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Randomized controlled trial of vitamin D supplementation in children with autism spectrum disorder

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Background: Autism spectrum disorder (ASD) is a frequent developmental disorder characterized by pervasive deficits in social interaction, impairment in verbal and nonverbal communication, and stereotyped patterns of interests and activities. It has been previously reported that there is vitamin D deficiency in autistic children; however, there is a lack of randomized controlled trials of vitamin D supplementation in ASD children. **Methods:** This study is a double-blinded, randomized clinical trial (RCT) that was conducted on 109 children with ASD (85 boys and 24 girls; aged 3–10 years). The aim of this study was to assess the effects of vitamin D supplementation on the core symptoms of autism in children. ASD patients were randomized to receive vitamin D3 or placebo for 4 months. The serum levels of 25-hydroxycholecalciferol (25 (OH)D) were measured at the beginning and at the end of the study. The autism severity and social maturity of the children were assessed by the Childhood Autism Rating Scale (CARS), Aberrant Behavior Checklist (ABC), Social Responsiveness Scale (SRS), and the Autism Treatment Evaluation Checklist (ATEC). Trial registration number: UMIN-CTR Study Design*:* trial number*:* UMIN000020281. **Results:** Supplementation of vitamin D was well tolerated by the ASD children. The daily doses used in the therapy group was 300 IU vitamin D3/kg/day, not to exceed 5,000 IU/day. The autism symptoms of the children improved significantly, following 4-month vitamin D3 supplementation, but not in the placebo group. This study demonstrates the efficacy and tolerability of high doses of vitamin D3 in children with ASD. **Conclusions:** This study is the first double-blinded RCT proving the efficacy of vitamin D3 in ASD patients. Depending on the parameters measured in the study, oral vitamin D supplementation may safely improve signs and symptoms of ASD and could be recommended for children with ASD. At this stage, this study is a single RCT with a small number of patients, and a great deal of additional wide-scale studies are needed to critically validate the efficacy of vitamin D in ASD.
Keywords: Autism spectrum disorder; vitamin D; children; clinical trial.

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental syndrome. ASD is characterized by pervasive deficits in social interaction, impairment in verbal and nonverbal communication, and stereotyped patterns of interests and activities. This syndrome usually occurs in children before 3 years. ASD has a complex and heterogeneous etiology, including both genetic and environmental factors (Saad, Abdel-Rahman, et al., 2016; Wang et al., 2016). Currently, there is no curative treatment for ASD, and control measures include education, supportive care, and behavioral management. Although pharmacological therapies provide an adjunct to behavioral therapy, they have no significant effects on improving the core symptoms of autism (Saad, Abdel-Rahman, et al., 2016; Saad, Eltayeb, et al., 2015; Wang et al., 2016).

Vitamin D has an important role in brain homeostasis, neurodevelopment, and aging, and it plays a significant role in gene regulation. Also, vitamin D has been shown to bind to more than 2,700 genes and regulate the expression of more than 200 of them (Cannell & Grant, 2013; Saad, Abdel-Rahman, et al., 2016). It has also been suggested that vitamin D acts as a neuroactive steroid, affecting neuronal differentiation, axonal connectivity, and brain structure and function. Furthermore, vitamin D deficiency during pregnancy is linked with several adverse effects in the fetus, such as intrauterine growth restriction and increasing the risk of autism

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development (Eyles, Burne, & McGrath, 2013; Grant & Sole, 2009; Noriega & Savelkoul, 2014). Studies have shown an association between the risk of ASD and vitamin D insufficiency. Previous data showed that 57% (70/122) of children with ASD had vitamin D deficiency, and another 30% of them (36/122) had vitamin D insufficiency (Saad, Abdel-Rahman, et al., 2016). A significant negative relationship was found between serum 25(OH)D levels and the severity of autism evaluated according to CARS scores ($p = .0001$) (Saad, Abdel-Rahman, et al., 2016). Maternal vitamin D deficiency during pregnancy and/or early childhood is considered as a possible risk factor in the development of ASD (Grant & Sole, 2009). In addition, another study has shown that ASD is more common in places with impaired solar UVB penetration (Wang et al., 2016). Considering the increasing prevalence of ASD and the promising results of our open-label study about the role of vitamin D in autistic children (Saad, Abdel-Rahman, et al., 2016), it is timely to evaluate the effect of vitamin D supplementation on ASD. In particular, this randomized clinical trial (RCT) aimed to evaluate the effect of vitamin D supplementation on the core symptoms of ASD in children. We hypothesize that vitamin D supplementation will decrease the intensity of the ASD symptoms in autistic children.

Methods

This study has been carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. All procedures are approved by the Ethical Committee of Qena Faculty of Medicine, South Valley University, Egypt. Participants were given a complete description of the study, and a written consent was obtained from the parents, in accordance with Ethical Committee guidelines (South Valley University).

Study design

This study was a 4-month, double-blinded, randomized, placebo-controlled trial undertaken in the outpatient clinics at the Assiut University Hospitals and five private autism treatment centers in Assiut city, Egypt, from June 2015 to October 2015.

Participants

Subjects were 120 children with ASD selected from a sample of 249 autistic children. All study populations were in a good nutritional state. In total, 129 children with ASD were excluded. Of these patients, 119 were excluded as they did not meet the inclusion criteria (summarized in Table 1) and 10 others were excluded as their families declined to participate in the study. All patients included in the study were recruited from the neuropediatric clinics at the Assiut University Hospitals and five private centers for ASD in Assiut city, Upper Egypt.

Initial clinical and psychiatric assessment

The ASD diagnosis was confirmed according to the *Diagnostic and Statistical Manual of Mental Disorders, 5th edition* (DSM-5) (American Psychiatric Association, 2013). Also, structured parent interviews of at least 2 hr proved the autistic manifestations. Later on, another 3-hour session was used for the assessment of autism severity according to the Childhood Autism Rating Scale (CARS) (Schopler, Van Bourgondien, Wellman, & Love, 2010). CARS evaluates behavior in 14 domains that are affected by ASD, plus one parameter of the general impression of autism. The 14 domains are as follows: (a) relating to people; (b) imitation, social-emotional understanding; (c) emotional response, emotional expression, and regulation of emotions; (d) body use; (e) object use, object use in play; (f) adaptation to change, adaptation to change/restricted interests; (g) visual response; (h) listening response; (i) taste, smell, and touch response and use; (j) fear or nervousness, fear or anxiety; (k) verbal communication; (l) nonverbal communication; (m) activity level, thinking/cognitive integration skills; and (n) level and consistency of intellectual response. The examiner assigned scores between 1 and 4 for each domain: 1 indicates normal behavior appropriate for age level (no signs of autism), while 4 indicates a severe deviance with respect to the normal behavior (severe symptoms of autism). The scores for the single items are added together into a total score. The maximum CARS score is 60, and the cutoff for autism is 30. A total score between 15 and 29.5 is considered nonautistic. Scores of 30.5–37 were rated as mildly–moderately autistic, while scores above 37. 5 were rated as severely autistic (Geier, Kern, & Geier, 2013; Schopler et al., 2010).

A carefully collected detailed history from the parents about each child was included in the study. The anamnesis included information about the family history of consanguinity, similar conditions of ASD in the family, social activities, self-care, and time of onset of the autistic manifestations. Also, the prime investigator carried out meticulous physical and neurological examinations (including sensory, motor, and autonomic evaluations) of all the patients.

Each family also completed a baseline dietary record, where food items consumed were classified into relevant food groups, such as cereals, legumes, vegetables, meat, dairy, and fruits. This was used to calculate food diversity. A minimum of four food groups was considered as adequate mixture. At each visit, a full physical and neurological examination was completed for patients who showed any negative symptoms.

Laboratory investigations

Prior to the randomization into a study group, each patient underwent laboratory screening tests which included complete blood analysis with differential counts, serum lead, calcium, phosphorus, potassium, glucose, and stool analysis (for the exclusion of parasitic, inflammatory, and infectious pathologies of the intestine). Liver and kidney function tests were also

Table 1 Exclusion criteria

Autistic children with vitamin D deficiency (<20 ng/ml), they received vitamin D directly by the authors
Significant hearing or vision loss
Other neurological disorders, e.g., cerebral palsy, phenylketonuria, tuberous sclerosis, neurofibromatosis, and seizure disorders
History of severe head trauma or stroke
Children with feeding problems or malnutrition
Genetic disorders
Prematurity
Subjects who had associated gastrointestinal problems, autoimmune disorders, and anemia
Children with known endocrine, cardiovascular, pulmonary, and liver or kidney disease
Subjects taking the following medications or supplements: cod liver oil, vitamin A, steroids, thiazide diuretics, digoxin, diltiazem, verapamil, cimetidine, heparin within the preceding 2 months before the study

performed. The serum level of 25-hydroxycholecalciferol (25 (OH)D) was estimated using a vitamin D ELISA kit (Immundiagnostik AG, Bensheim, Germany). All laboratory variables and tests were performed twice before the beginning of the study and after 4 months at the end of the trial. Normal level of vitamin D was defined as a 25-OHD concentration >30 ng/ml, vitamin D insufficiency was defined as when levels fall between 20 and 30 ng/ml, and vitamin D deficiency was defined as when levels were <20 ng/ml (Holick, 2007).

Assignment of the study procedures

After the baseline evaluation and random assignment to the vitamin D or placebo group, 120 subjects were contacted 1 month before the study by telephone and then returned for an in-person evaluation; the patients were divided into six groups (Figure 1). Every group consisted of 18–22 patients and evaluated by two pediatricians, one psychiatrist, and two experienced psychologists. Double blinding was maintained throughout the trial. Randomization was based on a random number generator in blocks of 10; the randomization process did not involve any stratifying variables. The project coordinator numbered the bottles and provided them (in complete blocks) to each of the six ASD centers. The bottle numbering (allocation) was performed distant from any enrolling site, with bottle status concealed from all children, parents, and enrolling/evaluating clinician investigators. Eleven patients dropped out, and 109 completed the 4-month study.

Group I consisted of 55 patients [43 boys (78.2%)] who were randomly allocated to receive vitamin D3 drops, 300 IU/kg/day not to exceed 5,000 IU/day (cholecalciferol drops; MUP Laboratory, Egypt; quality certified by the Egyptian Ministry of Health) for 4 months. Group II consisted of 54 patients [42 boys (77.8%)] who received a matching placebo drops with the same taste and color of vitamin D3 drops for 4 months. The placebo consisted of a combination of polysorbate 20, which is the same fragrance ingredient used in vitamin D drops in addition to glycerin, disodium edetate, and β-cyclodextrin in purified water. So children, parents, and researchers were unable to tell whether children were on vitamin D supplementation or placebo. Caregivers of participating children received a weekly telephone call from the research team who reminded them to administer the therapy. They were not allowed to change the provided dose or to add any supplements or pharmacotherapies throughout the study period. At follow-up visits, the parents were asked to return the empty bottles every month and to report any difficulties or adverse effects during the study period. Side effects were recorded throughout the study and were assessed using a checklist every month.

Outcome measures

The study examiners in the six groups evaluated the patients at the beginning and at the end of the study (4 months after the medication started). All the scales for each child were completed by two different psychologists and a senior psychiatrist. Four tools were used for psychiatric assessment of ASD patients.

Aberrant Behavior Checklist. The Aberrant Behavior Checklist (ABC) is a 58-item behavior rating scale used to measure behavior problems across five subscales. Items are rated on a 4-point scale, ranging from 0 (no problem) to 3 (severe problem), with higher scores indicating more severe problems (Aman, Singh, Stewart, & Field, 1985). ABC scores were calculated for all participants before and after the therapeutic intervention.

Childhood autism rating scale. The CARS is a well-established scale for the screening and classification of childhood autism with good agreement between DSM diagnostic criteria and CARS. The internal consistency reliability alpha coefficient is .94, the interrater reliability correlation coefficient is .71, and the test–retest correlation coefficient is .88. CARS is appropriate for use with any child over 2 years of age. Psychiatrists and psychologists reported that it is a consistent and stable indicator of ASD (Geier et al., 2013; Schopler et al., 2010). In this study, CARS scores were calculated for all

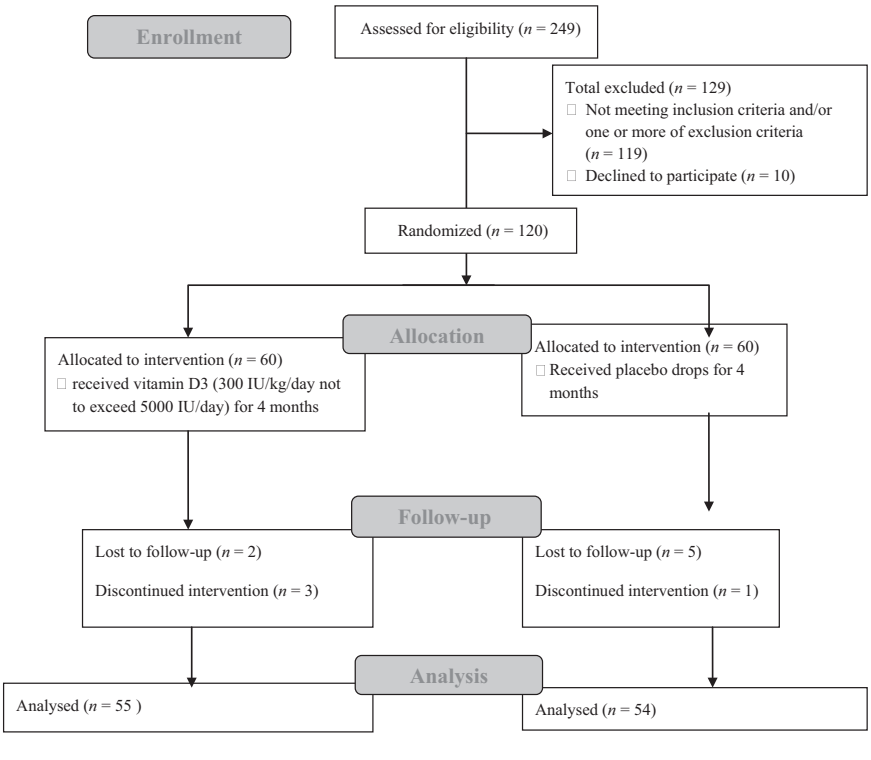

Figure 1 Consort flow diagram

participants before and after the therapeutic intervention (vitamin D and placebo).

Autism Treatment Evaluation Checklist. The Autism Treatment Evaluation Checklist (ATEC) is a questionnaire that was developed by the Autism Research Institute to evaluate the treatment efficacy in autistic individuals (Rimland & Edelson, 1999). The ATEC is suitable for ASD children 2 years of age and older. It consists of four subscales labeled: Speech/language/communication, sociability, sensory/cognitive awareness, and health/physical/behavior. The four subscale scores can be used to calculate a total score (total scores can range from 0 to 180). The higher the scores, the higher the ASD symptoms. ATEC is designed to allow the examiner to assess the outcomes of any treatments used in patients with ASD (Geier et al., 2013). In this study, ATEC scores were calculated for all participants before and after the therapeutic intervention (vitamin D and placebo).

Social Responsiveness Scale. The Social Responsiveness Scale (SRS) consists of 65 items, used for quantitative assessment of the severity of ASD symptoms. The SRS has been extensively validated and distinguishes ASD from other psychiatric disorders. The SRS is also sensitive to autistic traits and symptoms even in subthreshold ASD conditions (Constantino & Gruber, 2005; Frazier et al., 2014). This study used SRS scores for all patients before and after therapy.

Statistical analysis

Results were presented as mean ± standard deviation (SD). Statistical differences in the scores of each scale, before and after the 4-month vitamin D supplementation period, were determined by a paired *t*-test. Significance between groups was tested with linear regression analysis. Statistical Package for Social Sciences program (SPSS; Inc., Chicago, IL, USA) version 16 was used for data analysis. Results were considered statistically significant with p values ≤ .05.

Results

As shown in Figure 1, of the 120 patients who were eligible for this study, 109 completed the 4-month study. In the vitamin D-supplemented group (Group I), five patients dropped out, two of them were lost to follow-up after the baseline visit, and three patients discontinued the therapy: two of them because of skin rashes and one due to diarrhea. Regarding the placebo group (Group II), six patients were dropped out. Five patients were lost to follow-up, and one dropped out because his parents started another pharmacotherapy. The adverse effects reported by caregivers were mild, transient, and all other patients continued the study.

Tables 2 and 3 show demographic data and classification of autism of the two studied groups. Subjects were 3–10 years old (mean age 5.4 ± 2.5 years). The study included 85 males (78%) with male to female ratio 3.5:1. Eighty-one children (74.3%) were diagnosed before the age of 3 years, while 28 (25.7%) were diagnosed after 3 years.

The baseline CARS scores ranged from 30 to 54 (mean 36.9; SD 6.1). No significant differences were identified between the two randomized groups regarding the age, sex, weight, and classification of ASD. There was no significant difference between both groups in baseline CARS scores before intervention (mean ± SD, 36.8 ± 5.9 vs. 37.1 ± 4.7; p = .52).

Table 2 Demographic data and classification of autism of the studied group (N = 109)

Characteristics	
Age (years)	
Range	3–10
Mean ± SD	5.4 ± 2.5
Gender (number/%)	
Males	85 (78)
Females	24 (22)
Age at diagnosis	
Before 3 years (number/%)	81 (74.3)
After 3 years (number/%)	28 (25.7)
CARS classification of autism	
Severe (≥37) (%)	40 (36.7)
Mild/moderate (≤36.5) (%)	69 (63.3)

Table 3 Comparison between groups in some demographic data

Parameter	Group I (55 patients), vitamin D group	Group II (54 patients), placebo group	*p*-value
Age			
Range (years)	3–10	3–10	–
Mean age ± SD	5.3 ± 1.9	5.6 ± 2.7	NS
Gender			
Males (%)	43 (78.2)	42 (77.8)	NS
Females (%)	12 (21.8)	12 (22.2)	NS
Weight (Mean ± SD)	21.6 ± 6.7	20.9 ± 8.2	NS

NS = nonsignificant.

After the 4-month study duration, the total CARS scores were significantly decreased (improved) in the vitamin D-supplemented group (Group I) compared with the placebo group (Group II) (95% CI: 0.03–0.42; p = .02) (Table 4).

Table 4 shows the total CARS evaluation scoring system before and after the interventions. In the vitamin D-supplemented group (Group I), CARS scores were significantly improved after vitamin D therapy (mean score before = 36.8 ± 5.9, after = 30.3 ± 6.1, p < .001). There were no significant changes in the placebo group (mean score before = 37.1 ± 4.7, after = 36.4 ± 6.0, p = .34). The scores of CARS parameters were as follows: relating to people, emotional response, imitation, body use, object use, adaptation to change, listening response, visual response, and general autistic impression were significantly improved in the vitamin D-supplemented group (Group I). A decrease of 4–10 points in the total CARS scores (improvement) was found in 42 (76.4%) patients treated with vitamin D supplement, while other 10 (18.2%) patients had a 1- to 3-point improvement. Only three

Table 4 Classification of autism and outcome of psychiatric parameters of the two studied groups

	Group I (55 patients), vitamin D group	Group II (54 patients), placebo group	95% CI	*p*-value between groups
Total CARS scores				
Before therapy (vitamin D or placebo)	36.8 ± 5.9	37.1 ± 4.7	0.03 to 0.42	.02*
After therapy (vitamin D or placebo)	30.3 ± 6.1	36.4 ± 6.0		
Classification of autism				
Severe (CARS ≥ 37) (%)	21 (38.2)	19 (35.2)	−0.31 to 0.32	NS
Mild/moderate (CARS ≤ 36.5) (%)	34 (61.8)	35 (64.8)		
Autism Treatment Evaluation Checklist (ATEC) scores				
Before therapy (vitamin D or placebo)	71.9 ± 16.1	72.2 ± 18	35.2 to 50.9	<.01*
After therapy (vitamin D or placebo)	47.3 ± 6.5	73.1 ± 15.3		
Total Social Responsiveness Scale (SRS) scores				
Before therapy (vitamin D or placebo)	74.8 ± 3.3	75.6 ± 2.8	32.9 to 48.1	<.01*
After therapy (vitamin D or placebo)	71.1 ± 4.5	75 ± 2.7		

Data are expressed as mean ± SD, unless otherwise stated. Significance between groups was tested with linear regression analysis. *Significant values, NS = nonsignificant.

patients showed no improvements (5.4%). However, nine patients (16.7%) in the placebo group had a decrease of 4–10 points in the total CARS scores, 15 patients (27.8%) had a 1- to 3-point improvement, and 55.5% of the patients showed no improvement.

Regarding ABC scale, the mean ABC scores for the two randomized groups along with each subscale were calculated (Table 5). The scores of ABC subscales were significantly improved in the vitamin D-supplemented group (Group I) compared with the placebo group (Group II). There were statistically significant improvements in irritability (95% CI: 0.63–1.89, $p = .01$), hyperactivity (95% CI: 1.02–2.72, $p = .014$), social withdrawal (95% CI: 1.22–2.84, $p = .003$), stereotypic behavior (95% CI: −5.9 to −0.39, $p < .01$), and inappropriate speech (95% CI: −6.1 to −0.41, $p < .01$).

As regard the SRS scale, the mean scores for the two groups along with each subscale were calculated (Tables 4 and 5). The SRS evaluations showed a statistically significant improvement in the total mean SRS scores in the vitamin D-supplemented group (Group I) compared with the placebo group (Group II) (95% CI: 32.9–48.1, $p < .01$) (Table 4). After the 4-month study duration, there was a statistically significant improvement in SRS subscale scores in autistic mannerism (95% CI: 35.1–52.0, $p < .01$), social cognition (95% CI: 36.4–52.3, $p < .001$), and social awareness (95% CI: 1.17–1.60, $p < .001$) for the vitamin D-supplemented group (Group I); however, there were no significant changes in social communication (95% CI: −0.11 to 0.07, $p = .16$) and social motivation (95% CI: −12.0 to 7.2, $p = .56$) in the vitamin

Table 5 Outcome measures in ASD patients before and after vitamin D and placebo

	Group I (55 patients) Mean ± SD scores		Group II (54 patients) Mean ± SD scores			
Subscales	Before vitamin D therapy	After vitamin D therapy	Before placebo	After placebo	95% CI	*p*-value
Aberrant Behavior Checklist (ABC)						
Irritability	22.3 ± 5.9	9.5 ± 3.7	21.5 ± 4.6	22.8 ± 5.3	0.63 to 1.89	<.01*
Hyperactivity	26.1 ± 11.7	15.5 ± 7.8	27.2 ± 10.1	25.9 ± 9.9	1.02 to 2.72	.014*
Lethargy/social withdrawal	18.1 ± 6.2	10.6 ± 5.7	17.9 ± 10.3	18.2 ± 8.1	1.22 to 2.84	.003*
Inappropriate speech	6.6 ± 2.5	3.6 ± 2.8	6.4 ± 3.1	5.8 ± 3.5	−6.1 to −0.41	<.01*
Stereotypic behavior	6.1 ± 2.9	3.7 ± 4.2	5.9 ± 2.6	5.4 ± 4.0	−5.9 to −0.39	<.01*
Autism Treatment Evaluation Checklist (ATEC)						
Communication	12.3 ± 7.9	12.0 ± 6.8	13.3 ± 6.1	12.9 ± 7	−2.7 to 3.4	NS
Sociability	19.4 ± 8.5	12.1 ± 7.1	19.7 ± 6	20.9 ± 6.2	1.30 to 3.71	.004*
Cognitive awareness	19.5 ± 12.0	15.0 ± 3.7	20.2 ± 4.6	20 ± 3.3	1.16 to 1.92	<.05*
Behavior	21 ± 9.6	9.3 ± 7.9	20.7 ± 7.4	20.2 ± 8.3	1.06 to 1.96	<.05*
Social Responsiveness Scale (SRS)						
Social awareness	79 ± 6.9	68.7 ± 3.7	78 ± 5.6	78 ± 4.7	1.17 to 1.60	<.001*
Social cognition	76.3 ± 5.1	70.4 ± 4.8	75.2 ± 4.2	76 ± 3.7	36.4 to 52.3	<.001*
Social communication	74.3 ± 6.1	73.7 ± 5.2	75 ± 3.3	74.4 ± 5.3	−0.11 to 0.07	NS
Social motivation	70.4 ± 5.4	70.7 ± 7.2	71.1 ± 1.5	70.9 ± 2.2	−12.0 to 7.2	NS
Autistic mannerism	75.9 ± 7.2	70.4 ± 4.3	74.7 ± 3.4	74.3 ± 4.1	35.1 to 52.0	<.01*

Data are expressed as mean ± SD. Significance between groups was tested with linear regression analysis. *Significant values, NS = nonsignificant.

D-supplemented group when compared with placebo group (Table 5).

The ATEC (mean ± SD) scores of ASD patients of the vitamin D-supplemented and placebo groups are shown in Tables 4 and 5. The ATEC assessments showed statistically significant improvement in the total mean of the ATEC scores in Group I compared with Group II (95% CI: 35.2–50.9, $p < .01$) (Table 4). At the end of the study duration, there was a statistically significant improvement in the ATEC subscale scores for sociability ($p = .004$), cognitive awareness ($p < .05$), and behavior ($p < .05$) in Group I. No significant change was observed in communication parameter (95% CI: −2.7 to 3.4, $p = .74$) between the two groups (Table 5).

The mean baseline serum levels of 25 (OH)D were comparable between both randomized groups (mean ± SD, 26.3 ± 12.7 vs. 27.1 ± 15.1; $p = .43$). No significant difference was found in the serum levels of 25(OH) D between ASD boys and ASD girls. At the end of the study, the mean serum levels of 25 (OH)D were significantly elevated in the vitamin D-supplemented group (Group I) (mean ± SD, 45.9 ± 17.2 vs. 26.3 ± 12.7; $p = <.001$). However, no significant change for 25 (OH)D was found in the placebo group (mean ± SD, 27.1 ± 15.1 vs. 28.2 ± 13.8; $p = .41$).

Biological markers

Table 6 shows some biochemical parameters of the studied groups. There were no significant differences between the ASD groups in CBC variables, serum lead, calcium, potassium, phosphorus, glucose, alkaline phosphatase, alanine transaminase (ALT), aspartate transaminase (AST), blood urea nitrogen, and serum creatinine at baseline and after 4 months. Measurement of the biological markers was normal before and after vitamin D and placebo administration in either randomized group.

Adverse events

The high dose of vitamin D supplementation was well tolerated, with few adverse effects. Some transient side effects during the 4-month study period, such

Table 6 Biochemistry parameters among of the two studied groups before and after therapy

Biochemical parameters (units)	Group I (55 patients), vitamin D group	Group II (54 patients), placebo group	*p*-value
Vitamin D levels (ng/ml)			
Before therapy (vitamin D or placebo)	26.3 ± 12.7	27.1 ± 15.1	NS
After therapy (vitamin D or placebo)	45.9 ± 17.2	28.2 ± 13.8	<.001*
Calcium (mmol/L)			
Before therapy (vitamin D or placebo)	2.23 ± 0.2	2.12 ± 0.3	NS
After therapy (vitamin D or placebo)	2.25 ± 0.1	2.15 ± 0.2	NS
Phosphorous (mmol/L)			
Before therapy (vitamin D or placebo)	1.42 ± 0.4	1.45 ± 0.2	NS
After therapy (vitamin D or placebo)	1.45 ± 0.2	1.44 ± 0.3	NS
Magnesium (mmol/L)			
Before therapy (vitamin D or placebo)	0.83 ± 0.2	0.84 ± 0.2	NS
After therapy (vitamin D or placebo)	0.85 ± 0.2	0.84 ± 0.1	NS
Glucose (mmol/L)			
Before therapy (vitamin D or placebo)	4.38 ± 0.5	4.41 ± 0.4	NS
After therapy (vitamin D or placebo)	4.39 ± 0.3	4.37 ± 0.3	NS
Potassium (mmol/L)			
Before therapy (vitamin D or placebo)	5.41 ± 0.3	5.50 ± 0.3	NS
After therapy (vitamin D or placebo)	5.51 ± 0.5	5.48 ± 0.4	NS
Alkaline phosphate (U/L)			
Before therapy (vitamin D or placebo)	163.8 ± 69.1	159.2 ± 78.2	NS
After therapy (vitamin D or placebo)	160.3 ± 77.3	158.7 ± 72.4	NS
Lead (mcg/dl)			
Before therapy (vitamin D or placebo)	2.9 ± 0.8	3.1 ± 0.7	NS
After therapy (vitamin D or placebo)	3.0 ± 0.6	3.1 ± 0.8	NS
Blood urea nitrogen (BUN) (mg/dl)			
Before therapy (vitamin D or placebo)	10.1 ± 3.2	9.8 ± 2.5	NS
After therapy (vitamin D or placebo)	10.2 ± 3.6	10 ± 3.5	NS
Serum creatinine (mg/dl)			
Before therapy (vitamin D or placebo)	0.8 ± 0.2	0.7 ± 0.4	NS
After therapy (vitamin D or placebo)	0.9 ± 0.6	0.8 ± 0.3	NS
AST (U/L)			
Before therapy (vitamin D or placebo)	13.4 ± 2.9	12.5 ± 3.1	NS
After therapy (vitamin D or placebo)	12.7 ± 3.2	12.5 ± 1.9	NS
ALT (U/L)			
Before therapy (vitamin D or placebo)	9.9 ± 4.6	10.3 ± 6.1	NS
After therapy (vitamin D or placebo)	9.5 ± 5	9.6 ± 7.4	NS

Data are expressed as mean ± SD. *Significant values, NS = nonsignificant.

as skin rashes, itching, and diarrhea, were reported in five (8.3%) patients in Group I. All side effects were mild, transient, and only three patients discontinued the vitamin D treatment.

Discussion

There has been a dramatical increase in the prevalence of ASD all over the world during the last two decades. In 2010, the Centers for Disease Control and Prevention reported that one in every one hundred ten 8-year-old children in the United States was diagnosed with ASD. In 2014, the prevalence of ASD in the United States was estimated to be one of every forty-five 8-year-old children (2.24%) (Zablotsky, Black, Maenner, Schieve, & Blumberg, 2015). It is not known to what extent the exceptional growth in the prevalence of all forms of ASD combined is real or not. However, there can be no doubt that the registration of ASD must have been affected by a change in the concept regarding dimensional perspective, substitution, and 'addition' of diagnoses, as well as greater awareness, new therapeutic options, and specific approaches to the education of these children (Cannell & Grant, 2013; Saad, Eltayeb, et al., 2015).

New researches indicated that vitamin D insufficiency may be a significant risk factor in ASD. Vitamin D has a potential role in brain homeostasis and development, such as neuronal differentiation, neuronal migration and growth, neurotransmission, and synaptic function (Harms, Burne, Eyles, & McGrath, 2011; Wang et al., 2016). Research has also linked other neurological and neuropsychiatric conditions, such as multiple sclerosis and schizophrenia, to prenatal vitamin D deficiency (Wang et al., 2016).

Pharmacological interventions in ASD are mainly aimed to reduce commonly associated symptoms, including inattention, impulsivity, hyperactivity, compulsions, anxiety, sleep disturbances, irritability, self-injury, and aggression. Currently, there is no recognized effective approved pharmacotherapy for the core symptoms of ASD. Therefore, many research groups all over the world try to reach a safer drug modality for ASD.

In this study, vitamin D supplementation revealed significant effects on the core manifestations of ASD. The scores of ABC subscales significantly improved in the group that received vitamin D (Group I) but not in the placebo group (Group II). The parents of the children in Group I rated significant improvement in irritability, hyperactivity, social withdrawal, stereotypic behavior, and inappropriate speech (Table 5).

The total scores from the evaluations with SRS and ATEC improved significantly in Group I compared with Group II (Table 4). The participants in Group I showed significantly fewer autistic mannerisms in the SRS evaluation compared with Group II ($p < .01$); 61.8% (34/55) of the ASD patients in Group I showed significantly decreased repetitive hand movements, creation of noises, jumping, and restricted interests as compared with 25.9% (14/54) in the placebo group. Furthermore, significant improvements in social cognition and social awareness ($p < .001$ for both) were found in Group I compared with Group II.

The ATEC evaluation displayed significant improvement in the scores for sociability ($p = .004$), cognitive awareness ($p < .05$), and behavior ($p < .05$) in Group I compared with Group II. In Group I, the total CARS scores were significantly improved after vitamin D therapy ($p = .02$). Furthermore, significant improvements were found in nine domains of the CARS in Group I after vitamin D3 therapy.

During the last decade, researchers have demonstrated significant advances in clarifying the biochemical mechanisms of vitamin D in the brain, especially its role in neurodevelopmental and degenerative processes. Vitamin D plays a role in cell differentiation, axonal growth, stimulation of neurotrophic factor expression, regulation of calcium signaling, modulation of the production of brain-derived reactive oxygen species, stimulation of glutathione (GSH), and down-regulation of excitotoxicity (Eyles et al., 2013; Kočovská et al., 2014; Wang et al., 2016). Oxidative stress may be a common feature in ASD. Antioxidants, especially GSH, are essential for neural survival during the early critical period (Rossignol & Frye, 2012). Vitamin D can increase the cellular level of GSH, which is crucial for the control of detoxification processes in the brain (Garcion, Wion-Barbot, Montero-Menei, Berger, & Wion, 2002; Wang et al., 2016).

Recently, Wang et al. (2016) performed a systematic review and meta-analysis of all studies on serum concentration of 25 (OH)D in ASD (Wang et al., 2016). Eleven studies were included, accounting for a total of 870 ASD patients and 782 healthy controls. Serum levels of 25 (OH)D in participants with ASD were significantly lower than those in controls. They concluded that low vitamin D might serve as a risk factor for autism spectrum disorder (Wang et al., 2016). However, data on vitamin D supplementation for children with ASD are still very limited.

In a recent survey, our research group measured 25 (OH)D in 122 ASD children (3–9 years old) and 100 healthy children as controls (Saad, Abdel-Rahman, et al., 2016). The ASD group showed a significantly lower level of serum 25 (OH)D compared with the control group ($p < .0001$). The study found highly significant inverse correlations between serum 25 (OH)D levels and autism rating scales. In the second part of the previous study (Saad, Abdel-Rahman, et al., 2016), an open-label trial of 83 subjects who completed a 3-month therapy with high daily doses of vitamin D (300 IU/kg/day) was performed. Collectively, 80.7% of the children with ASD had significantly improved outcome, which was mainly in the sections of the CARS and ABC

subscales that measure behavior, stereotypy, eye contact, and attention span (Saad, Abdel-Rahman, et al., 2016).

In a recent case report, a 32-month-old Chinese toddler with severe ASD was tested before and after vitamin D treatment (Jia et al., 2015). Before vitamin D treatment, the patient scores on autism assessments were 80 for the Autism Behavior Checklist, 35 for CARS, and 6 for the Severity of Illness of Clinical Global Impression. The serum vitamin D level was 12.5 ng/ml. The child was given vitamin D3 150,000 IU every month intramuscularly for 2 months and 400 IU/day orally for 2 months. After vitamin D therapy, the patient's parents reported a significant improvement in behavioral problems and core symptoms of ASD (Jia et al., 2015). The same group of Chinese scientists performed a very recent open-label trial of vitamin D3 in ASD children (Feng et al., 2017). The study found highly significant inverse correlations between serum 25 (OH)D levels and ABC total scores and language subscale scores. Vitamin D3 was intramuscularly administered at a dosage of 150,000 IU per month (in total three injections) and orally administered at a dosage of 400 IU/day (in total 3 months). After vitamin D3 supplementation, the symptom scores were significantly reduced on the CARS and ABC. In addition, the study suggested that treatment effects were more pronounced in younger children with ASD (Feng et al., 2017).

Research progressively indicates that vitamin D deficiency may be a risk factor for the development and progressing of ASD (Cannell & Grant, 2013; Grant et al., 2015; Saad, Abdel-Rahman, et al., 2016; Wang et al., 2016). A study of the prevalence of ASD in children aged 6–17 years in the United States showed a significant inverse correlation between the incidence of ASD and the exposure to ultraviolet B (shortwave) rays, as measured by the level of ultraviolet (UV) radiation in the child's state of birth (Grant & Cannell, 2013). It is possible that vitamin D insufficiency during pregnancy could account for this outcome, although also childhood vitamin D deficiency may contribute to the condition (Cannell & Grant, 2013; Grant & Cannell, 2013). Research has also shown that children born in overcast and rainy counties of Oregon, Washington, and California are twice as likely to be diagnosed with ASD compared with children born in sunnier parts of these states (Waldman, Nicholson, Adilov, & Williams, 2008). Consequently, there is an inverse correlation between the rapid rise in ASD incidence and the percentage of the US population with low plasma concentrations of 25 (OH)D (Patrick & Ames, 2014). Patrick and Ames (2014) suggested a mechanism linking vitamin D deficiency to the risk of ASD (Patrick & Ames, 2014). They proposed that sufficient levels of vitamin D hormone may be essential for activation of the serotonin-synthesizing gene tryptophan hydroxylase 2 (TPH2) and accordingly elevation of serotonin concentrations in the brain and inversely affects serotonin production in peripheral tissues. Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter and plays a major role in regulating emotions during social decision-making. Patients with ASD may show lower concentrations of brain serotonin and have higher serotonin levels in the blood (Crockett, Clark, Tabibnia, Lieberman, & Robbins, 2008; Patrick & Ames, 2014). The vitamin D-mediated production of serotonin would be critical to produce serotonergic signals during neurodevelopment, thus shaping the developing brain, and throughout adulthood, where it plays a crucial role in regulating multiple brain functions including social behavior. Also, adequate vitamin D hormone levels would suppress the expression of tryptophan hydroxylase 1 (TPH1), which has important implications for lowering gastrointestinal inflammation, increasing bone mineral density, and keeping autoimmunity at bay (Patrick & Ames, 2014). The same study indicated that to increase the concentration of 25 (OH)D might improve some core symptoms of ASD (Patrick & Ames, 2014).

Vitamin D plays a role in the regulation of the synthesis and response to oxytocin, the regulation of oxytocin-related genes, and the response to vasopressin (Patrick & Ames, 2014; Wang et al., 2016). Positive therapeutic results of oxytocin have been reported in ASD patients, including improved retention of speech comprehension and enhanced mind-reading performance, social communication, and interaction (Aoki et al., 2015; Guastella et al., 2015). Research has also shown that ASD children have lower plasma concentrations of oxytocin as compared with nonautistic children (Ashwood et al., 2011). In dark-skinned mothers living in northern latitudes, an increased incidence of ASD has been related to maternal vitamin D insufficiency. Furthermore, research indicates that low levels of maternal 25 (OH)D increase the risk of having a child with behavioral problems associated with ASD, such as language impairment and attention-switching problems (Patrick & Ames, 2014; Whitehouse et al., 2013).

Activated vitamin D (1, 25-dihydroxyvitamin D), a secosteroid, upregulates the DNA repair genes. Therefore, vitamin D deficiency during early development may inhibit the repair of de novo DNA mutations in fetuses and contribute to the risk of ASD (Cannell & Grant, 2013). Vitamin D might also decrease the severity of ASD through its anti-inflammatory effects. It inhibits the synthesis of proinflammatory prostaglandins, which are elevated in ASD. Also, vitamin D inhibits NF-κB, which is involved in aberrant signaling in the brain of individuals with ASD (Feng et al., 2017; Tamiji & Crawford, 2010).

Vitamin D deficiency may alter the immune responses in individuals with ASD (Ashwood et al.,

2011). ASD patients often have elevated levels of autoimmune markers, such as anti-nuclear antibodies, anti-ganglioside M1 antibodies, anti-MBP autoantibodies, and antinucleosome-specific antibodies (Cannell & Grant, 2013; Rossignol & Frye, 2012; Wang et al., 2016). Some studies have shown that the levels of these markers significant positively correlate with the severity of autism (Cannell & Grant, 2013; Rossignol & Frye, 2012; Wang et al., 2016). Vitamin D can decrease inflammation and produce immune protective effects (Jones, Tulic, Rueter, & Prescott, 2012). Vitamin D-1,25$(OH)_2$D3 can activate helper T cells, regulatory T cells, and activate B cells and dendritic cells. The anti-autoimmune effects of vitamin D may explain the reported epidemiological associations between vitamin D status and many autoimmune disorders. Therefore, vitamin D may have a potential role in treating diseases with autoimmune involvement, such as ASD (Cannell & Grant, 2013).

Conclusion

ASD is a severe, lifelong disorder with serious emotional and financial consequences. This study is the first double-blinded RCT proving the efficacy of vitamin D3 in ASD patients. Depending on the parameters measured in the study, oral vitamin D supplementation may safely improve signs and symptoms of ASD and could be recommended for children with ASD. At this stage, this study is a single RCT with a small number of patients and a great deal of additional wide-scale studies is needed to critically validate the efficacy of vitamin D in ASD. We recommended further studies to investigate the correlations between the clinical response of vitamin D and the biochemical changes in ASD patients.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. CONSORT 2010 checklist of information.

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Key points

- It has been previously reported that there is vitamin D deficiency in autistic children; however, there is a lack of randomized controlled trials of vitamin D in ASD children.
- This study is the first double-blinded, randomized clinical trial that was conducted on children with ASD.
- 300 IU vitamin D3/kg/day of vitamin D was generally well tolerated by the ASD children.
- The autism symptoms improved significantly, following 4 months of vitamin D3 supplementation, but not in the placebo group. This study demonstrates the efficacy and tolerability of high doses of vitamin D3 in children with ASD.

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