ONLINE FIRST

ORIGINAL ARTICLE

Comparison of Efficacy and Safety of Vita 6 versus Zinc along with Applied Behavioural Therapy in Management of Autism Spectrum Disorder in Children aged 3-14 Years

Nadeem Iqbal Qaisrani[1*], Erum Afzal[2], Mubina Ifat[3], Masoom Ifat[4], Hafiz Muhammad Ishfaq[5], Maimoona Arshad[6]

[1]*Postgraduate Resident, Department of Developmental and Behaviour Paediatrics, The Children's Hospital, and Institute of Child Health, Multan, Pakistan*
[2]*Associate Professor, Department of Developmental and Behaviour Paediatrics, The Children's Hospital, and Institute of Child Health, Multan, Pakistan*
[3]*Senior Registrar, Department of Developmental and Behaviour Paediatrics, The Children's Hospital, and Institute of Child Health, Multan, Pakistan*
[4]*Women Medical Officer, Department of Paediatrics, DHQ Hospital, South City, Multan, Pakistan*
[5]*Occupational Therapist, Department of Developmental and Behaviour Paediatrics, The Children's Hospital, and Institute of Child Health, Multan, Pakistan*
[6]*Developmental Therapist, Department of Developmental and Behaviour Paediatrics, The Children's Hospital, and Institute of Child Health, Multan, Pakistan*
Correspondence: *nadeemqaisrani88@gmail.com*
doi: 10.22442/jlumhs.2026.01515

ABSTRACT

OBJECTIVE: To compare the efficacy and safety of Vita 6 versus zinc, both administered alongside applied behavioural therapy (ABT) in children aged 3–14 years with autism spectrum disorder (ASD).

METHODOLOGY: This randomized controlled trial was conducted at the Department of Paediatrics, The Children's Hospital and Institute of Child Health, Multan, Pakistan, from October 2025 to March 2026. Children aged 3 to 12 years with newly diagnosed ASD were enrolled and randomly assigned to receive either Vita 6 tablet 50 mg twice daily or zinc sulphate syrup 2 mg/kg/day, along with ABT in both groups, for 12 weeks. Outcomes were assessed using Childhood Autism Rating Scale (CARS) scores at baseline, 6 weeks, and 12 weeks. Data were analyzed in IBM-SPSS Statistics, version 26, with $p<0.05$ as significant.

RESULTS: Among 98 children, the median age was 6.0 (4.0–9.0) years, and 70 (71.4%) were boys. The median symptom duration was 17.5 (12.0–25.0) months, and the median baseline CARS score was 37.5 (34.0–41.0). At 6 weeks, median CARS score was 34.0 (31.0–38.0) in the Vita 6 group and 35.0 (32.0–40.0) in the zinc group (p=0.173). At 12 weeks, median CARS scores were 30.0 (27.0–35.0) in the Vita 6 group and 33.0 (30.0–38.0) in the zinc group (p=0.028). Adverse effects occurred in 7 (14.3%) children in the Vita 6 group and 12 (24.5%) children in the zinc group (p=0.194).

CONCLUSION: Adjunctive Vita 6 given alongside ABT was associated with greater reduction in autism severity than zinc sulphate with ABT, while both interventions showed acceptable tolerability.

KEYWORDS: Autism spectrum disorder, behaviour therapy, children, safety, zinc.

Trial Registration: NCT07383363 (clinicaltrials.gov)

INTRODUCTION

Autism spectrum disorder (ASD) typically manifests in early childhood and significantly affects language, cognition, adaptive functioning, and social integration, often requiring long-term multidisciplinary care[1,2]. WHO estimates that approximately 1 in 100 children worldwide has ASD. In comparison, recent surveillance data from the United States report a prevalence as high as 1 in 31 children, with a male predominance of nearly 3–4:1. [3,4] In low- and middle-income countries, the burden generally remains under-recognized due to delayed diagnosis, stigma, and limited access to specialized care, as many children with ASD present late with significant functional impairment, highlighting the need for effective and accessible therapeutic strategies[5].
Applied Behavioural Therapy (ABT) remains the cornerstone of ASD management and has been shown to improve communication, adaptive skills, and behavioural outcomes[6]. However, treatment response varies, and adjunctive interventions are frequently explored to enhance outcomes. Nutritional and micronutrient-based therapies have gained increasing attention due to the high prevalence of selective eating and nutritional deficiencies in children with ASD. Zinc plays an important role in synaptic function, neurotransmission, and neurodevelopment, while vitamin-based therapies such as Vita 6 (including vitamin B6) are involved in neurotransmitter synthesis and brain metabolism[7]. A randomized controlled trial evaluating a comprehensive vitamin and mineral supplementation regimen demonstrated significant improvement in non-verbal IQ and autism-related symptoms compared to controls ($p=0.009$)[8]. A randomized controlled trials of other biologically active compounds such as sulforaphane have demonstrated improvements in behavioral measures in children with ASD[9].
Whatever evidence remains, it is heterogeneous with limited direct comparisons between different micronutrient interventions and insufficient data from low-resource settings about the effectiveness of various strategies in ASD. Safety profiles and tolerability also require evaluation, particularly in pediatric populations receiving prolonged supplementation. Given the increasing burden of ASD, variability in response to ABT alone and the lack of locally generated comparative evidence, it is important to evaluate adjunctive treatment strategies in real-world settings. Therefore, this study aimed to compare the efficacy and safety of Vita 6 versus zinc, both administered alongside ABT in children aged 3–14 years with ASD.

METHODOLOGY

This randomized controlled trial (NCT07383363 at clinicaltrials.gov) was conducted at the Department of Paediatrics, The Children's Hospital and Institute of Child Health, Multan, Pakistan, from October 2025 to March 2026, after obtaining approval from the institutional ethical review committee (No. 1895, dated: 22-09-2025). Children aged 3-12 years with newly diagnosed ASD were enrolled. Only newly diagnosed patients who had not received any prior pharmacological, nutritional, or behavioural treatment for ASD were included. Children with coexisting psychiatric disorders, movement disorders, or those already receiving treatment for ASD were excluded. The diagnosis of ASD was established by a pediatric neurologist, developmental paediatrician, or child psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria. A sample size of 98 (49 in each group) was calculated using the Open Epi online calculator to detect a 20% difference, with a 95% significance level and 80% power of the study.
Children were recruited using non-probability consecutive sampling. Baseline data included age, gender, weight, and baseline severity of ASD. Randomization was performed using a simple

randomization technique based on a computer-generated random sequence. Allocation concealment was ensured using sealed opaque envelopes that were opened at the time of assignment. Due to differences in the formulation of the interventions, blinding was not feasible. Participants in the Vita 6 + ABT group received tablet Vita 6 at a dose of 50 mg twice daily, while participants in the Zinc + ABT group received zinc sulphate syrup at a dose of 2 mg/kg/day. In addition to the assigned intervention, all children in both groups received ABT according to the standard protocol.

The severity of ASD and treatment response were assessed using the Childhood Autism Rating Scale (CARS), a validated and clinically feasible tool widely used in children with ASD. Each child was assessed at baseline before initiation of therapy, at 6 weeks, and again at 12 weeks after treatment initiation. The primary outcome was improvement in autism severity, measured as the change in total CARS score over time within and between the two groups. Response to treatment was evaluated by comparing baseline CARS scores with those recorded at 6 weeks and 12 weeks. Clinical improvement was defined as a reduction of at least 5 points in CARS score from baseline. Safety was assessed by monitoring adverse events during the study period. In the Vita 6 group, adverse effects included hypersensitivity reactions and sensory neuropathy, while in the zinc sulphate group, adverse effects included hypersensitivity, vomiting, and diarrhea. Caregivers were instructed to report any adverse events, and these were documented at follow-up visits. Compliance with treatment was assessed through caregiver reporting at each visit. All participants were followed throughout the study period, and any losses to follow-up or discontinuations were recorded with reasons. The flow of participants, including enrollment, allocation, follow-up, and analysis, was documented according to CONSORT guidelines. (**Figure 1**).

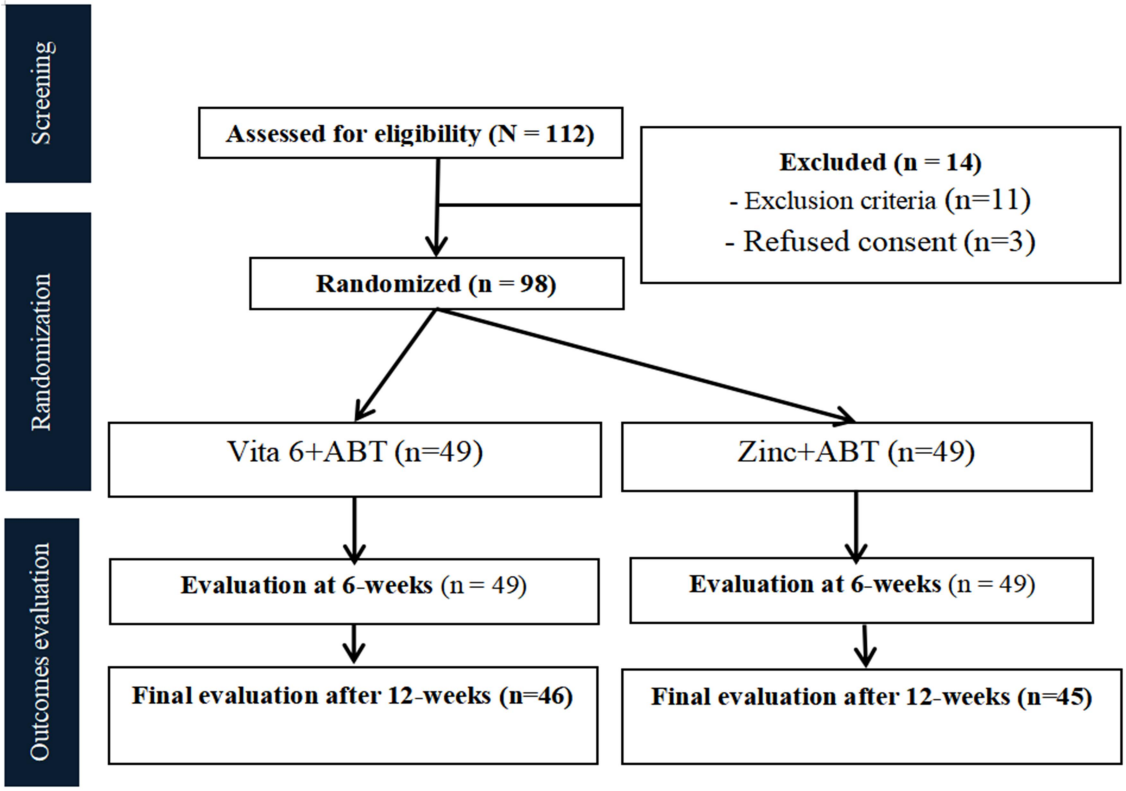

Figure 1: Study flow diagram

Data were entered and analyzed using IBM SPSS Statistics version 26. Quantitative variables were expressed as mean and standard deviation (SD), or median and interquartile range (IQR) depending on data distribution, while categorical variables were presented as frequencies and percentages. For comparison of continuous variables, an independent samples t-test or Mann–Whitney U test was applied. Categorical outcomes were compared between groups using the chi-square test or Fisher's exact test where appropriate. Effect estimates were reported with 95% confidence intervals (CI). A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 112 children were assessed for eligibility; of these, 98 fulfilled the inclusion criteria and were randomized, with 49 children allocated to the Vita 6 plus ABT group and 49 to the zinc sulphate plus ABT group (**Figure 1**). A total of 98 children were enrolled; the median age was 6.0 years (4.0–9.0), and 70 (71.4%) were boys. The median weight was 20.5 kg (16.0–25.0), and the median duration of symptoms was 17.5(12.0–25.0) months. The median baseline CARS score was 37.5 (34.0–41.0). Baseline characteristics were comparable between the Vita 6 and zinc groups, with no statistically significant differences across age (p=0.438), gender distribution (p=0.648), weight (p=0.561), duration of symptoms (p=0.671), or baseline CARS score (p=0.694), as detailed in **Table I**.

Table I: Baseline characteristics of study participants (N=98)

Characteristics		Vita 6 + ABT (n=49)	Zinc + ABT (n=49)	P-value
Age (years), median (IQR)		6.0 (4.0–8.0)	7.0 (4.0–9.0)	0.438
Male gender		34 (69.4%)	36 (73.5%)	0.648
Weight (kg)		20.0 (16.0–25.0)	21.0 (16.0–26.0)	0.561
Duration of symptoms (months), median (IQR)		17.0 (11.0–25.0)	18.0 (12.0–26.0)	0.671
ASD severity	Mild to moderate	31 (63.3%)	30 (61.2%)	0.832
	Severe	18 (36.7%)	19 (38.8%)	
Baseline CARS score, median (IQR)		37.0 (34.0-41.0)	38.0 (34.0-42.0)	0.694

At 6 weeks, the median CARS score decreased to 34.0(31.0–38.0) in the Vita 6 group, and 35.0(32.0–40.0) in the zinc group (p=0.173). By 12 weeks, the median CARS score was 30.0 (27.0–35.0) in the Vita 6 group and 33.0 (30.0–38.0) in the zinc group (p=0.028). The median reduction in CARS score from baseline to 12 weeks was greater in the Vita 6 group than in the zinc group, at 7.0 (4.0–10.0) points versus 4.0 (2.0–7.0) points, respectively (p=0.011). At 6 weeks, clinical improvement was observed in 19 (38.8%) children in the Vita 6 group and 14(28.6%) in the zinc group (p=0.279). At 12 weeks, 30 (65.2%) children in the Vita 6 group achieved clinical improvement, compared with 19 (42.2%) in the zinc group (p=0.032). **Table II** and **Figure 2** show details of efficacy outcomes during the study period between groups.

Table II: Comparison of efficacy outcomes at 6 and 12 weeks

Outcomes	Vita 6 + ABT	Zinc + ABT	P-value
CARS score at 6 weeks, mean ± SD	34.0 (31.0–38.0)	35.0 (32.0–40.0)	0.173
CARS score at 12 weeks, mean ± SD	30.0 (27.0–35.0)	33.0 (30.0–38.0)	0.028
Mean reduction in CARS score at 12 weeks	7.0 (4.0–10.0)	4.0 (2.0–7.0)	0.011
Clinical improvement at 6 weeks	19/49 (38.8%)	14/49 (28.6%)	0.279
Clinical improvement at 12 weeks	30/46 (65.2%)	19/45 (42.2%)	0.032
Severe ASD at 12 weeks	6/46 (13.0%)	10/45 (22.2%)	0.250

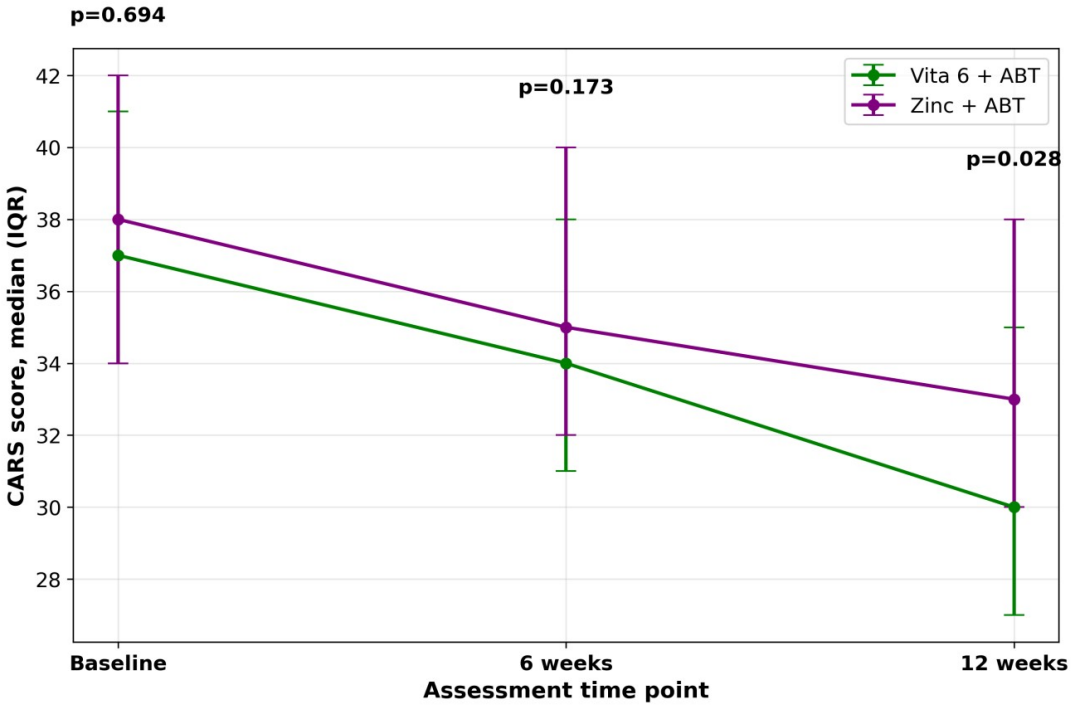

Figure 2: Comparison of median CARS scores during study period between study groups

Overall, both interventions were generally well tolerated, and adverse effects were reported in 7 (14.3%) children in the Vita 6 group and 12 (24.5%) in the zinc group (p=0.194). In the Vita 6 group, hypersensitivity reaction was noted in 2(4.1%) children and symptoms suggestive of sensory neuropathy in 2(4.1%). In the zinc group, vomiting was reported in 6(12.2%) children, diarrhea in 4(8.2%), and hypersensitivity in 2(4.1%). No serious adverse events or treatment discontinuation due to toxicity were observed (**Table III**).

Table III: Adverse effects and treatment compliance

Safety and compliance variables	Vita 6 + ABT (n=32)	Zinc + ABT (n=32)	p-value
Any adverse effect	7 (14.3%)	12 (24.5%)	0.194
Hypersensitivity	2 (4.1%)	2 (4.1%)	1.000
Sensory neuropathy symptoms	2 (4.1%)	-	0.153
Good compliance	41 (83.7%)	39 (79.6%)	0.596

DISCUSSION

Direct randomized comparisons between different micronutrient strategies in children with ASD remain scarce in recent literature, particularly in settings where behavioural therapy is delivered concurrently. This limits the ability to determine the relative effectiveness of commonly used adjunctive interventions. This study revealed that both Vita 6 with ABT and Zinc with ABT were effective; children receiving Vita 6 had a greater reduction in CARS scores by 12 weeks and a higher proportion achieving clinical improvement. Both interventions were generally well tolerated, though GI complaints were relatively more frequent in the zinc group and no serious adverse event or treatment discontinuation occurred in any of the patients. A study from China found that an ABA-based program delivered in eight one-hour sessions significantly improved social, communication, and daily life skills ($p<0.05$) [10]. Pooled evidence of ABA-based interventions identified 25 studies and concluded that such interventions improved communication, adaptive skills, and cognitive outcomes while also reducing challenging characteristics [11].

The greater reduction in CARS score in the Vita 6 group compared with the zinc group is in line with growing interest in broad micronutrient strategies rather than single nutrient replacement alone. A study evaluating vitamin-mineral micronutrient formulation in 161 participants with ASD reported that 73% of respondents rated overall benefit as moderate, good, or great, with a low adverse effect score of 0.25 out of 3.0 [12]. Multi-nutrient approaches may produce broader symptomatic benefit than narrower supplementation. Most of the benefits from comprehensive nutritional treatment could start appearing in the first few months of treatment, which closely matches the 12-week window of the present trial[13].

In this trial, 65.2% of children in the Vita 6 group achieved clinical improvement compared with 42.2% in the zinc group. This difference is clinically meaningful, considering that families often seek practical gains in communication, interaction, and behaviour over relatively short follow-up periods[14]. In 2023, it was estimated that the evidence base for early childhood autism interventions has approximately doubled in the previous four years[15]. That review also warned that adverse events were poorly monitored in many trials. The present study therefore contributes not only efficacy data but also structured tolerability reporting, which improves its clinical relevance.

Adverse effects were reported in 14.3% of the Vita 6 group and 24.5% of the zinc group, with vomiting and diarrhea concentrated in the zinc arm, and only two children in the Vita 6 arm showing symptoms suggestive of sensory neuropathy. No child required discontinuation, and this pattern is broadly similar to a micronutrient survey in which the overall adverse effect score was low and far lower than ratings reported for psychiatric and seizure medicines[12]. The present findings also parallel another trial in which compliance exceeded 90%, and no major safety signal dominated the report[16]. The higher frequency of GI complaints with zinc in the present trial is clinically anticipated as zinc formulations are commonly associated with nausea, abdominal discomfort, and loose stools in pediatric practice[17,18]. Vita 6 may be easier for some families to continue, especially when behavioural therapy schedules and travel burden already challenge adherence[19,20].

Children with ASD often have restricted food choices and multiple nutritional gaps rather than isolated deficiency in a single trace element[21]. A broader supplement may therefore address a wider nutritional profile than zinc alone. Every child in this study received ABT, so the observed difference likely reflects an added effect over a behavioural baseline rather than a stand-alone drug response. The present study included newly diagnosed and treatment-naive children aged 3 to 12 years, which may have reduced some confounding from prior therapies and made early change easier to detect.

This study has several limitations. The trial was conducted at a single tertiary care hospital, which limits generalizability to community settings and to populations with different access to therapy. The sample size was adequate for the primary comparison but still modest for subgroup analyses. The study was open-label because the formulations differed, which may have influenced caregiver expectations and reporting. Nutrient levels were not measured before or after treatment, so it was not possible to identify which children were truly deficient or whether biochemical correction tracked clinical change. Follow-up ended at 12 weeks, so persistence of benefit beyond the short-term remains uncertain. Longer follow-up with repeated CARS assessments and functional measures such as language and adaptive behaviour would strengthen the evidence base.

CONCLUSION

Adjunctive Vita 6 given alongside ABT was associated with greater reduction in autism severity than zinc sulphate with ABT, while both interventions showed acceptable tolerability. These findings support the need for larger multicenter trials in low and middle-income settings to clarify which children benefit most from targeted nutritional support and how these treatments can be integrated into routine autism care.

Acknowledgments: The authors would like to thank Muhammad Aamir Latif (RESnTEC) for his valuable assistance in the trial registration of this research.
Ethical Permission: Children's Hospital and Institute of Child Health, Multan, Pakistan, ERC approval letter No. 1895.
Conflict Of Interest: No conflicts of interest, as stated by authors.
Financial Disclosure / Grant Approval: No funding agency was involved in this research.
Data Sharing Statement: The corresponding author can provide the data on reasonable request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Qaisrani NI: Data collection, drafting, responsible for data, proofreading, approved for publication.
Afzal E: Conception and design, critical revisions, approved for publication.
Ifat M1: Data collection, drafting, critical revisions, responsible for data, approved for publication.
Ifat M2: Literature review, drafting, critical revisions, proofreading, approved for publication.
Ishfaq HM: Data analysis, data Interpretation, proofreading, critical revision, approved for publication.
Arshad M: Data collection, data synthesis, proofreading, approved for publication.

REFERENCES

1. Leisman G, Melillo R. Autism Spectrum Disorder: What Do We Know and Where Do We Go? Brain Sci. 2025; 15(9): 1010. doi: 10.3390/brainsci15091010
2. Okoye C, Obialo-Ibeawuchi CM, Obajeun OA, Sarwar S, Tawfik C, Waleed MS et al. Early Diagnosis of Autism Spectrum Disorder: A Review and Analysis of the Risks and Benefits. Cureus. 2023; 15(8): e43226. doi: 10.7759/cureus.43226.
3. WHO. Autism. Updated: 17th September, 2025. Available from: https://www.who.int/news-room/fact-sheets/detail/autism-spectrum-disorders.
4. CDC. Data and Statistics on Autism Spectrum Disorder. Updated: 27th May, 2025. Available from: https://www.cdc.gov/autism/data-research/index.html.
5. Saifuddin T, Aqeel A, Afzal A. Exploring autism spectrum disorder (ASD) in Pakistan: Contextual challenges and barriers to diagnosis and support for inclusive educators and mothers. J Curr Sign. 2025; 3: 89-101.
6. Gitimoghaddam M, Chichkine N, McArthur L, Sangha SS, Symington V. Applied Behavior Analysis in Children and Youth with Autism Spectrum Disorders: A Scoping Review. Perspect Behav Sci. 2022; 45(3): 521-557. doi: 10.1007/s40614-022-00338-x.
7. Li Z, Liu Y, Wei R, Yong VW, Xue M. The Important Role of Zinc in Neurological Diseases. Biomolecules. 2022; 13(1): 28. doi: 10.3390/biom13010028.
8. Adams JB, Audhya T, Geis E, Gehn E, Fimbres V, Pollard EL, Mitchell J, et al. Comprehensive Nutritional and Dietary Intervention for Autism Spectrum Disorder-A Randomized, Controlled 12-Month Trial. Nutrients. 2018; 10(3): 369. doi: 10.3390/nu10030369.
9. Zimmerman AW, Singh K, Connors SL, Liu H, Panjwani AA, Lee LC et al. Randomized controlled trial of sulforaphane and metabolite discovery in children with Autism Spectrum Disorder. Mol Autism. 2021; 12(1): 38. doi: 10.1186/s13229-021-00447-5.
10. Du G, Guo Y, Xu W. The effectiveness of applied behavior analysis program training on enhancing autistic children's emotional-social skills. BMC Psychol. 2024; 12(1): 568. doi: 10.1186/s40359-024-02045-5.
11. Han DG, Lee Y, Kim HS, Suh HW, Lee J, Shin SH et al. Effectiveness and experiences of early intensive behavioral and naturalistic developmental behavior interventions for autism spectrum disorders: a mixed-methods systematic review and meta-analysis. Child Adolesc Psychiatry Ment Health. 2025; 20(1): 14. doi: 10.1186/s13034-025-00997-z.
12. Adams JB, Kirby J, Audhya T, Whiteley P, Bain J. Vitamin/mineral/micronutrient supplement for autism spectrum disorders: a research survey. BMC Pediatr. 2022; 22(1): 590. doi: 10.1186/s12887-022-03628-0.
13. Al-Beltagi M. Nutritional management and autism spectrum disorder: A systematic review. World J Clin Pediatr. 2024; 13(4): 99649. doi: 10.5409/wjcp.v13.i4.99649.
14. Brignell A, Chenausky KV, Song H, Zhu J, Suo C, Morgan AT. Communication interventions for autism spectrum disorder in minimally verbal children. Cochrane Database Syst Rev. 2018; 11(11): CD012324. doi: 10.1002/14651858.CD012324.pub2.
15. Sandbank M, Bottema-Beutel K, Crowley LaPoint S, Feldman J I, Barrett D J, Caldwell N et al. Autism intervention meta-analysis of early childhood studies (Project AIM): updated systematic review and secondary analysis. BMJ. 2023; 383: 076733 doi:10.1136/bmj-2023-076733.
16. Javadfar Z, Abdollahzad H, Moludi J, Rezaeian S, Amirian H, Foroughi AA et al. Effects of vitamin D supplementation on core symptoms, serum serotonin, and interleukin-6 in children

with autism spectrum disorders: A randomized clinical trial. Nutrition. 2020; 79-80: 110986. doi: 10.1016/j.nut.2020.110986.
17. do Nascimento PKDSB, Oliveira Silva DF, de Morais TLSA, de Rezende AA. Zinc Status and Autism Spectrum Disorder in Children and Adolescents: A Systematic Review. Nutrients. 2023; 15(16): 3663. doi: 10.3390/nu15163663.
18. Arif MI, Ru L, Maimaiti R. Effect of different nutritional interventions in children with autism spectrum disorder: A systematic review. Res Autism. 2025; 120: 202535. doi: 10.1016/j.reia.2025.202535.
19. Obara T, Ishikuro M, Tamiya G, Ueki M, Yamanaka C, Mizuno S et al. Potential identification of vitamin B6 responsiveness in autism spectrum disorder utilizing phenotype variables and machine learning methods. Sci Rep. 2018; 8(1): 14840. doi: 10.1038/s41598-018-33110-w.
20. Sato K. Why is vitamin B6 effective in alleviating the symptoms of autism? Med Hypotheses. 2018; 115: 103-106. doi: 10.1016/j.mehy.2018.04.007.
21. Alhrbi A, Vlachopoulos D, Healey EM, Massoud AT, Morris C, Revuelta Iniesta R. Nutritional Status of Children Diagnosed with Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. J Hum Nutr Diet. 2025; 38(4): e70099. doi: 10.1111/jhn.70099.