Investigating The Effect of Combined Nutritional Supplementation in the Management of Autism Spectrum Disorder and the Progression of Social and Communication Skills: A Nonrandomized Interventional Study

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ABSTRACT
In recent years, there has been an increase in the use of nutritional supplementation as a method of treating autism spectrum disorder (ASD). The aim of our study is to investigate the effect of using combined supplementation on treating the symptoms of Autism. In this study, we conducted a nonrandomized, nonblinded trial where we followed up with patients with ASD who were diagnosed through the CARS2-ST scale and evaluated through the Vineland-3 scale within two visits. The patients were divided into control and intervention groups where training only and supplementation with training were used, respectively. Results: The study included a total of 115 participants, out of which 95 (82.6%) were assigned to the intervention group and 20 (17.4%) were assigned to the control group. Significant improvement was observed in the intervention group compared to the control group. It was observed that the CARS2 score for the intervention group was reduced by a mean of 16.1 points compared to only 4.45 mean points reduced in the control group. On the other hand, the Vineland-III scale was increased by a mean of 350.23 points in the intervention group, while the control group had an increase of only 41.3 points. Conclusion: Our study concludes that the use of combined supplementation along with behavioral training greatly reduces autism symptoms compared to training alone. Thus, our study can recommend the use of combined supplementation as a form of treatment for Autism. Further studies with randomization and blinding will be helpful in confirming the findings of our study.

1. Introduction

Autism is defined as a neurodevelopmental disorder in which a child's communicative, emotional, and symbolic development, as well as their ability to establish relationships with others, is impaired [1,2]. In autism, both the receptive and expressive levels of verbal language are affected, particularly in relation to the pragmatic and sematic code, as well as the nonverbal communication systems [3]. The neurodevelopmental problems in people with autism spectrum disorder (ASD) begins at an early age and persist to a greater or lesser extent throughout life. Children diagnosed with autism display atypical variations in child development. Occasionally, symptoms of forthcoming issues may be apparent from the time of birth. Others have typical development initially, however during their ages between 18 to 36 months, the circumstances start to shift. Parents may see that their children begin showing social estrangement, demonstrate abnormal behavior, and experience regression in linguistic and social skills acquired at earlier developmental stages. alternatively in certain cases, the distinction between children diagnosed with autism spectrum condition and their peers of similar age is more apparent by careful observation. [4–6].

The treatment of autism spectrum disorder can take many forms. Although the forms are different, the symptoms to be treated are the same. The main approach to treating the fundamental signs of autism involves psychosocial interventions that include applied behavior analysis. [7]. Other forms of treatment include Pharmacotherapy with atypical antipsychotics [7], diet and nutritional status, and dietary supplementation [8].

Recent studies have shown a strong connection between people having autism and the presence associated digestive issues. As a result, there has been a rise in the use in dietary supplements as a treatment option for autism. Recent studies have provided mounting evidence in favor of this treatment strategy. [9].

Due to the frequent occurrence of gastrointestinal symptoms such as diarrhea, constipation, and irritable bowel syndrome (IBS) in autistic children, almost 20% of doctors in the United States started using probiotics as a form of treatment for autism. This comes due to the beneficial function of probiotics at restoring the balance of the intestinal microflora as well as the rebuilding of the intestinal mucosa [8].

A study performed in 2006 showed a connection between a lack of Omega-3 fatty acids as well as the development of psychological disorders, including autism [10]. Additional evidence was provided by case-control research conducted in 2007. The study revealed that a group taking Omega-3 fatty acid supplements showed improved speech and articulation, and with greater social openness, in comparison to the control group [11].

A previous study conducted in 1985 investigated the potential therapeutic impact of administering Vitamin B6 as well as magnesium supplements to individuals with autism. The study found that the intervention group, which took the supplements, had enhanced behavior comparison with the control group, which did not take the supplements. [12].

Moreover, research has revealed that the administration of vitamin D supplements also enhances the symptoms associated with autism. In a research conducted in 2016, it was shown that almost 80 percent of the individuals demonstrated enhancements in the behaviors, stereotypes, eye contact, and attention span categories of the CARS2-ST scale as well as Aberrant Behavior Checklist, which are used for evaluating autism [13].

In addition, it has been found that folate concentrations in the cerebrospinal fluid were low in children with low-functioning autism. The supplementation of these children with oral folate was found to have positive effects on their general health after 12 months [14].

Moreover, Zinc has also been used as a form of supplemental treatment in patients with autism [15]. Studies show that the Zinc-to-copper ratio is lower than normal in children with autism, thus indication a deficiency of zinc and an overload of copper, in so supporting the treatment with supplemental Zinc [16].

In addition, several investigations have shown a clear connection among autism with the Glutathione cycle. Study has shown which the use of N-acetyl cysteine (NAC), a substance that precedes glutathione, may potentially alleviate symptoms of autism [17,18]. In a 2012 randomized controlled experiment, the utilization of N-acetylcysteine (NAC) showed a positive effect on the irritability portion of the Aberrant Behavior Checklist (ABC) in children diagnosed with autism [19]. In the same year, a case study was conducted which likewise revealed enhancements in the social impairments along with aggressive symptoms of the autistic youngster being monitored. [20].

Similarly, research has indicated a lack of Choline in kids with autism in comparison with kids without autism [21]. This raises the prospect of using choline as a therapeutic intervention. A study conducted in 2019 utilized a combination of Donepezil Hydrochloride and a Choline supplement to examine its impact on receptive language abilities in children diagnosed with autism spectrum disorder. The study found that this therapy resulted in a lasting improvement in language skills over a period of six months. This phenomenon was particularly found in youngsters aged below 10 years. [22].

Alternatively, it has been suggested that there is a dysregulation of excitatory and inhibitory neurotransmitters in individuals with autism spectrum condition. A research was conducted to assess the concentration of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter, in autistic kids compared to normally developing guys. The study found that people having autism spectrum disorder have lower levels of Gaba compared with people with regular development. In addition, they found a correlation among lower amounts of GABA and poorer IQ as well as more social impairment within the studied groups. [23].

As a possible cause of autism, it has been proposed that oxidative stress in genetically vulnerable individuals leads to impaired methylation and, thus, neurological deficits [24]. S-adenosyl-methionine (SAMe), on the other hand, is a major methyl group doner and exerts influence on the central nervous system through the cellular transmethylation pathway [25]. Studies on mice injected with valproic acid to simulate symptoms of autism spectrum disorder demonstrate that the coadministration of SAMe with Valproic acid alleviates these symptoms, thus opening the door for research to use SAMe supplementation on human subjects [26].

Lastly, in addition to the supplements mentioned above, studies have also shown the use of digestive enzymes as supplements to be effective in alleviating autism symptoms. A randomized controlled trial on 101 autistic children found that supplementation with digestive enzymes showed improvement in emotional response, general impression, and general behavior, as well as gastrointestinal symptoms in children with autism [27].

The aim of our study is to investigate the effect of combined supplementation to improve mitochondrial function, Gut function, Oxidative stress, Methylation, and relaxation on alleviating the symptoms of children with autism spectrum disorder [ASD) as observed through Childhood Autism Rating Scale 2nd edition (CARS2-ST) and Vineland Adaptive Behavior Scale 3rd edition (Vineland-3).

Methodology

Study design

We used a nonblinded, nonrandomized controlled trial design in our study as the choice of enrolment was made by the parents. This design allows for a more feasible conduction of the study, considering the sensitivity of the topic.

Settings, locations, and relevant dates

Recruitment process occurred in Barr Al Aman Institute of Autism, Baghdad, Iraq. Enrolment occurred throughout the year of 2020. The timing of the follow-up visit was determined by the parent. Data collection occurred during the visit, and the assessment was conducted by a professional.

Participants

The target population was patients diagnosed with autism using the CARS2-ST scale. Exclusion criteria were patients below and above two years and 15 years of age, respectively, patients with concomitant cerebral palsy, Fragile X syndrome, or Down syndrome, and patients receiving medications for other neurological disorders were also excluded. These criteria were utilized, as concomitant disorders or medication use might affect the outcome. Severity category and gender were not used to exclude participants to appreciate differences in outcomes in these two variables. Methods of recruitment included patients referred from other medical institutions after receiving the diagnosis or parents visiting the clinic to

diagnose their children. Assignment to treatment or control group was the decision of the parents. Patients in the control group had applied behaviour analysis training, while patients in the treatment group had nutritional supplementation in addition to applied behaviour analysis training. Combined supplementation consisted of supplements as follows:

- Mitochondrial Function (L. Carnitine, co-enzyme Q 10)
- Gut Function (probiotic, pancreatic enzyme)
- Antioxidants (N- acetyl cystine, glutathione)
- Nootropic supplements (omega 3, choline)
- Methylation, Vitamins, and minerals (methyl folate, SAME, B group, D3, Zinc)
- Relaxation supplements (GABA, Mg citrate)

Variables and assessment tools

The primary outcome of the study is improving the severity of autism measured with the CARS2-ST tool. The CARS2-ST is a 15-item tool that aims to identify patients with ASD and determine the severity with two categories, either mild-moderate or severe, using ratings that are quantifiable and based on direct observation by a professional. Items are rated on a 4-point scale. Given the variations in sensitivities and specificities observed among different suggested cut-off points by various studies concerning the diagnosis of ASD [28,29], this study has adopted the subsequent cut-off points to address this heterogeneity: <30, 30-36, and 37-60, were assigned for no autism, mild-moderate autism, and severe autism, respectively. A study aimed to assess the diagnostic predictability of the CARS2-ST scale found that it is both sensitive and specific for the diagnosis of ASD [30]. A systematic review and meta-analysis found that the CARS2-ST tool's internal validity and sensitivity to be acceptable, although specificity was not, indicating its accuracy in detecting ASD accompanied with other diagnostic tools [31]. The secondary outcomes of the study were improving the daily living, socialization, communication, and motor skills measured using the Vineland-3 scale and exploring the effect of age and gender on the efficacy of nutritional supplementation in autism patients. The Vineland adaptive behaviour scale is a psychometric tool used to assess intellectual disability and developmental delays. The original version comprised of the three domains of adaptive functioning specified by the American Association on Intellectual and Developmental Disabilities and by DSM-5: daily living skills, communication, and socialization. Subsequent versions have expanded to include optional domains including motor skills and maladaptive behaviours. We included the three original domains in addition to the motor skills domain in this study.

Additionally, demographic data were included to derive additional associations. The scales were administered in the first clinic visit and in the follow up clinic visit, and interventions were applied in between. Scales were administered by a professional in the presence of the parent. An informed consent to enrol was taken from the parents prior to the initiation of the study, and confidentiality and anonymity were assured.

Data analysis and statistical methods

Data entry and data cleaning were done using Excel 365, and data analysis was conducted using SPSS V26. An Independent T-test was utilized to measure the primary outcome. For

secondary outcomes, Independent T-test, chi-square test, and correlation coefficient were utilized.

Results

Demographics statistics:

The study included a total of 115 participants. 82.6% (95) of the study participants were enrolled in the intervention group, while the control group composed 17.4% (20) of the sample. The sample of the study included 86 males (74.8%) compared to 29 females (25.2%). The mean age at diagnosis was 5.4 years, with 2.4 years and 13 years being the minimum and maximum ages in the entire study sample. The mean follow-up period is 0.79 years, with one month and three years being the minimum and maximum follow-up periods, respectively. Chi-square test found no significant association between the gender and treatment group. Table 1 demonstrates the baseline characteristics of the entire sample.

Primary outcomes

According to the CARS2-ST scale, 68.7% and 31.3% of the entire sample were diagnosed with severe autism and mild to moderate autism, respectively, in the first visit. In the follow-up visit, however, 76.5% of the sample had no autism, 15.7% had severe autism, and 7.8% had mild to moderate autism. Mean CARS2-ST score in the first visit for the entire sample was 41.63, with a minimum of 30 and a maximum of 60. Mean CARS2-ST score in the follow-up visit for the entire sample was 27.56, with a minimum of 17 and a maximum of 57. Mean CARS2-ST 2 score in the first visit was 40.42 and 47.4 for the intervention and control groups, respectively. Mean CARS2-ST score in the follow-up visit was 24.3 and 42.6 for the intervention and control groups, respectively. CARS2-ST score was reduced by a mean of 16.10 points for the intervention group compared to only 4.45 points for the control group between the two visits, leaving a difference of 11.65 points, which was statistically significant [P = <0.01]. Figure 1 demonstrates the change in diagnosis and severity of autism between the first and follow-up visits for the intervention and control groups. Figure 2 demonstrates the change in CARS2-ST score frequencies between the first and follow-up visits for the intervention and control groups. Figure 3 demonstrates the change in Vineland-3 score frequencies between the first and follow-up visits for the intervention and control groups.

Secondary outcomes

The Vineland-3 mean score for the first visit for the entire sample was 127.36, with a minimum score of 0 and a maximum score of 526.0. The mean Vineland-3 score in the follow-up visit for the entire sample was 423.86, with a minimum of 8 and a maximum of 664. Mean Vineland-3 scores in the first visit were 134.1 and 95.5 for the intervention and control groups, respectively. Mean Vineland-3 scores in the follow-up visit were 484 and 137 for the intervention and control groups, respectively. An independent T-test comparing the Vineland-3 means of the two groups in the first visit yielded a T-score of 1.91 (df=113; p=0.058), indicating the nonsignificant difference between the two groups. An independent T-test comparing the Vineland-3 means of the two groups in the follow-up visit yielded a T-score of 16.79 (df=113; p<0.001), indicating that the intervention group's Vineland-3 mean in the follow-up visit was significantly higher than the control group's mean. Vineland-3 scale score was increased by a mean of 350.23 points for the intervention group compared to only 41.3 points for the control group between the two visits, leaving a difference of 308.93 points, which was statistically significant [P = <0.001]. Table 2 summarizes the means and

standard deviations for CARS2-ST and Vineland-3 based on the assigned treatment group and gender variables, respectively. Daily living skills subsection score was increased by a mean of 89.89 points for the intervention group compared to only 12.25 points for the control group between the two visits, leaving a difference of 77.64 points between the means of the two groups, which was statistically significant [P = <0.001]. Socialization subsection score was increased by a mean of 105.45 points for the intervention group compared to only 5.85 points for the control group between the two visits, leaving a difference of 99.6 points between the means of the two groups, which was statistically significant [P = <0.001]. Communication subsection score was increased by a mean of 94.3 points for the intervention group compared to only 9.4 points for the control group between the two visits, leaving a difference of 84.9 points between the means of the two groups, which was statistically significant [P = <0.001]. Motor skills subsection score was increased by a mean of 60.58 points for the intervention group compared to only 13.8 points for the control group between the two visits, leaving a difference of 46.78 points between the means of the two groups, which was statistically significant [P = <0.001]. The correlation coefficient between age at the first visit and CARS2-STscore at the first visit was 0.064 (P=0.494), indicating a non-significant and very weak positive relationship. The correlation coefficient between age at the follow-up visit and CARS2-STscore at the follow-up visit is 0.288 (P=0.002), indicating a significant and weak positive relationship between the two variables. The correlation coefficient between age at the first visit and Vineland-3 score at the first visit was 0.262 (P=0.005), indicating a significant and weak positive relationship between the two variables. The correlation coefficient between age at the follow-up visit and Vineland-3 score at the follow-up visit was -0.176 (P=0.06), indicating a nonsignificant relationship between the two variables. The correlation coefficients between age at the first visit and Vineland-3 subsections score at the first visit were 0.288 (P=0.002), -0.003 (P=0.974), 0.359 (P<0.001), and 0.252 (P=0.007) for daily living skills, socialization, communication, and motor skills, respectively. The correlation coefficients between age at the follow-up visit and Vineland-3 subsections score at the follow-up visit were -.176 (P=0. 06), -0.088 (P=0.352), -0.305 (P=0.001), and -0.123 (P=0.189) for daily living skills, socialization, communication, and motor skills, respectively. An independent t-test was conducted specifically on the intervention group to compare the difference in CARS2-STscores and the difference in Vineland-3 scores of males and females between the first and follow-up visits. The results indicated that there were no significant differences between the two genders (P=0.7 for the difference in CARS2-ST and P=0.118 for the difference in Vineland-3 scores).

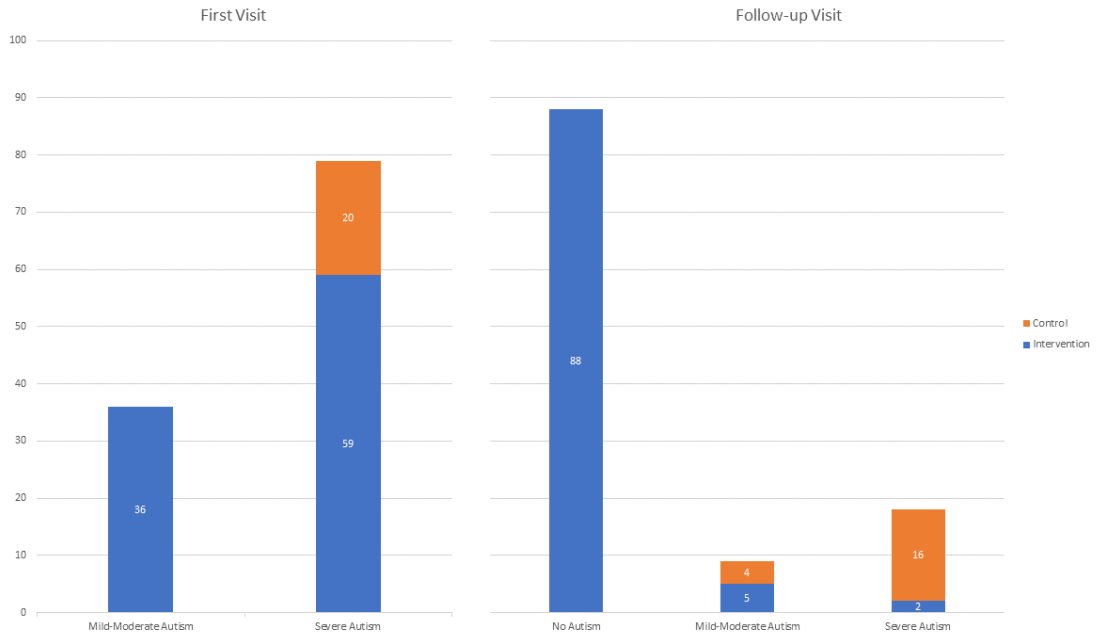

Figure 1. The frequency percentage of the three categories of autism diagnosis based on the CARS2-ST scale for the intervention and control groups between the first and follow-up visits

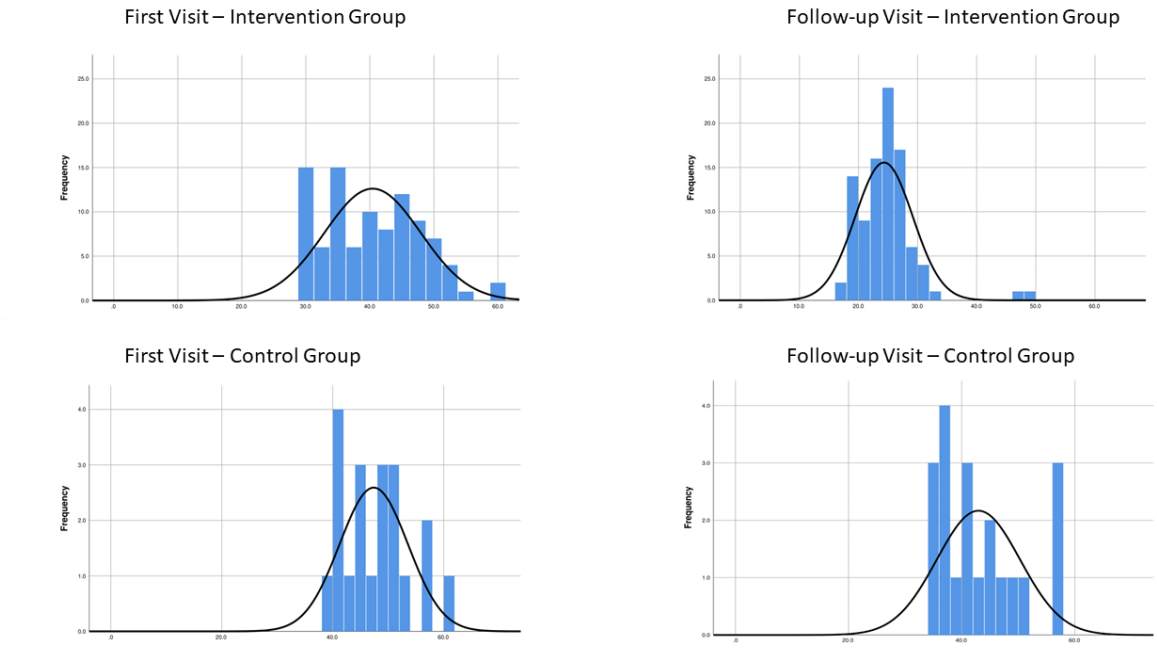

Figure 2. Histograms of CARS2-ST results for the intervention and control groups between the first and follow-up visits

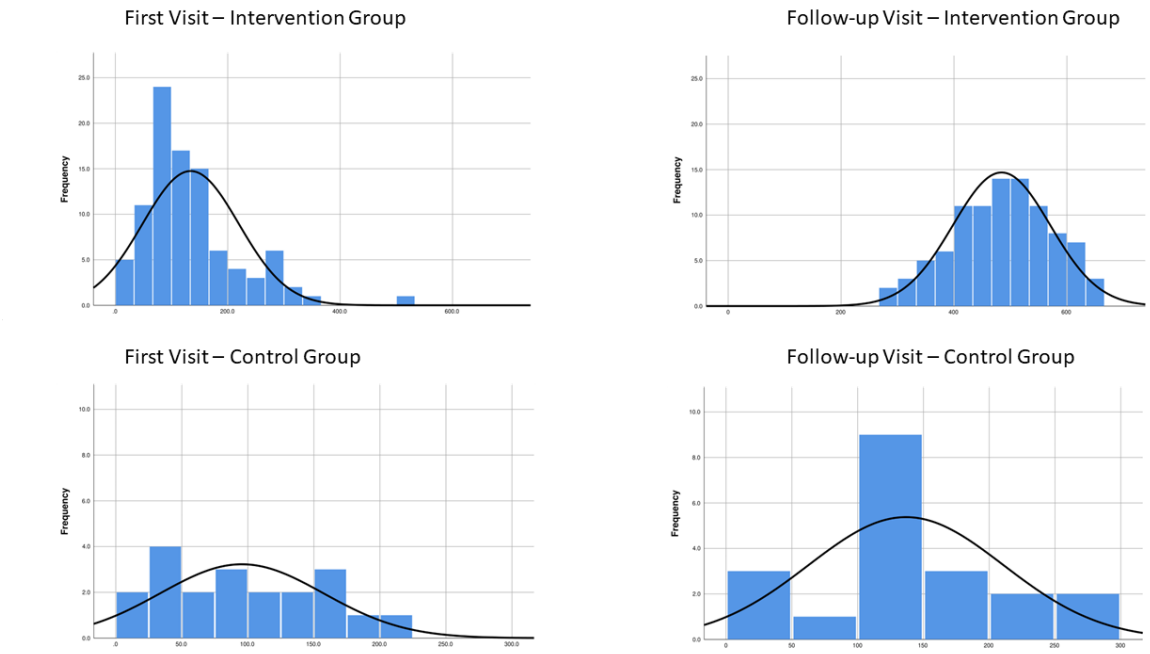

Figure 3. Histograms of Vineland-3 results for the intervention and control groups between the first and follow-up visits

		N	N %	Mean	Standard Deviation	Minimum	Maximum
Gender	female	29	25.2%				
	male	86	74.8%				
Age at first visit*				5.42	2.14	2.42	13.00
Age at Follow-up visit*				6.21	2.24	3.00	14.00
Follow-up Period*				.79	.60	1**	3.00
*: age in years **: age in months							

Table 1. Baseline demographic statistics for the entire sample

	Assigned Treatment Group						Gender					
	intervention		control		Total		female		male		Total	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
CARS2-ST for the First Visit	40.4	7.5	47.4	6.2	41.6	7.7	40.2	7.7	42.1	7.7	41.6	7.7
CARS2-ST for the follow-up visit	24.3	4.9	43	7.4	27.6	8.9	26.1	9.2	28	8.8	27.6	8.9
Vineland-3 for	134.1	85.7	95.5	61.8	127.4	83.1	118.7	71.4	130.3	86.8	127	83.1

the first visit												
Vineland-3 for the follow-up visit	484	86	137	74	424	157	414	120	427	168	424	157

Table 2. Mean and SD of CARS2-ST and Vineland-3 scales for assigned treatment group and gender variables between the first and second visits

Discussion:

Our research found that the use of combined supplementation along with training yielded higher improvement results among children with autism compared to training alone. This allows us to reject the null hypothesis and conclude that the use of combined supplementation greatly improves patients with Autism Spectrum Disorder (ASD). This agrees with the literature that suggested the use of a variety of supplements we used to improve autism symptoms [9,11–14,19,27].

This was demonstrated through a higher decrease of the mean of the CARS2-ST scale between the first and the follow-up visit in the intervention group that took the supplementation compared to the control group that only did training. However, the study did not prove any difference in changes in the CARS2-ST scale between males and females or between different ages.

Furthermore, our conclusion was also demonstrated through the Vineland-3 scale. It showed that the mean increase in the intervention group was much higher when compared to the control group. Once more, the study could not demonstrate a differences in changes between males and females or between the different ages.

During the study, and through our history taking prior to inclusion in the study, we observed that children with previous gestational problems, such as asphysxia neonatorum, intrauterine growth restriction, oligo or polyhydramnios, or other placental issues, had a better response to the supplementation compared to those with no previous gestational issues. This could indicate the presence of an underlying biological damage in such cases that was corrected using supplementation. More studies are needed to confirm such a hypothesis.

Moreover, we found through the history that those who had a previous history of gastrointestinal symptoms, including constipation, diarrhea, bloating, and repeated abdominal pain, had better responses compared to those with no such prior history. This might be attributed to the use of probiotics thus correcting underlying microflora disturbances. However, further studies are needed to prove this hypothesis.

In addition, we also noted that patients who have a positive family history of autism, speech delay, or other psychiatric disorders generally had a worse response than those with isolated cases in the family. This cannot be deomnstrated through our study, so a follow-up study is crucial to investigate this hypothesis.

Also, during our study, we noticed that some patients developed symptoms of agitation, especially with mitochondrial suppelemts such as Co-enzyme Q10, Niacin, and Omega-3. For these patients, we either reduced the dose or stopped the supplementation for one to two weeks and then returned it; the patients responded well to this strategy and had no further agitation, as per the parents.

The research, however, is subject to limitations. Firstly, there is a high discrepency between the size of the intervention group compared to the control group. This stems from the collection method as we let the parents decide wehter they would like to include their child in the supplementation group or the training-only group, and most parents chose the former. This collection method additionally contributed to the large gap in the male-to-female ratio; thus, generalzation to a larger population might be affected. Moreover, confounding variables were not accounted for in the design of the study and in the statistical analysis. Lastly, the study was not randomized, nor was it blinded, further adding to the limitation of the study.

In light of these limitations, we recommend a future study that addresses these concerns with a larger smaple, more equally distributed groups, and a balance between males and females. We further recommend the study to be randomized and blinded to standarize and generalize the study with more confidence.

Conclusion

In light of the results of our study, we conclude that the use of combined supplementation greatly improves the symptoms of those afflicted with autism spectrum disorder. This was evident through noticeable improvements across both the CARS2-ST and the Vineland-3 scales. Considering this conclusion, the study can suggest the use of combined supplementation as an effective method of treating autism. However, bearing in mind the limitations of the study, additional studies might be necessary to further the findings on this topic.

Declarations:

We declare that the work in this research, titled "Investigating the Effect of Combined Nutritional Supplementation in the Management of Autism Spectrum Disorder and the Progression of Social and Communication Skills: A Nonrandomized Interventional Study" was novel work carried out by us, the mentioned authors. Furthermore, all stated information taken from the literature has been acknowledged and referenced at the end of the paper.

Competing interest:

We declare that us, the authors, have no financial, professional, or personal interests that might influence the work presented in this manuscript.

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Ethical approval:

This study received ethical approval from the ethical committee of the Iraqi ENT Society based on their ethical criteria and regulations regarding scientific research.

Consent to participate and consent to publish:

All participants in this study were informed about the content and process of the study, along with clarifications of their role and participation. They were then made to sign a consent form willingly by their choosing. Furthermore, the participants were informed and consented to the publication of the study they enrolled in.

Authors Contributions:

Ahmad Sh. Muhialdin: Conceived and designed the research idea, collected the data, and contributed to writing the paper.

Muhamed Shamsaldin: performed the analysis and wrote the paper (wrote the introduction, part of the results, the discussion, and the conclusion).

Omar Alrefai: performed the analysis and wrote the paper (wrote the results, methodology, and arranged the references).

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Reference

[1] Daniels AM, Mandell DS. Explaining differences in age at autism spectrum disorder diagnosis: a critical review. Autism [Internet]. 2014 [cited 2023 Aug 17];18(5):583–97. Available from: https://pubmed.ncbi.nlm.nih.gov/23787411/

[2] Guinchat V, Chamak B, Bonniau B, Bodeau N, Perisse D, Cohen D, et al. Very early signs of autism reported by parents include many concerns not specific to autism criteria. Res Autism Spectr Disord. 2012 Apr 1;6(2):589–601.

[3] Larsen K, Aasland A, Diseth TH. Identification of Symptoms of Autism Spectrum Disorders in the Second Year of Life at Day-Care Centres by Day-Care Staff: Step One in the Development of a Short Observation List. J Autism Dev Disord [Internet]. 2018 Jul 1 [cited 2023 Aug 17];48(7):2267–77. Available from: https://pubmed.ncbi.nlm.nih.gov/29423606/

[4] Kern JK, Trivedi MH, Garver CR, Grannemann BD, Andrews AA, Savla JS, et al. The pattern of sensory processing abnormalities in autism. Autism [Internet]. 2006 Sep [cited 2023 Aug 17];10(5):480–94. Available from: https://pubmed.ncbi.nlm.nih.gov/16940314/

[5] Piek JP, Dyck MJ. Sensory-motor deficits in children with developmental coordination disorder, attention deficit hyperactivity disorder, and autistic disorder. Hum Mov Sci [Internet]. 2004 Oct [cited 2023 Aug 17];23(3–4):475–88. Available from: https://pubmed.ncbi.nlm.nih.gov/15541530/

[6] Green SA, Ben-Sasson A, Soto TW, Carter AS. Anxiety and sensory over-responsivity in toddlers with autism spectrum disorders: Bidirectional effects across time. J Autism Dev Disord [Internet]. 2012 Jun [cited 2023 Aug 17];42(6):1112–9. Available from: https://pubmed.ncbi.nlm.nih.gov/21935727/

[7] De Filippis M, Wagner KD. Treatment of Autism Spectrum Disorder in Children and Adolescents. Psychopharmacol Bull [Internet]. 2016 Aug 8 [cited 2023 Aug 17];46(2):18. Available from: /PMC/articles/PMC5044466/

[8] How nutritional status, diet, and dietary supplements can affect autism. A review - PubMed [Internet]. [cited 2023 Aug 17]. Available from: https://pubmed.ncbi.nlm.nih.gov/23789306/

[9] Gogou M, Kolios G. The effect of dietary supplements on clinical aspects of autism spectrum disorder: A systematic review of the literature. Brain Dev [Internet]. 2017 Sep 1 [cited 2023 Aug 17];39(8):656–64. Available from: https://pubmed.ncbi.nlm.nih.gov/28438367/

[10] Tsalamanios E, Yanni AE, Koutsari C. Omega-3 Fatty Acids: Role in the Prevention and Treatment of Psychiatric Disorders. Curr Psychiatry Rev. 2006 Apr 28;2(2):215–34.

[11] Amminger GP, Berger GE, Schäfer MR, Klier C, Friedrich MH, Feucht M. Omega-3 fatty acids supplementation in children with autism: a double-blind randomized, placebo-controlled pilot study. Biol Psychiatry [Internet]. 2007 Feb 15 [cited 2023 Aug 17];61(4):551–3. Available from: https://pubmed.ncbi.nlm.nih.gov/16920077/

[12] Martineau J, Barthelemy C, Garreau B, Lelord G. Vitamin B6, magnesium, and combined B6-Mg: therapeutic effects in childhood autism. Biol Psychiatry [Internet]. 1985 [cited 2023 Aug 17];20(5):467–78. Available from: https://pubmed.ncbi.nlm.nih.gov/3886023/

[13] 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 [Internet]. 2016 Sep 13 [cited 2023 Aug 17];19(8):346–51. Available from: https://pubmed.ncbi.nlm.nih.gov/25876214/

[14] Ramaekers VT, Blau N, Sequeira JM, Nassogne MC, Quadros E V. Folate receptor autoimmunity and cerebral folate deficiency in low-functioning autism with neurological deficits. Neuropediatrics [Internet]. 2007 Dec [cited 2023 Aug 17];38(6):276–81. Available from: https://pubmed.ncbi.nlm.nih.gov/18461502/

[15] Issacson HR, Moran MM, Hall A, Harmon BJ, Prekosovich MA. Autism: a retrospective outcome study of nutrient therapy. Journal of Applied Nutrition. 1996;48(4):110–8.

[16] Faber S, Zinn GM, Kern JC, Skip Kingston HM. The plasma zinc/serum copper ratio as a biomarker in children with autism spectrum disorders. Biomarkers [Internet]. 2009 May [cited 2023 Aug 17];14(3):171–80. Available from: https://pubmed.ncbi.nlm.nih.gov/19280374/

[17] Main PAE, Angley MT, O'Doherty CE, Thomas P, Fenech M. The potential role of the antioxidant and detoxification properties of glutathione in autism spectrum disorders: a systematic review and meta-analysis. Nutr Metab (Lond) [Internet]. 2012 [cited 2023 Aug 17];9. Available from: https://pubmed.ncbi.nlm.nih.gov/22524510/

[18] Bjørklund G, Doşa MD, Maes M, Dadar M, Frye RE, Peana M, et al. The impact of glutathione metabolism in autism spectrum disorder. Pharmacol Res [Internet]. 2021 Apr 1 [cited 2023 Aug 17];166. Available from: https://pubmed.ncbi.nlm.nih.gov/33493659/

[19] Hardan AY, Fung LK, Libove RA, Obukhanych T V., Nair S, Herzenberg LA, et al. A randomized controlled pilot trial

of oral N-acetylcysteine in children with autism. Biol Psychiatry [Internet]. 2012 Jun 1 [cited 2023 Aug 17];71(11):956–61. Available from: https://pubmed.ncbi.nlm.nih.gov/22342106/

[20] N-acetylcysteine for treatment of autism, a case report - PubMed [Internet]. [cited 2023 Aug 17]. Available from: https://pubmed.ncbi.nlm.nih.gov/23826003/

[21] Jennings L, Basiri R. Amino Acids, B Vitamins, and Choline May Independently and Collaboratively Influence the Incidence and Core Symptoms of Autism Spectrum Disorder. Nutrients [Internet]. 2022 Jul 1 [cited 2023 Aug 17];14(14). Available from: https://pubmed.ncbi.nlm.nih.gov/35889852/

[22] Gabis L V., Ben-Hur R, Shefer S, Jokel A, Shalom D Ben. Improvement of Language in Children with Autism with Combined Donepezil and Choline Treatment. J Mol Neurosci [Internet]. 2019 Oct 1 [cited 2023 Aug 17];69(2):224–34. Available from: https://pubmed.ncbi.nlm.nih.gov/31230222/

[23] Cochran DM, Sikoglu EM, Hodge SM, Edden RAE, Foley A, Kennedy DN, et al. Relationship among Glutamine, γ-Aminobutyric Acid, and Social Cognition in Autism Spectrum Disorders. J Child Adolesc Psychopharmacol [Internet]. 2015 May 1 [cited 2023 Aug 17];25(4):314–22. Available from: https://pubmed.ncbi.nlm.nih.gov/25919578/

[24] Deth R, Muratore C, Benzecry J, Power-Charnitsky VA, Waly M. How environmental and genetic factors combine to cause autism: A redox/methylation hypothesis. Neurotoxicology [Internet]. 2008 Jan [cited 2023 Aug 17];29(1):190–201. Available from: https://pubmed.ncbi.nlm.nih.gov/18031821/

[25] Gao J, Cahill CM, Huang X, Roffman JL, Lamon-Fava S, Fava M, et al. S-Adenosyl Methionine and Transmethylation Pathways in Neuropsychiatric Diseases Throughout Life. Neurotherapeutics [Internet]. 2018 Jan 1 [cited 2023 Aug 17];15(1):156–75. Available from: https://pubmed.ncbi.nlm.nih.gov/29340929/

[26] Ornoy A, Weinstein-Fudim L, Tfilin M, Ergaz Z, Yanai J, Szyf M, et al. S-adenosyl methionine prevents ASD-like behaviors triggered by early postnatal valproic acid exposure in very young mice. Neurotoxicol Teratol [Internet]. 2019 Jan 1 [cited 2023 Aug 17];71:64–74. Available from: https://pubmed.ncbi.nlm.nih.gov/29343446/

[27] Saad K, Eltayeb AA, Mohamad IL, Al-Atram AA, Elserogy Y, Bjørklund G, et al. A Randomized, Placebo-controlled Trial of Digestive Enzymes in Children with Autism Spectrum Disorders. Clin Psychopharmacol Neurosci [Internet]. 2015 Aug 1 [cited 2023 Aug 17];13(2):188–93. Available from: https://pubmed.ncbi.nlm.nih.gov/26243847/

[28] Dawkins T, Meyer AT, Van Bourgondien ME. The Relationship Between the Childhood Autism Rating Scale: Second Edition and Clinical Diagnosis Utilizing the DSM-IV-TR and the DSM-5. J Autism Dev Disord [Internet]. 2016 Oct 1 [cited 2023 Aug 17];46(10):3361–8. Available from: https://pubmed.ncbi.nlm.nih.gov/27422400/

[29] Chlebowski C, Green JA, Barton ML, Fein D. Using the Childhood Autism Rating Scale to Diagnose Autism Spectrum Disorders. J Autism Dev Disord [Internet]. 2010 Jul [cited 2023 Aug 17];40(7):787. Available from: /PMC/articles/PMC3612531/

[30] Dawkins T, Meyer AT, Van Bourgondien ME. The Relationship Between the Childhood Autism Rating Scale: Second Edition and Clinical Diagnosis Utilizing the DSM-IV-TR and the DSM-5. J Autism Dev Disord [Internet]. 2016 Oct 1 [cited 2023 Aug 17];46(10):3361–8. Available from: https://pubmed.ncbi.nlm.nih.gov/27422400/

[31] Moon SJ, Hwang JS, Shin AL, Kim JY, Bae SM, Sheehy-Knight J, et al. Accuracy of the Childhood Autism Rating Scale: a systematic review and meta-analysis. Dev Med Child Neurol [Internet]. 2019 Sep 1 [cited 2023 Aug 17];61(9):1030–8. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/dmcn.14246