12), Tianshu (ST25,

bilateral), Guanyuan (CV4), Zusanli (ST36, bilateral), Xinshu (BL15, bilateral), Ganshu (BL18, bilateral), Pishu (BL20, bilateral), and Shenshu (BL23, bilateral). Acupoint location was based on *Acupuncture Therapeutics* [11]. After routine disinfection of acupoints and selection of appropriate positions for children, 0.2 mm × 0.9 mm disposable sterile press-needles (Sichuan Yuanquan Medical Device Co., Ltd.) were pressed completely into the acupoints, retained for 24 hours, with appropriate pressing during this period to enhance needle sensation. Treatment was administered 5 times/week for 3 months/course. Press-needle procedures were performed by acupuncturists with more than 10 years of experience.

(2) VitD₂ intramuscular injection: For ASD children with 25-(OH)D deficiency or insufficiency, VitD₂ (manufactured by Jiangxi Gannan Haixin Pharmaceutical Co., Ltd., National Drug Approval Number H20054433, 5 mg (200,000 units)/vial) was administered via intramuscular injection at 5 mg/dose (ages 1–2) or 10 mg/dose (ages 2–7), once every 2 weeks until 25-(OH)D levels returned to normal, with a maximum of 12 injections; for ASD children with 30 ng/mL < 25-(OH)D < 50 ng/mL, VitD₂ was administered intramuscularly twice at the same dosage. Treatment duration was 3 months/course.

1.4 Outcome Measures

1.4.1 General data: General information of ASD children meeting inclusion criteria was collected through the electronic medical record HIS system of Gansu Province Hospital Rehabilitation Center and Affiliated Hospital of Gansu University of Chinese Medicine, including gender, age, and disease course.

1.4.2 Autism Behavior Checklist (ABC) score [12]: Contains 57 items covering behavior, communication, language, sensation, and self-care abilities, with a total score of 158 points. Higher total scores indicate more severe behavioral problems.

1.4.3 Childhood Autism Rating Scale (CARS) score [13]: Contains 15 aspects including behavior, language, and emotion, with a total score of 60 points. Higher total scores indicate more severe symptoms.

1.4.4 Social Responsiveness Scale (SRS) score [14]: Contains 65 items mainly covering functional development, with a total score of 195 points. Higher total scores indicate poorer social ability.

1.4.5 TCM gastrointestinal symptom score [15]: Contains 14 items including stomach pain, nausea, poor appetite, constipation, and insomnia, with a total score of 24 points. Higher total scores indicate more severe gastrointestinal symptoms.

1.4.6 Serum 25-(OH)D level: 3 mL of fasting venous blood was drawn in the morning, centrifuged to separate serum, and serum 25-(OH)D levels were detected by high-performance liquid chromatography-tandem mass spectrometry.

statistically significant differences in ABC, CARS, SRS, or TCM gastrointestinal symptom scores between the two groups ($P > 0.05$). After treatment, these scores in the observation group were lower than those in the control group, with statistically significant differences ($P < 0.05$), as shown in Table 2 .

Table 2 Comparison of ABC, CARS, SRS, and TCM gastrointestinal symptom scores between two groups of ASD children before and after treatment ($\bar{x} \pm s$, points)

Group	Cases	ABC score	CARS score	SRS score	TCM gastrointestinal symptom score
Control (Before)	40	95.38 ± 19.83	34.33 ± 3.98	96.03 ± 10.12	6.25 ± 1.43
Control (After)	40	80.58 ± 20.80	31.80 ± 3.81	95.10 ± 19.29	34.03 ± 3.70
Observation (Before)	40	95.10 ± 19.29	34.03 ± 3.70	96.45 ± 10.12	6.18 ± 1.01
Observation (After)	40	80.58 ± 20.80	31.80 ± 3.81	95.10 ± 19.29	34.03 ± 3.70
t (paired)		<0.001	<0.001	<0.001	<0.001
t (between groups, after)		2.2	2.2	2.2	2.2
P (between groups, after)		<0.001	<0.001	<0.001	<0.001

Note: ABC = Autism Behavior Checklist, CARS = Childhood Autism Rating Scale, SRS = Social Responsiveness Scale.

2.3 Serum 25-(OH)D Levels

Compared with before treatment, serum 25-(OH)D levels in the control group decreased after treatment, while levels in the observation group increased, with statistically significant differences ($P < 0.05$). Before treatment, there was no statistically significant difference in serum 25-(OH)D levels between the two groups ($P > 0.05$). After treatment, serum 25-(OH)D levels in the observation group were higher than those in the control group, with a statistically significant difference ($P < 0.05$), as shown in Table 3 .

Table 3 Comparison of serum 25-(OH)D levels between two groups of ASD children before and after treatment ($\bar{x} \pm s$, ng/mL)

Group	Cases	Before treatment	After treatment	t (paired)	P (paired)
Control	40	26.28±7.28	24.42±7.40	16.290	<0.001
Observation	40	26.30±7.19	62.80±13.94	15.6	<0.001
t (between groups)		0.013			
P (between groups)		0.990			<0.001

2.4 Comparison of Clinical Efficacy

The total effective cases were 36 (90.0%) in the observation group and 30 (75.0%) in the control group. The total effective rate of the observation group was higher than that of the control group, with a statistically significant difference ($t=16.290$, $P<0.001$), as shown in Table 4.

Table 4 Comparison of efficacy between the two groups after treatment [cases (%)]

Group	Cases	Markedly effective	Effective	Ineffective	Total effective
Control	40	3 (7.5)	27 (67.5)	10 (25.0)	30 (75.0)
Observation	40	6 (15.0)	30 (75.0)	4 (10.0)	36 (90.0)

3. Discussion

ASD is a complex and severe neurodevelopmental disorder whose pathogenesis remains incompletely understood. Current research suggests that multiple mechanisms are involved, including oxidative stress imbalance, genetic factors, neurotransmitter metabolism disorders, inflammatory responses, and autoimmune abnormalities [17]. The human gut microbiota, as an important endogenous ecosystem, not only plays a key role in nutrient metabolism but also regulates brain development, gene expression patterns, and behavioral development trajectories through the immune system, neuroendocrine system, and enteric nervous system. These microorganisms can promote fermentation and decomposition of dietary fiber, synthesize essential nutrients such as vitamins, and maintain energy homeostasis and immune function balance [18]. Recent research has revealed that the gut and central nervous system are closely connected through a bidirectional “brain-gut axis.” This complex network not only participates in human emotional, cognitive, and behavioral regulation [19] but

also is closely related to the occurrence and development of autism spectrum disorder and functional gastrointestinal diseases through abnormal signal transduction [20]. According to clinical investigations and literature review, most ASD children have comorbid gastrointestinal dysfunction such as constipation, abdominal pain, and nausea [3,21], suggesting that gut microbiota dysbiosis may be one of the important pathological features of ASD and is significantly associated with core disease symptoms [21-22]. Therefore, “brain-gut axis” dysfunction has been included in the research scope of potential pathogenesis of ASD [23]. Gut microbiota can affect the clinical manifestations of ASD children, including social interaction, language communication, executive function, and stereotyped behaviors, through metabolite-mediated, immune regulation, neuroendocrine signal transduction, and vagus nerve pathways [24-25].

This study used press-needle combined with VitD to treat ASD children. The results showed that compared with before treatment, ABC, CARS, SRS, and TCM gastrointestinal symptom scores in both groups decreased after treatment, with observation group scores significantly lower than control group scores, indicating that the observation group could significantly relieve behavioral and gastrointestinal symptoms in ASD children. Compared with before treatment, serum 25-(OH)D levels in the control group decreased after treatment, while levels in the observation group significantly increased, and observation group serum 25-(OH)D levels were significantly higher than control group levels, indicating that the observation group played a role in regulating serum 25-(OH)D levels. The total effective rate of the observation group was 90.00%, higher than the control group’s 75.00%, indicating that press-needle combined with VitD has significant therapeutic efficacy in treating ASD children.

Traditional Chinese medicine does not have the disease name “autism,” but since ancient times, many practitioners have classified ASD under categories such as “delayed speech,” “lack of intelligence,” “fetal weakness,” and “childhood stupor.” Its etiology and pathogenesis are mostly caused by insufficient essence and marrow, with the disease location in the brain and close relationships with the heart, liver, spleen, and kidney [21]. This study used press-needle therapy, which has dual functions of acupuncture and massage. It uses finger pressure on press-needles buried in skin acupoints to achieve persistent and effective stimulation for disease treatment. Because the pain is minimal, it reduces children’s fear and is easily accepted by children. As stated in *Suwen: Yin-Yang Yingxiang Dalun*, “Those who are good at treatment treat the skin and hair.” Press-needle stimulates the skin surface to mobilize the movement of defensive qi. Defensive qi travels outside the vessels, warming the skin, regulating sweat pore opening and closing, and resisting external pathogens. This study selected Zhongwan, Tianshu, Guanyuan, Zusanli, Xinshu, Ganshu, Pishu, and Shenshu for the observation group. Their functions are as follows: Zhongwan and Guanyuan are important acupoints on the Ren meridian. Stimulating Zhongwan can regulate Ren meridian qi and blood, thereby affecting qi and blood movement in all yin meridians. As the mu point of the stomach, it can regulate spleen and stomach function and treat gastrointestinal symptoms. Guanyuan is an important health

preservation point that can cultivate original qi and regulate qi and blood function of the liver, spleen, kidney, and other organs. As the mu point of the small intestine, it regulates gastrointestinal function. Tianshu belongs to the Foot-Yangming Stomach Meridian and is also the mu point of the large intestine. Mu points are where visceral meridian qi converges in the chest and abdomen, so stimulating Tianshu can effectively conduct and regulate gastrointestinal qi movement. Sufficient qi and blood in the body provide important support for brain development and mental regulation. Zusanli is an important acupoint of the Foot-Yangming Stomach Meridian and the lower he-sea point of the stomach. Lower he-sea points are key locations where qi and blood of the six fu organs converge. The classic Chinese medicine statement “for abdominal problems, retain Zusanli” indicates that stimulating Zusanli can fortify the spleen and stomach, harmonize qi and blood, and improve discomfort symptoms. Xinchu, Ganshu, Pishu, and Shenshu are back-shu points that can regulate the function of each organ and harmonize yin and yang.

VitD is an essential nutrient closely related to the function of the central nervous system [5]. Research by L Bishop et al. [26] showed that VitD has immune regulatory functions. When serum VitD < 20 ng/mL, it cannot meet the functional needs of the brain, immune system, and gene regulation. Serum VitD > 38 ng/mL is required to meet neuroimmune functions [24], and experts recommend serum VitD ≥ 40 ng/mL [25]. Studies have shown that neurotransmitter system dysregulation including serotonin (5-HT), oxytocin, and dopamine is associated with ASD [27], playing key roles in neural transmission, brain development and maturation, cortical organization, social behavior, and repetitive behaviors. VitD can facilitate the transcription of tryptophan hydroxylase 2, thereby promoting the synthesis of 5-HT synthetic enzymes. Meanwhile, 5-HT is a monoamine neurotransmitter that plays an important regulatory role in emotions during social decision-making [28]. Increasing research indicates that ASD has obvious inflammatory pathological characteristics. VitD demonstrates unique regulatory effects in this pathological process [29-30]: on one hand, it can reshape the inflammatory-immune balance by downregulating pro-inflammatory cytokine levels and optimize protective immune response mechanisms; on the other hand, VitD supplementation can inhibit abnormal secretion of autoantibodies, promote biosynthesis of the antioxidant substance glutathione in the brain by enhancing γ -glutamyl transpeptidase activity, enhance detoxification metabolism of neural tissue, and thereby reduce oxidative stress damage to brain tissue. VitD can reduce the severity of ASD by utilizing its anti-inflammatory effects, promoting regulatory T cell proliferation, regulating autoimmunity, and enhancing glutathione system activity [23]. Research by Yin Heng et al. [5] showed that VitD treatment for ASD children can improve core symptoms. Gould et al. [31] found that higher VitD levels in cord blood were associated with more ideal early language development in children.

Research by Huang Zhaohua [1] showed that VitD is one of the important nutrients affecting clinical symptoms in ASD children. VitD plays a key role in regulating intestinal health. By modulating intestinal microbiota composi-

tion, repairing intestinal barrier function, and inhibiting local inflammatory responses, VitD can effectively improve the intestinal immune microenvironment and relieve various intestinal discomfort symptoms. Its mechanism is mainly manifested in two aspects: optimizing intestinal mucosal barrier function and regulating the biological behavior of immune cells to reshape the microbiota ecology [22]. In terms of barrier function regulation, the active metabolite 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] can specifically bind to the VitD receptor and maintain the integrity of the intestinal epithelial barrier by regulating the expression and distribution of tight junction proteins [22]. At the immune regulation level, 1,25(OH)₂D₃ can activate various immune cells such as epithelial cells and monocytes, promote the synthesis and secretion of antimicrobial peptides, and enhance the phagocytic activity of macrophages. Additionally, this active ingredient can bidirectionally regulate the adaptive immune process by promoting directional differentiation of immune cells and inhibiting excessive cytokine release and regulating cell proliferation and differentiation processes, thereby maintaining intestinal immune homeostasis [32-34]. Thus, VitD plays an indispensable role in maintaining intestinal barrier function and immune regulation.

In summary, in ASD children with gastrointestinal symptoms, VitD levels are generally deficient, indicating that VitD levels are closely related to ASD and its gastrointestinal symptoms. This study utilized the traditional Chinese medicine theory of “zang-fu meridians”—press-needle combined with Western medicine VitD—to treat behavioral and gastrointestinal symptoms in ASD children, demonstrating the advantages of integrated traditional Chinese and Western medicine. This combined treatment can effectively improve children’s social interaction and behavioral abilities, relieve gastrointestinal discomfort symptoms, and improve rehabilitation training outcomes. However, this study has certain limitations, including a small sample size, and should be expanded in future research. Additionally, follow-up studies should investigate the effects of press-needle combined with VitD on specific individual gastrointestinal symptoms and the severity of ASD to provide a more refined treatment method for ASD children.

Author contributions: GU Jianhui was responsible for study implementation, manuscript writing, and statistical analysis; JING Yuzhen and WEI Qinglin provided technical guidance and supervision; LU Junfeng and YANG Lina were responsible for data collection and collation; JIA Yongnan was responsible for study design and quality control.

Conflict of interest: None declared.

ORCID iDs: GU Jianhui <https://orcid.org/0009-0003-7471-1489>; JIA Yongnan <https://orcid.org/0009-0008-2419-7393>

References

- [1] Huang Zhaohua. Analysis of vitamin D levels in children with autism spectrum disorder and their correlation with clinical symptoms [J]. *Primary Medical Forum*, 2023, 27(19): 61-63. DOI: 10.19435/j.1672-1721.2023.19.020.
- [2] Ministry of Health of the People's Republic of China. Guidelines for diagnosis and treatment of childhood autism (Weiban Yizhengfa [2010] No. 123) [J]. *Chinese Journal of Child Health*, 2011, 19(3): 289-294.
- [3] Identification and management principles of common comorbidities in children with autism spectrum disorder [J]. *Chinese Journal of Pediatrics*, 2018, 56(3): 174-178. DOI: 10.3760/cma.j.issn.0578-1310.2018.03.004.
- [4] Chen Dongmei, Chen Yanxia, Xie Panpan, et al. Clinical study of press-needle treatment for gastrointestinal dysfunction after endoscopic resection of colorectal polyps based on "twelve skin region theory" [J]. *Inner Mongolia Journal of Traditional Chinese Medicine*, 2024, 43(4): 103-105. DOI: 10.16040/j.cnki.cn15-1105.2024.04.033.
- [5] Yin Heng, Xie Jiang. Research progress on vitamin D and autism spectrum disorder [J]. *Journal of Bio-Education*, 2023, 11(1): 70-74.
- [6] Zhang Huifeng, Han Xiao, Wu Shanshan. Significance and cutoff values of serum 25(OH)D levels for assessing vitamin D nutritional status in children [J]. *Chinese Journal of Pediatrics*, 2015(3): 4. DOI: 10.3760/cma.j.issn.0578-1310.2015.03.002.
- [7] American Psychiatric Association. *The Diagnostic and Statistical Manual of Mental Disorders* [M]. 5th ed. Washington, DC: American Psychiatric Publishing Inc, 2013.
- [8] Yang Hui, Yan Siyu, Cao Jinghua, et al. Vitamin D nutritional status among freshmen in a university [J]. *Chinese Journal of School Health*, 2019, 40(1): 119-121. DOI: 10.16835/j.cnki.1000-9817.2019.01.033.
- [9] Li Xiaosong. *Medical Statistics* [M]. 3rd ed. Beijing: Higher Education Press, 2014: 259.
- [10] Feng Xiang, Ge Junyun, Jiang Quanrui, et al. Clinical observation of traditional Chinese massage intervention in children with autism spectrum disorder [J]. *Clinical Journal of Traditional Chinese Medicine*, 2020, 32(2): 358-361. DOI: 10.16448/j.cjtc.2020.0242.
- [11] Gao Shuzhong, Yang Jun. *Acupuncture Therapeutics* [M]. 4th ed. Beijing: China Press of Traditional Chinese Medicine, 2016: 33-35.
- [12] Haem E, Doostfatemah M, Firouzabadi N, et al. A longitudinal item response model for Aberrant Behavior Checklist (ABC) data from children with autism [J]. *J Pharmacokinet Pharmacodyn*, 2020, 47(3): 241-253. DOI: 10.1007/s10928-020-09686-0.

- [13] Yang Yufeng, Du Yasong. Rehabilitation Training Guidance for Children with Autism Spectrum Disorder [M]. Beijing: People's Medical Publishing House, 2020.
- [14] Tian Li, Wang Chen, Song Xiaorong, et al. Efficacy analysis of repetitive transcranial magnetic stimulation for sleep problems in children with autism spectrum disorder [J]. *Journal of Neuropsychiatry and Mental Health*, 2022, 22(1): 40-46. DOI: 10.3969/j.issn.1009-6574.2022.01.008.
- [15] Zhang Shengshun, Liu Fengbin, Hou Zhengkun. Expert consensus on symptom quantification standards for spleen and stomach diseases (2017) [J]. *China Journal of Traditional Chinese Medicine and Pharmacy*, 2017, 32(8): 3590-3596.
- [16] Wang Guan. Intervention study of acupuncture and massage combined with Chinese medicine five elements on rehabilitation of autistic children [J]. *World Journal of Integrated Traditional and Western Medicine*, 2016, (7): 968-971. DOI: 10.13935/j.cnki.sjzx.160721.
- [17] Ren Shuang, Li Chenyang, Qiao Chong, et al. Necessity of routine screening for inherited metabolic diseases in children with autism spectrum disorder [J]. *Chinese Journal of Child Health*, 2019, 27(4): 397-399. DOI: 10.11852/zgetbjzz2018-1367.
- [18] Zhang Chunyan, Zhu Luwen, Tang Qiang. Research progress on the relationship between gut microbiota and autism spectrum disorder [J]. *Chinese Journal of Rehabilitation Theory and Practice*, 2019, 25(3): 319-323. DOI: 10.3969/j.issn.1006-9771.2019.03.013.
- [19] Grenham S, Clarke G, Cryan J F, et al. Brain-gut-microbe communication in health and disease [J]. *Front Physiol*, 2011, 2: 94. DOI: 10.3389/fphys.2011.00094.
- [20] Carabotti M, Scirocco A, Maselli M A, et al. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems [J]. *Ann Gastroenterol*, 2015, 28(2): 203-209.
- [21] Jia Yongnan, Gu Jianhui, Wei Qinglin, et al. Effects of scalp point-through-point acupuncture on emotion and sleep in children with autism spectrum disorder [J]. *Acupuncture Research*, 2021, 46(11): 948-952. DOI: 10.13702/j.1000-0607.20210276.
- [22] Zhang Jing, Wang Kaixuan, Sheng Fang, et al. Effect of vitamin D on serum 25(OH)D level, intestinal symptoms, and intestinal flora dysbiosis in children with autism spectrum disorder [J]. *Maternal and Child Health Care of China*, 2020, 35(23): 4493-4495. DOI: 10.19829/j.zgfybj.issn.1001-4411.2020.23.030.
- [23] Cannell J J. Autism, will vitamin D treat core symptoms? [J]. *Med Hypotheses*, 2013, 81(2): 195-198. DOI: 10.1016/j.mehy.2013.05.004.
- [24] Bischoff-Ferrari H A, Dietrich T, Orav E J, et al. Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both

active and inactive persons aged ≥ 60 y [J]. *Am J Clin Nutr*, 2004, 80(3): 752-758.

[25] Bischoff-Ferrari H A, Giovannucci E, Willett W C, et al. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes [J]. *Am J Clin Nutr*, 2006, 84(1): 18-28. DOI: 10.1093/ajcn/84.1.18.

[26] L Bishop E, Ismailo A, Dimeloe S, et al. Vitamin D and immune regulation: antibacterial, antiviral, anti-inflammatory [J]. *JBMR Plus*, 2021, 5(1): e10405. DOI: 10.1002/jbm4.10405.

[27] Li Kangwei, Yan Ling, Zhang Cai, et al. Effects of hippocampus on depressive behavior and neurotransmitter content in chronic stress model mice [J]. *Journal of Guangdong Ocean University*, 2018, 38(6): 7. DOI: 10.3969/j.issn.1673-9159.2018.06.012.

[28] Liang Xi, Yang Ting, Li Ting, et al. Study on correlation between 25OH vitamin D level detection and Childhood Autism Rating Scale (CARS) score and prediction model [J]. *Modern Progress of Biomedicine*, 2023, 23(1): 98-102. DOI: 10.13241/j.cnki.pmb.2023.01.019.

[29] Guo Min, Li Tingyu. Research progress on dietary behavior and nutrient problems in children with autism spectrum disorder [J]. *Chinese Journal of Child Health*, 2018, 26(6): 634-637. DOI: 10.11852/zgetbjzz2018-26-06-15.

[30] Basheer S, Natarajan A, Van Amelsvoort T, et al. Vitamin D status of children with autism spectrum disorder: case-control study from India [J]. *Asian J Psychiatr*, 2017, 30: 200-201. DOI: 10.1016/j.ajp.2017.10.031.

[31] Gould J F, Anderson A J, Yelland L N, et al. Association of cord blood vitamin D with early childhood growth and neurodevelopment [J]. *J Paediatr Child Health*, 2017, 53(1): 75-83. DOI: 10.1111/jpc.13308.

[32] Singh P, Kumar M, Al Khodor S. Vitamin D deficiency in the Gulf Cooperation Council: exploring the triad of genetic predisposition, the gut microbiome and the immune system [J]. *Front Immunol*, 2019, 10: 1042. DOI: 10.3389/fimmu.2019.01042.

[33] Yamamoto E, Jørgensen T N. Immunological effects of vitamin D and their relations to autoimmunity [J]. *J Autoimmun*, 2019, 100: 7-16. DOI: 10.1016/j.jaut.2019.03.002.

[34] Yamamoto E A, Jørgensen T N. Relationships between vitamin D, gut microbiome, and systemic autoimmunity [J]. *Front Immunol*, 2019, 10: 3141. DOI: 10.3389/fimmu.2019.03141.

(Received: June 9, 2025; Revised: July 12, 2025)

(Editor: Kang Yanhui)

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv — Machine translation. Verify with original.