Review Article

Role of Micronutrients in the Management of Autism Spectrum Disorders: A Systematic Review and Meta-Analysis

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Abstract

Autism spectrum disorders (ASDs) are a group of neurodevelopmental disorders characterized by increased developmental, educational, and social demands. Recent evidence suggests that up to 40%–50% of symptom variability may be determined by environmental factors including nutritional deficiency of folate, omega-3 fatty acids, and Vitamin D. Studies exist which advocate the use of micronutrient therapy to improve brain function. However, there is no consensus on their use in ASD and opinions remain divisive. This study aims to identify the role of micronutrients in ASD. We searched PubMed, Google Scholar, and Cochrane Library from the period of January 2010 to January 2022. We excluded animal studies, cross-sectional studies, and less 10 participants in a study. An initial literature search yielded a total of 666 studies, out of which 26 studies were included in the systematic review with a pooled sample of 12086 patients. Twelve studies showed an improvement in the symptoms of ASD patients when they were treated with micronutrients and seven studies found an association between micronutrient levels and ASD symptoms. The results of the meta-analysis in seven studies show that micronutrient therapy has a beneficial effect in reducing the severity of ASD, albeit being statistically insignificant (log odds ratio = −1.03, 95% confidence interval: −2.11–0.05). Our study suggests that in spite of low quality of evidence and randomized data, universal micronutrient supplementation may be started in children diagnosed with ASD due to the potential of reducing the severity of ASD along with a low risk of side effects.

Keywords: Autism spectrum disorders, folic acid, micronutrient therapy, omega-3 fatty acids, Vitamin D

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by increased developmental, educational, and social demands.[1] Patients with ASD exhibit impairments in communication and social interactions, and those affected have difficulty controlling their behavior and emotions.[2] The prevalence of ASD has steadily increased over the past decades, with current estimates of prevalence being around 20/10,000.[3]

The clinical symptoms of individuals with ASD vary widely, suggesting that it is multifactorial in nature.[4] With the exact etiology unknown, it is postulated that ASD has genetic and environmental origins.[5] Recent evidence suggests that up to 40%–50% of ASD symptom variability may be determined

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by environmental factors such as heavy metal exposure, nutritional deficiency of folic acid, omega-3 and Vitamin D, advanced parental age, maternal obesity, and maternal diabetes.[6]

At present, there is no cure for the aforementioned group of disorders. Reduction of symptoms that interfere with daily functioning and quality of life remains the mainstay of current treatments for ASD. These are mostly counseling centric, focusing on cognitive, behavioral, and speech therapy to aid with social and behavioral impairments associated with ASD.[7]

In 2010, there were an estimated 52 million cases of ASDs, equating to a prevalence of 7.6/1000 or one in 132 persons. Globally, autistic disorders accounted for more than 58 disability-adjusted life years (DALYs)/100,000 population and other ASDs accounted for 53 DALYs/100,000.[8]

Reports advocate the use of high-potency, high-dose micronutrient therapy for the management of a variety of neuropsychiatric and genetic disorders. To explain the benefit of enhancing brain function, mechanisms such as improved metabolic efficiency at the level of neurotransmitters, enhanced antioxidant activity, improved bioenergetic function, improved methylation, and facilitation of mitochondrial function have been proposed.[9] The usage of nutritional therapy as an adjunct in the management of ASD has been quite divisive. Although many dietary interventions have been studied, there has been a lack of conclusive scientific data about the effects of therapeutic diets on autism, and as such, no definitive recommendation can be made for a specific nutritional therapy as a standard treatment for ASD.[10] Similarly, Vitamin D, omega-3, zinc, iron, copper, Vitamin B6, Vitamin B12, folate, calcium, and probiotics were found to be notably associated with autism by big data analysis of abstracts on autism disorder and nutrition research.[11] However, there is also speculation of ineffectiveness, like this meta-analysis which does not support nonspecific dietary interventions to be ineffective in the management of ASD.[12]

Given the lack of a cure, various researchers point toward the benefit of nutritional and dietary interventions as a pivotal tool in curbing the symptoms of ASD and assisting with cognitive development and overall functioning. Keeping this in view, this systematic research aims to identify the role of micronutrients in ASDs.[13]

Materials and Methods

Search strategy

Databases including PubMed, Google Scholar, and Cochrane Library from the period of January 2010 to January 2022 were searched using a combination of controlled vocabulary such as words like Autism/ASD, micronutrient deficiency, nutritional supplements, and developmental delay. Articles addressing recent therapies and the reference lists of the included articles in the quest of identifying relevant data for the study sourcing were hand searched. In consideration of avoiding bias, the following inclusion and exclusion criteria were formulated.

Inclusion criteria

Inclusion criteria are provided in Table 1.

Exclusion criteria

Exclusion criteria are provided in Table 2.

Study selection

The initial literature search yielded a total of 666 studies in accordance with the topic. A total of 110 records were screened. The remaining potential studies were evaluated as per a systematic literature search strategy using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. With the application of the inclusion and exclusion criteria, 26 potential studies were obtained.

The studies were chosen on the basis of their year of publication, country where the study was conducted, study design, sample size, the method of intervention, and the micronutrients assessed in ASD patients.

Data extraction

Data were extracted from each eligible study and double checked the same. Data extracted included publication year, country where the study was conducted, sample characteristics such as clinical diagnosis, age group, age at baseline, functional and/clinical severity at baseline, and intellectual functioning.

Quality assessment

The quality of all the included studies was assessed using the Cochrane risk-of-bias assessment tool 2 [Figure 1]. The assessment evaluation included the following categories:

1. Deviations from the intended interventions
2. Missing outcome data
3. Measurement of the outcome
4. Selection of the reported result.

Table 1: Inclusion criteria of the studies selected

Category	Criteria
Study population	Children aged between 1 and 18
	Children diagnosed with ASD
Number of participants	>10 per study
Publication language	English
Study design	Randomized control study
	Meta-analysis study
	Case–control study
	Prospective cohort study

ASD: Autism spectrum disorder

Table 2: Exclusion criteria of the studies selected

Category	Criteria
Study	Animal studies
	Unpublished articles
	Preprints

Results

Study characteristics

The initial literature search yielded a total of 666 studies in accordance with the topic. A total of 110 records were eliminated in the screening. The remaining potential studies were evaluated as per a systematic literature search strategy using the PRISMA guidelines [Figure 2]. With the application of exclusion and inclusion criteria, we have been able to narrow down 26 studies.

Synthesis of results

A total of 26 studies were selected, with 12,086 patients being assessed and their characteristics stated in the Table 3. Out of these in 9 studies, 7 studies[14-20] found the relationship of micronutrient level with ASD symptoms, while 2 studies[10,21] did not find any change in the level of micronutrients by supplementation in ASD patients. In the remaining 17 studies, 12 studies[5,11,13,22-30] showed improvements in the symptoms of ASD patients when they were treated with micronutrient supplements, while 4 studies[31-34] disagreed. The result was obtained after statistical analysis of these studies.

Statistical methods

We used R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (URL https://www.R-project.org/), version 4.2.2 for MS Windows for statistical analysis using the "metaphor" statistical package.[35] The analysis was carried out using the log odds ratio as the outcome measure. A random-effects model was fitted to the data. The amount of heterogeneity (i.e., τ^2) was estimated using the restricted maximum likelihood estimator. In addition to the estimate of τ^2, the Q-test for heterogeneity and the I^2 statistic are reported (I^2 < 25%: weak heterogeneity, I^2 = 25%–50%: moderate heterogeneity, and I^2 > 50%: large or extreme heterogeneity). In case any amount of heterogeneity is detected (i.e., $\tau^2 > 0$, regardless of the results of the Q-test), a prediction interval for the true outcomes is also provided. Studentized residuals and Cook's distances are used to examine whether studies may be outliers and/or influential in the context of the model. Studies with a studentized residual larger than the $100 \times (1 - 0.05/[2 \times 7])^{th}$ percentile (=99.96%) of a standard normal distribution are considered potential outliers (i.e., using a Bonferroni correction with two-sided alpha = 0.007 for 7 studies included in the meta-analysis). Studies with a Cook's distance larger than the median plus six times the interquartile range of the Cook's distances are considered to be influential. The rank correlation test and the regression test, using the standard error of the observed outcomes as a predictor, are used to check for funnel plot asymmetry.

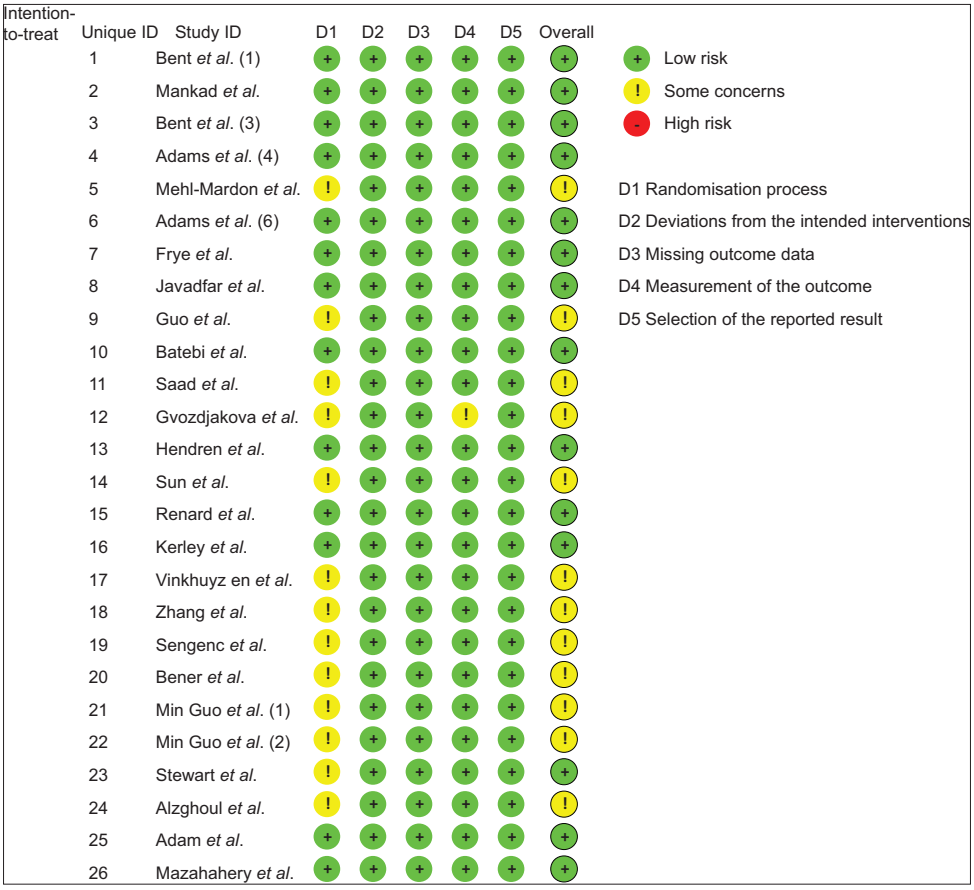

Figure 1: Risk-of-bias assessment table from Cochrane's risk-of-bias assessment tool 2

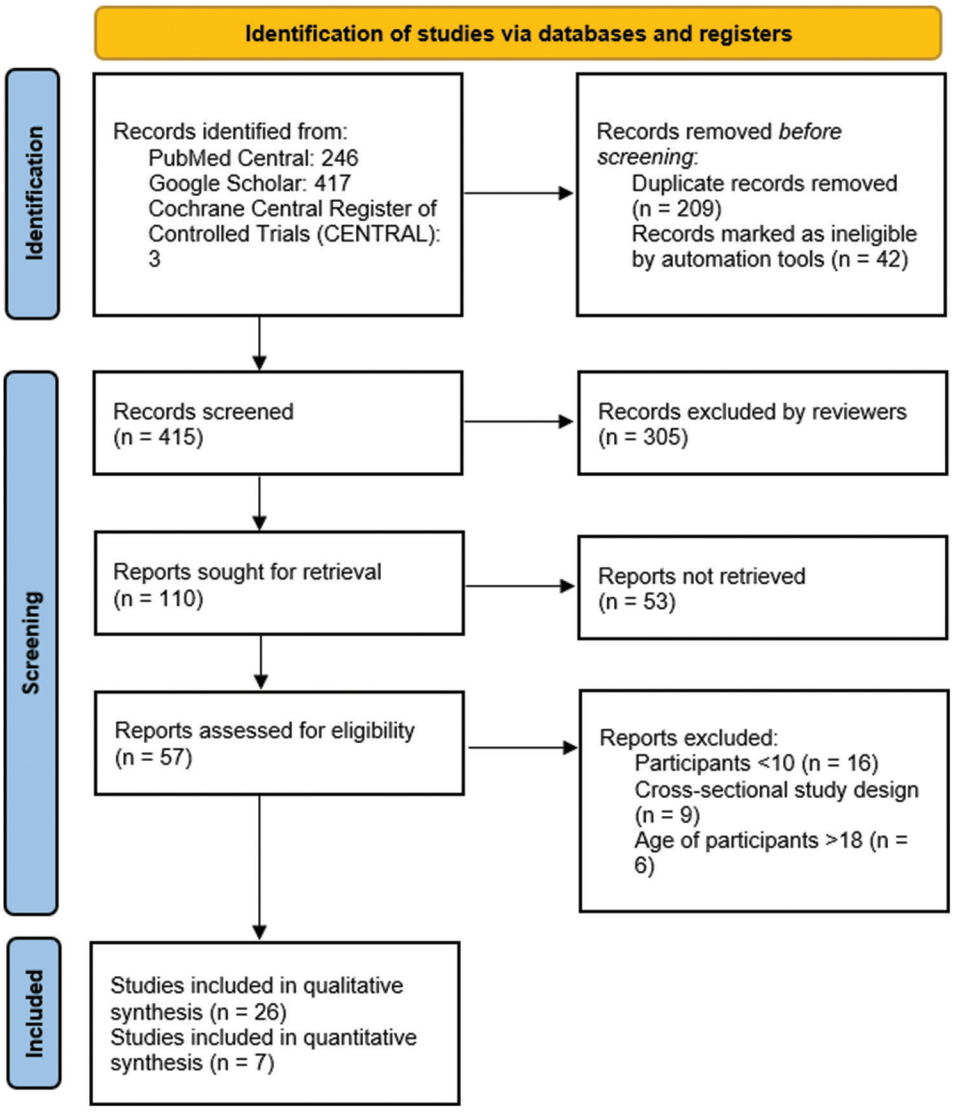

Figure 2: PRISMA (2020) flowchart of the Studies Selected for the Systematic Review and Meta-analysis. PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-analysis

Study characteristics

The studies were chosen on the basis of year of publication, country where the study was conducted, study design, sample size, the method of intervention, and the micronutrients assessed in ASD patients [Table 3].

Meta-analysis results [Figure 3]

A total of $k = 7$ studies were included in the analysis. The outcome assessed was increased severity of ASD. The observed log odds ratios ranged from − 7.1074 to 0.6061, with the majority of estimates being negative (71%). Negative estimates indicate the beneficial effect of micronutrient therapy. The estimated average log odds ratio based on the random-effects model was − 1.0295 (95% confidence interval: −2.1061–0.0471). Therefore, the average outcome did not differ significantly from zero ($z = -1.8742$, $P = 0.0609$). According to the Q-test, the true outcomes appear to be heterogeneous ($Q[6] = 18.1776$, $P = 0.0058$, tau² = 1.2428, $I^2 = 66.0250\%$). A 95% prediction interval for the true outcomes is given by − 3.4653–1.4063. Hence, although the average outcome is estimated to be negative, in some studies, the true outcome may in fact be positive. An examination of the studentized residuals revealed that one study (Saad *et al*.) had a value larger than ± 2.6901 and may be a potential outlier in the context of this model.[36] According to Cook's distances, none of the studies could be considered to be overly influential. The regression test indicated funnel plot asymmetry ($P = 0.0381$) but not the rank correlation test ($P = 0.3813$) [Figure 4].

Discussion

The problem of the paucity of available treatments in alleviating ASD has been compounded by substantial challenges in accessing evidence-based treatment approaches, compelling patients to pursue purportedly therapeutic dietary manipulations as components of treatment.[12,39] Children with ASD often

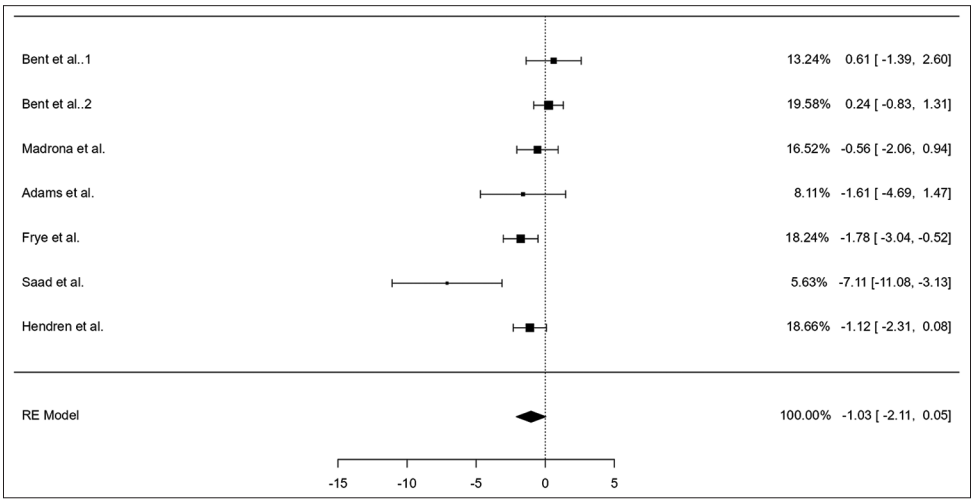

Figure 3: Forest plot for the studies analyzed

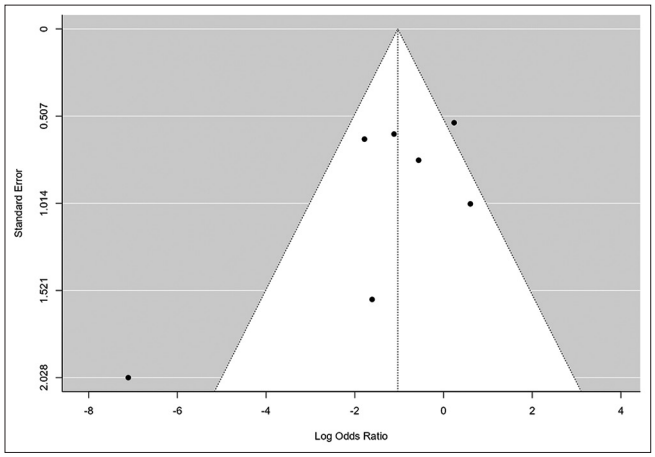

Figure 4: Funnel plot for the studies analyzed

demonstrate selective eating behaviors that may place them at risk for inadequate vitamin and mineral intake (Vitamin D, calcium, potassium, and choline).[13,40] The present review identified the most frequently encountered micronutrient deficiencies along with nutritional interventions implemented in the treatment of children, adolescents, and adults with ASD and evaluated the quality and effectiveness of vitamin/mineral supplementation as a tool in the armamentarium for combatting core and associated symptoms of ASD.

Findings

Most of the studies reported in this review found positive associations between nutritional interventions and autism symptoms, i.e., tailoring a specially designed balanced diet with appropriate micronutrient supplementation may ameliorate the severity of autism symptoms and related abnormal behaviors.[22,23] Although the long-term effects of these therapies are not well understood, some studies have contradictory findings.[31,32]

Our analysis concludes that although there does exist a positive correlation between the usage of micronutrients and amelioration of ASD symptoms, the relationship is statistically insignificant ($P > 0.05$). Specifically, it means that the data analyzed are not strong enough to infer the rejection of the null hypothesis. It is important to note that an absence of evidence does not directly correspond to evidence of absence.[41] Furthermore, Saad *et al*. has a standardized residual variable >± 2.6901 and is a potential outlier to the model.[36]

Constraints in existing literature

The reported use of alternative treatments for the improvement of ASD symptoms is widespread. However, there is little evidentiary support to validate its efficacy as a reasonable adjunct therapy. Thus, comprehensive investigations on micronutrient levels are warranted.[42] Several limitations were also identified in the design of the research. Reduced sample size, the presence of heterogeneous (nonstratified) populations, clinical measures and outcomes where there are significant differences in micronutrient levels with respect to gender, age and degree of autism, interventions with variable and usually short duration, lack of pre- and postintragroup comparisons are a few of limitations identified on critical analysis of each individual study.

It is worth noting that there was a lack of agreement regarding the supplement doses to be administered to patients with ASD. Furthermore, there was an inability to establish which dosage caused symptoms to improve or the time when the supplement should be administered in order to obtain favorable results. These findings are concurrent with current reviews on the topic. In 2017, Sathe *et al*. systematically reviewed the effectiveness of different nutritional interventions in individuals with autistic spectrum disorders and found inadequacy of data to make conclusions about the body of evidence.[43] Another systematic review investigating the effects of gluten- and/or casein-free diets on the treatment of autism did not identify any therapeutic benefits of a gluten-free, casein-free diet in the treatment of ASD.[44]

Questions remaining for the future

This poses a vexing question for future researchers to ponder upon – Is there a possibility that certain micronutrients

Table 3: Summary of studies analyzed

Study ID	Country	Study design	Sample size	Intervention	Results	Description
Bent *et al*., 2014[33]	USA	Internet-based randomized control trial	57	Children were randomly assigned to treatment with omega-3 fatty acids or identical placebo	Omega-3 fatty acids were well tolerated and no statistical difference was seen in the total number of adverse events (P=0.25)	Omega-3 fatty acids did not lead to a statistically significant reduction in hyperactivity
Mankad *et al*., 2015[31]	Canada	Randomized placebo control trial	38	Participants were randomized into 2 arms in a 1:1 manner by pharmacy	Participants with placebo Showed a mild improvement (mean change =−3.0 points from baseline). The treatment group demonstrated worsening scores	Supplementation of EPA and DHA in preschoolers with ASD does not provide any efficacy related to the core symptoms
Bent *et al*., 2011[32]	USA	Pilot randomized control trial	27	Children with satisfied eligibility were provided omega-3 fatty acids or identical placebo at a random selection for 12 weeks	Hyperactivity as measured by the ABC improved 2.7±4.8 points in the omega-3 group compared to 0.3±7.2 points in the placebo group but this was not statistically proven	Omega-3 fatty acids did not lead to a statistically significant improvement in hyperactivity in children diagnosed with ASD
Adams *et al*., 2011[24]	USA	Randomized control trial	141	Pre- and postsymptoms of autism were assessed. For 53 children pre- and postmeasurements of nutritional and metabolic status were conducted	Significant improvements in metabolic status: Total sulfate (+17%, P=0.001), SAM (+6%, P=0.003), reduced glutathione (+17%, P=0.0008), ratio of oxidized to reduced glutathione, NADH and NADPH	Oral vitamin/mineral supplementation is beneficial in improving the nutritional and metabolic status of autistic children including improvements in methylation, glutathione, oxidative stress, and sulfation
Mehl Madrona *et al*., 2010[29]	Canada	Case-control study	88	One group was provided a micronutrient-based regimen while the other group was on pharmaceuticals	The groups exhibited significant decreases in ABC scores, but the micronutrient groups were significantly greater (P<0.0001)	Lower activity level, less social withdrawal, and less anger were seen in the group taking micronutrients
Adams *et al*., 2018[13]	USA	Randomized control trial	117	Treatment with a special vitamin/mineral supplement, and additional treatments were added including essential fatty acids, Epsom salt baths, carnitine, digestive enzymes, and a HGCSF diet	The intervention group compared to the nontreatment group, had significantly greater improvement in autism symptoms	Comprehensive nutritional and dietary intervention are effective at improving nutritional status, nonverbal IQ and autism symptoms in individuals with ASD
Frye *et al*., 2018[22]	USA	Randomized placebo-controlled trial	48	Children with ASD received high-dose folinic acid or placebo per day	Improvement in VABS, ABC, the autism symptom Questionnaire and the behavioral assessment system for children were significantly greater in the folinic acid group as compared with the placebo	Improvement in verbal communication as compared with placebo
Javadfar *et al*., 2020[23]	Iran	Randomized clinical trial	43	Children were randomly selected to receive either Vitamin D drop (300 IU/kg up to a maximum of 6000 IU daily) or placebo	The symptoms of autism measured by CARS and ATEC scales were alleviated significantly (P=0.021 and P=0.020)	Vitamin D supplementation improves ASD symptoms
Guo *et al*., 2018[25]	China	Randomized clinical trial	33	The DSM-V criteria and CARS score were used for symptom assessment of the patients, respectively, before and after Vitamin A supplementation	After Vitamin A supplementation, children with ASD showed significant improvement in autism symptoms	VA supplementation is a reasonable therapy for a subset of children with autism

Contd...

Table 3: Contd...

Study ID	Country	Study design	Sample size	Intervention	Results	Description
Batebi *et al*., 2021[26]	Iran	Randomized placebo-controlled trial	55	Folinic acid was given to the intervention group while placebo to the control group	Significant effect for time × treatment interaction on inappropriate speech (F=3.51; df=1.61; P=0.044), stereotypic behavior and hyperactivity/ noncompliance (F=6.79; df=1.66; P=0.003) subscale scores	Folinic acid is recommended as a beneficial complementary supplement for allaying speech and behavioral symptoms in children with ASD
Saad *et al*., 2016[36]	Egypt	Case-controlled cross-sectional analysis	122	Subscales filled for pre- and postsupplementation of Vitamin D3	The group receiving Vitamin D3 supplementation had improved score on subscales	Vitamin D beneficial effects in ASD Patients when the final serum level is>40 ng/mL
Gvozdjáková *et al*., 2014[27]	Slovakia	Cross-sectional study	24	Children were supplemented with a daily dose of 50 mg ubiquinol	Significant improvement in autistic symptomatology was observed of ubiquinol supplementary therapy in children prevailingly at over 2.5 uCoQ10-total plasma concentration	Ubiquinol decreases symptoms in children with autism
Hendren *et al*., 2016[30]	USA	Randomized placebo-controlled trial	57	Children with ASD were assigned to treatment with methyl B12 (75 lg/kg) or 7saline placebo every 3 days in a subcutaneous injection	The clinician-rated CGI-I score was significantly improved in methyl B12 group (2.4) than in the placebo group	Methyl B12 treatment significantly improved clinician-rated symptoms of ASD
Sun *et al*., 2016[28]	China	Open-label trial	66	44 autistic children were given folic acid supplementation while 22 acted as a control group	Vitamin B9 intervention improved symptoms toward sociability, cognitive verbal/ preverbal, receptive language, and effective expression and communication	Folic acid supplementation has a role in the treatment of children with autism
Renard *et al*., 2020[37]	USA	Placebo-controlled randomized trial	19	9 patients received folinic acid supplementation while 10 received placebo	Greater change of (ADOS) global score (−2.78 vs. −0.4 points) and (−1.78 vs. 0.20 points) in the folinic acid group, compared to the placebo	Folinic acid treatment in contrast with other treatments used in ASD does not have any serious adverse effects
Kerley *et al*., 2017[34]	Ireland	Placebo-controlled RCT	42	2000 IU Vitamin D3 supplementation or placebo daily for 20 weeks	Improvement in self-care on DD-CGAS (P=0.02) is seen	Vitamin D supplementation has limited and inconsistent effects in children with ASD
Vinkhuyzen *et al*., 2018[20]	Australia	Population based-cohort study	4229	Examination of the 2 time-point measures of Vitamin D deficiency in gestational mother	Mother–infant pairs with deficiency at both times had higher scores on the SRS	25OHD deficiency either at mid-gestation or at birth was related with an increase in autism-related traits in children
Zhang *et al*., 2021[17]	China	Multi-center survey	2058	Measurement of serum levels of magnesium, iron, copper, and zinc of 1020 children with ASD and 1038 healthy children	Children with ASD had significantly lower serum levels of magnesium, copper, and zinc than healthy children (P<0.05)	The serum levels of magnesium and zinc was related with core symptoms in children with ASD
Şengenç *et al*., 2020[16]	Turkey	Cross-sectional study	1529	Serum 25-OHD levels in patients with ASD aged 3–18 years were measured	Vitamin D deficiency or insufficiency was found in approximately 95% of all patients	Serum 25-OHD levels were lower and alkaline phosphatase levels were higher compared to healthy children

Contd...

Table 3: Contd...

Study ID	Country	Study design	Sample size	Intervention	Results	Description
Bener *et al*., 2017[15]	Turkey	Case-control study	616	The ADOS-generic was the instrument used for diagnosis of Autism. (308 autistic and 308 control)	The mean of serum iron levels in autistic children was severely reduced than in control children (74.13±21.61 µg/dL (P=0.003). Vitamin D deficiency was common among autistic children (18.79±8.35 ng/mL) as compared to healthy children	Deficiency of iron and Vitamin D as well as anemia were more common in autistic compared to control children
Guo *et al*., 2019[19]	China	Case-controlled study	529	Serum retinol levels and serum levels of 25-OH Vitamin D in 332 autistic children with 197 control children	Serum retinol and 25-OHD levels in autistic children were significantly lower than those in the control children	Children with autism have more Vitamin A and Vitamin D deficiencies than control children
Guo *et al*., 2020[18]	China	Cross-sectional study	377	ABC, SRS, and GDS were completed 274 for children diagnosed with ASD. Vitamins and minerals were compared for ASD and 97 age-matched TD children	Vitamin D, folate, calcium, magnesium, iron, and zinc were lower in children with ASD compared to those with TD	Children with autism had more vitamin and mineral insufficiencies than TD children. Their levels were associated with ASD symptoms
Stewart *et al*., 2015[21]	USA	Cross-sectional study	288	Use of a gluten/ casein-free diet was documented in ASD children aged 2–11 years	One-third of children remained deficient for Vitamin D and 54% for calcium	Common micronutrient deficits were not corrected (Vitamin D, calcium, potassium and choline) by supplements
Alzghoul *et al*., 2020[14]	Jordan	Case-controlled cross-sectional study	189	Assessment of vitamin levels in 83 children with ASD aged less than 8 years compared to 106 healthy Controls	Vitamin D levels in ASD patients had a significant correlation with GI complaints	Vitamin D levels in ASD patients were significantly lower
Fraguas *et al*., 2019[12]	Spain	Meta-analysis	1028	Examination of 27 double-blind, randomized clinical trials of ASD patients: 542 in the intervention group and 486 within the placebo	Dietary supplementation (omega-3, vitamin supplementation, and/or other supplementation) was more efficacious at improving several symptoms, functions, and clinical domains	This meta-analysis does not support nonspecific dietary interventions as treatment of ASD
Mazahery *et al*., 2019[38]	New Zealand	Randomized control trial	111	Children with ASD (aged 2.5–8 years) participated in a trial of receiving either Vitamin D, omega-3 LCPUFA or both	Children receiving OM (5.0±5.0, P=0.001) and VID (−4.0±4.9, P=0.01) had greater reduction in irritability than placebo	Vitamin D and omega-3 LCPUFA reduced irritability in children with ASD. Vitamin D reduced hyperactivity symptoms within the children

HGCSF: Healthy gluten-free, casein-free, soy-free, ASD: Autism spectrum disorder, ABC: Autism Behaviour Checklist, SRS: Social Responsiveness Scale, GDS: Griffiths Development Scale, GI: Gastrointestinal, DD-CGAS: Developmental Disabilities Modification of Children's Global Assessment Scale, ADOS: Autism Diagnostic Observation Schedule, CGI-I: Clinical Global Impression Improvement Score, ATEC: Autism Treatment Evaluation Checklist, VABS: Vineland Active Behavior Scale, CARS: Childhood Autism Rating Scale, LCPUFA: Omega-3 long-chain polyunsaturated fatty acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid, RCT: Randomized controlled trial, SAM: Severe acute malnutrition, DSM-V: Diagnostic and Statistical Manual of Mental Disorders, TD: Tourette's disease, VID: Vitamin D OM: Omega-3, NADPH: Nicotinamide adenine dinucleotide phosphate, NADH: Nicotinamide adenine dinucleotide IQ: intelligence quotient, VA: vitamin A

have a conclusive benefit to ASD management while the rest are inconsequential? Moreover, are there baseline phenotypes for the patients to respond to micronutrient treatment? A cross-sectional study found that although 56% of ASD patients consume micronutrient supplements, the underlying deficit is not being corrected. Rather, it led to an excess in certain nutrients across the participating children.[21] This leads to the query on whether most of the micronutrients that are commonly administered are necessary for children. Answers to these questions may pioneer the burgeoning field of alternative treatment methods for ASD.

Limitations

This research did not include unpublished papers and only randomized clinical trials and prospective cohort studies were included in the review, which may have restricted and negatively affected the number of references analyzed.

In summary, although some authors report progress in symptoms associated with autism in individuals with ASD undergoing nutritional interventions and its potential role in alleviating clinical symptoms of ASD, there is still insufficient scientific evidence to conclusively support its use.[34,37] Therefore, studies with rigorous methodologies covering a large intervention period, an adequate sample size, and a well-considered set of evaluation measures and results must be developed to allow for a proper understanding of the consistency and precision of the impact of nutritional intervention on these disorders, which has larger, more important public health implications.

Authors' conclusions

The existing studies conducted to expound on the role of micronutrients in the management of ASD are inadequate and suffer from methodological constraints. Nevertheless, our systematic review finds evidence that a positive effect exists in the management of ASD using micronutrients. Clinicians should include micronutrient supplementation in the diets of children affected with ASD. Due to low costs incurred to patients and low potential of side effects with micronutrient supplementation, universal micronutrient therapy in children with ASD will help to reduce morbidity and improve the quality of life of these patients.

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Conflicts of interest

There are no conflicts of interest.

References

1. What Does ASD Look Like? | Ministry of Health NZ. Available from: https://www.health.govt.nz/publication/what-does-asd-look. [Last accessed on 2022 Nov 23].
2. Lord C, Brugha TS, Charman T, Cusack J, Dumas G, Frazier T, *et al.* Autism spectrum disorder. Nat Rev Dis Primers 2020;6:5.
3. Fombonne E. Epidemiology of pervasive developmental disorders. Pediatr Res 2009;65:591-8.
4. Mazurek MO, Vasa RA, Kalb LG, Kanne SM, Rosenberg D, Keefer A, *et al.* Anxiety, sensory over-responsivity, and gastrointestinal problems in children with autism spectrum disorders. J Abnorm Child Psychol 2013;41:165-76.
5. Rylaarsdam L, Guemez-Gamboa A. Genetic causes and modifiers of autism spectrum disorder. Front Cell Neurosci 2019;13:385.
6. Modabbernia A, Velthorst E, Reichenberg A. Environmental risk factors for autism: An evidence-based review of systematic reviews and meta-analyses. Mol Autism 2017;8:13.
7. Hyman SL, Levy SE, Myers SM, Council on Children with Disabilities, Section on Developmental and Behavioral Pediatrics. Identification, evaluation, and management of children with autism spectrum disorder. Pediatrics 2020;145:e20193447.
8. Baxter AJ, Brugha TS, Erskine HE, Scheurer RW, Vos T, Scott JG. The epidemiology and global burden of autism spectrum disorders. Psychol Med 2015;45:601-13.
9. Ames BN, Elson-Schwab I, Silver EA. High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased K(m)): Relevance to genetic disease and polymorphisms. Am J Clin Nutr 2002;75:616-58.
10. Karhu E, Zukerman R, Eshraghi RS, Mittal J, Deth RC, Castejon AM, *et al.* Nutritional interventions for autism spectrum disorder. Nutr Rev 2020;78:515-31.
11. Choe SY, Lee DW, Jeong HS. Data analysis of autism disorder and micronutrition using pubmed abstracts. Int J Nutr Pharmacol Neurol Dis 2021;11:148. Available from: https://www.ijnpnd.com/article.asp?issn=2231-0738;year=2021;volume=11;issue=2;spage=148;epage=153;aulast=Choe. [Last accessed on 2022 Nov 23].
12. Fraguas D, Díaz-Caneja CM, Pina-Camacho L, Moreno C, Durán-Cutilla M, Ayora M, *et al.* Dietary interventions for autism spectrum disorder: A meta-analysis. Pediatrics 2019;144:e20183218.
13. Adams JB, Audhya T, Geis E, Gehn E, Fimbres V, Pollard EL, *et al.* Comprehensive nutritional and dietary intervention for autism spectrum disorder-a randomized, controlled 12-month trial. Nutrients 2018;10:369.
14. Alzghoul L, Al-Eitan LN, Aladawi M, Odeh M, Abu Hantash O. The association between serum Vitamin D3 levels and autism among Jordanian boys. J Autism Dev Disord 2020;50:3149-54.
15. Bener A, Khattab AO, Bhugra D, Hoffmann GF. Iron and vitamin D levels among autism spectrum disorders children. Ann Afr Med 2017;16:186-91.
16. Şengenç E, Kıykım E, Saltik S. Vitamin D levels in children and adolescents with autism. https://doi.org/101177/0300060520934638 [Internet]. 2020;48(7). Available from: https://journals.sagepub.com/doi/10.1177/0300060520934638?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed. [Last cited 2023 May 11].
17. Zhang XH, Yang T, Chen J, Chen L, Dai Y, Jia FY, *et al.* Association between serum trace elements and core symptoms in children with autism spectrum disorder: A national multicenter survey. Zhongguo Dang Dai Er Ke Za Zhi 2021;23:445-50.
18. Guo M, Li L, Zhang Q, Chen L, Dai Y, Liu L, *et al.* Vitamin and mineral status of children with autism spectrum disorder in Hainan Province of China: Associations with symptoms. Nutr Neurosci 2020;23:803-10.
19. Guo M, Zhu J, Yang T, Lai X, Lei Y, Chen J, *et al.* Vitamin A and vitamin D deficiencies exacerbate symptoms in children with autism spectrum disorders. Nutr Neurosci 2019;22:637-47.
20. Vinkhuyzen AA, Eyles DW, Burne TH, Blanken LM, Kruithof CJ, Verhulst F, *et al.* Gestational vitamin D deficiency and autism-related traits: The generation R study. Mol Psychiatry 2018;23:240-6.
21. Stewart PA, Hyman SL, Schmidt BL, Macklin EA, Reynolds A, Johnson CR, *et al.* Dietary supplementation in children with autism spectrum disorders: Common, insufficient, and excessive. J Acad Nutr Diet 2015;115:1237-48.
22. Frye RE, Slattery J, Delhey L, Furgerson B, Strickland T, Tippett M, *et al.* Folinic acid improves verbal communication in children with autism and language impairment: A randomized double-blind placebo-controlled trial. Mol Psychiatry 2018;23:247-56.
23. 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.
24. Adams JB, Audhya T, McDonough-Means S, Rubin RA, Quig D, Geis E, *et al.* Effect of a vitamin/mineral supplement on children and adults with autism. BMC Pediatr 2011;11:111.
25. Guo M, Zhu J, Yang T, Lai X, Liu X, Liu J, *et al.* Vitamin A improves the symptoms of autism spectrum disorders and decreases 5-hydroxytryptamine (5-HT): A pilot study. Brain Res Bull 2018;137:35-40.
26. Batebi N, Moghaddam HS, Hasanzadeh A, Fakour Y, Mohammadi MR, Akhondzadeh S. Folinic acid as adjunctive therapy in treatment of

inappropriate speech in children with autism: A double-blind and placebo-controlled randomized trial. Child Psychiatry Hum Dev 2021;52:928-38.
27. Gvozdjáková A, Kucharská J, Ostatníková D, Babinská K, Nakládal D, Crane FL. Ubiquinol improves symptoms in children with autism. Oxid Med Cell Longev 2014;2014:798957.
28. Sun C, Zou M, Zhao D, Xia W, Wu L. Efficacy of folic acid supplementation in autistic children participating in structured teaching: An open-label trial. Nutrients 2016;8:337.
29. Mehl-Madrona L, Leung B, Kennedy C, Paul S, Kaplan BJ. Micronutrients versus standard medication management in autism: A naturalistic case-control study. J Child Adolesc Psychopharmacol 2010;20:95-103.
30. Hendren RL, James SJ, Widjaja F, Lawton B, Rosenblatt A, Bent S. Randomized, placebo-controlled trial of methyl B12 for children with autism. J Child Adolesc Psychopharmacol 2016;26:774-83.
31. Mankad D, Dupuis A, Smile S, Roberts W, Brian J, Lui T, *et al.* A randomized, placebo controlled trial of omega-3 fatty acids in the treatment of young children with autism. Mol Autism 2015;6:18.
32. Bent S, Bertoglio K, Ashwood P, Bostrom A, Hendren RL. A pilot randomized controlled trial of omega-3 fatty acids for autism spectrum disorder. J Autism Dev Disord 2011;41:545-54.
33. Bent S, Hendren RL, Zandi T, Law K, Choi JE, Widjaja F, *et al.* Internet-based, randomized, controlled trial of omega-3 fatty acids for hyperactivity in autism. J Am Acad Child Adolesc Psychiatry 2014;53:658-66.
34. Kerley CP, Power C, Gallagher L, Coghlan D. Lack of effect of vitamin D(3) supplementation in autism: A 20-week, placebo-controlled RCT. Arch Dis Child 2017;102:1030-6.
35. Viechtbauer W. Meta-analysis package for R [R package metafor version 3.8-1]. Stat Softw 2022;36. Available from: https://CRAN.R-project.org/package=metafor. [Last accessed on 2022 Nov 23].
36. Saad K, Abdel-Rahman AA, Elserogy YM, Al-Atram AA, Cannell JJ, Bjørklund G, *et al.* Vitamin D status in autism spectrum disorders and the efficacy of vitamin D supplementation in autistic children. Nutr Neurosci 2016;19:346-51.
37. Renard E, Leheup B, Guéant-Rodriguez RM, Oussalah A, Quadros EV, Guéant JL. Folinic acid improves the score of Autism in the EFFET placebo-controlled randomized trial. Biochimie 2020;173:57-61.
38. Mazahery H, Conlon CA, Beck KL, Mugridge O, Kruger MC, Stonehouse W, *et al.* A randomised controlled trial of vitamin D and omega-3 long chain polyunsaturated fatty acids in the treatment of irritability and hyperactivity among children with autism spectrum disorder. J Steroid Biochem Mol Biol 2019;187:9-16.
39. Onaolapo OJ, Onaolapo AY, Onaolapo OJ, Onaolapo AY. Nutrition in autism spectrum disorders: A review of evidences for an emerging central role in aetiology, expression, and management. AIMS Med Sci 2018;5:122-44. Available from: http://www.aimspress.com/article/doi/10.3934/medsci. 2018.2.122. [Last accessed on 2022 Nov 23].
40. Bandini LG, Curtin C, Phillips S, Anderson SE, Maslin M, Must A. Changes in food selectivity in children with autism spectrum disorder. J Autism Dev Disord 2017;47:439-46.
41. Singh AK, Kelley K, Agarwal R. Interpreting results of clinical trials: A conceptual framework. Clin J Am Soc Nephrol 2008;3:1246-52.
42. Monteiro MA, Santos AA, Gomes LM, Rito RV. Autism spectrum disorder: A systematic review about nutritional interventions. Rev Paul Pediatr 2020;38:e2018262.
43. Sathe N, Andrews JC, McPheeters ML, Warren ZE. Nutritional and dietary interventions for autism spectrum disorder: A systematic review. Pediatrics 2017;139:e20170346.
44. Mulloy A, Lang R, O'Reilly M, Sigafoos J, Lancioni G, Rispoli M. Gluten-free and casein-free diets in the treatment of autism spectrum disorders: A systematic review. Res Autism Spectr Disord 2010;4:328-39. https://www.ncbi.nlm.nih.gov/books/NBK79740/. [Last accessed on 2022 Nov 23].