Vitamin D status in autism spectrum disorders and the efficacy of vitamin D supplementation in autistic children

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Objectives: Autism spectrum disorder (ASD) is a developmental disorder characterized by pervasive deficits in social interaction, impairment in verbal and non-verbal communication, and stereotyped patterns of interests and activities. Vitamin-D deficiency was previously reported in autistic children. However, the data on the relationship between vitamin D deficiency and the severity of autism are limited.
Methods: We performed a case–controlled cross-sectional analysis conducted on 122 ASD children, to assess their vitamin D status compared to controls and the relationship between vitamin D deficiency and the severity of autism. We also conducted an open trial of vitamin D supplementation in ASD children.
Results: Fifty-seven percent of the patients in the present study had vitamin D deficiency, and 30% had vitamin D insufficiency. The mean 25-OHD levels in patients with severe autism were significantly lower than those in patients with mild/moderate autism. Serum 25-OHD levels had significant negative correlations with Childhood Autism Rating Scale (CARS) scores. Of the ASD group, 106 patients with low-serum 25-OHD levels (<30 ng/ml) participated in the open label trial. They received vitamin D3 (300 IU/kg/day not to exceed 5000 IU/day) for 3 months. Eighty-three subjects completed 3 months of daily vitamin D treatment. Collectively, 80.72% (67/83) of subjects who received vitamin D3 treatment had significantly improved outcome, which was mainly in the sections of the CARS and aberrant behavior checklist subscales that measure behavior, stereotypy, eye contact, and attention span.
Conclusion: Vitamin D is inexpensive, readily available and safe. It may have beneficial effects in ASD subjects, especially when the final serum level is more than 40 ng/ml.
Trial registration number: UMIN-CTR Study Design: trial Number: R000016846.

Keywords: Autism, Neurodevelopmental, Children, Vitamin D

Introduction

Autism spectrum disorder (ASD) is a developmental disorder characterized by pervasive deficits in social interaction, impairment in verbal and non-verbal communication, and stereotyped patterns of interests and activities. The previous version of the manual (DSM-IV-TR) listed three distinct subgroups for ASD: autistic disorder, Asperger's syndrome, and pervasive developmental disorder not otherwise specified.[1] In 2013 were the separate diagnostic labels replaced by one umbrella term ASD in the new version of the manual (DSM-V).[2]

ASD has a complex and heterogeneous etiology, including both genetic and environmental factors. The interplay between genetic and environmental factors has become the subject of intensified research in the last several years.[3–6] Despite early evidence for heritability of ASD, the largest twin population-based studies have found that concordance rates for dizygotic twins were actually higher than previously reported and that shared prenatal environment

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DOI 10.1179/1476830515Y.0000000019

accounted for the bulk of ASD risk in twins, with the genetic contribution being only modest.[7,8]

During the last decades, the reported incidence and prevalence for autism have drastically increased. Todays 14.7 per 1000 (one in 68) children aged 8-year-old in the USA are diagnosed with ASD.[9]

There is no agreement as to whether autism prevalence is truly on the rise or if a higher reported rate in recent years might be secondary to better awareness, and more sensitive diagnostic tools.[10–13] However, as ASD children are so remarkable it is unlikely that doctors, parents, and teachers would have missed a majority of these patients 30 years ago. Therefore, it is likely that a substantial proportion of the change in the prevalence of ASD is real.[14]

Many different well-defined biological disturbances occur in ASD patients. The problem is that some of the most commonly found disturbances, like evidence demonstrating enhanced oxidative stress or impaired antioxidant defense, are very non-specific and common in many other diseases as well. Other difference epidemiological and clinical found in ASD individuals are very specific, but not universal since they are found only in a subgroup of ASD patients.[15] Recently, Patrick and Ames[16] used a genetic database to demonstrate how something as simple as vitamin D deficiency explains some of these differences. For example, they found vitamin D deficiency explains six mysteries of ASD: the low concentrations of serotonin in the brain and its elevated concentrations in serum; the low concentrations of the vitamin D hormone precursor 25-hydroxyvitamin D (25-OHD); the high male prevalence of autism; the presence of maternal antibodies against fetal brain tissue; and the abnormalities in ASD of two peptide hormones, oxytocin and vasopressin.[16]

Vitamin D has a unique role in brain homeostasis, neurodevelopment, immunological modulation, ageing, and also, importantly, in gene regulation. In addition, it has been shown to bind to more than 2700 genes and to regulate the expression of more than 200 of them. In children (1–18 years), the recommended daily requirement of vitamin D ranged from 200 to 600 IU per day.[5,17,18] Vitamin D deficiency has recently been proposed as a potential environmental risk factor for ASD.[4,18–20] Vitamin-D deficiency was previously reported in autistic children.[4,21–24] However, the data on the relationship between vitamin D deficiency and the severity of autism are limited; also, the previous studies had several drawbacks, such as absence of controls, small sample sizes, and some were not conducted on children.[18] We attempted to remedy some of these deficits in the present study by using a case–controlled cross-sectional analysis conducted on a large patient group of ASD children to assess their vitamin D status compared to controls and the relationship between vitamin D deficiency and the severity of autism. We also conducted an open trial of vitamin D supplementation in ASD children.

Materials and methods

The Ethical Committee of Assiut University, Assiut, Egypt, approved the study. All methods and procedures used in this study were approved by the Institutional Review Board at Assiut University, Egypt. Informed consent followed the guidelines set forth by the Institutional Review Board at Assiut University and included a brief description of study procedures, potential benefits and risks, a discussion of the voluntary nature of the study, right to withdraw without consequences, and confidentiality of information. Informed consents were written in Arabic language that was age appropriate for all participants in the study. Prior to participation in the study, children were required to give their assent and parents were required to give consent for participation. Finally, participants were given a Participant's Bill of Rights.

Patients

The present study included 122 Egyptian children with ASD (aged from 3 to 9 years) participated in this case–control, cross-sectional study. All patients were recruited from the neuropediatric clinics Assiut university hospitals and five private centers for autism in Assiut city, Upper Egypt. A total of 204 patients were screened for eligibility, and 122 were enrolled (20 failed to provide consent and 62 did not meet eligibility criteria and/or one or more of exclusion criteria). ASDs were diagnosed according to DSM-IV-TR diagnostic criteria.[1] Structured interviews of at least 1 hour each both with the parents and the child were performed, in a room equipped with play material appropriate for age level. Later on, another 2 hours session was conducted for classification of ASD patients by using the Childhood Autism Rating Scale (CARS).[25] CARS is a well-established scale for the screening and classification of childhood autism with good agreement between DSM-IV-TR diagnostic criteria and CARS.[26] The scale assesses behavior in 14 domains that are affected by severe problems in ASD, plus one general category of impressions of ASD, with the aim of identifying children with ASD, as differentiated from the other developmental disorders. The examiner assigned a score of 1–4 for each item: one indicates behavior appropriate for age level, while four indicates a severe deviance with respect to the normal behavior for age level. The scores for the single items are added together into a total score, which classifies the child as not autistic (below 30), mild or moderately autistic (30–36.5) or severely autistic (above 36.5).[25,26] One hundred typically developing

apparently healthy Egyptian children of comparable age and sex (75% males) were included as a control group; the social status of the control group was compatible with cases. All control cases were recruited from outpatient clinics Assiut university hospitals and from healthy siblings of the patients. The dietary intake of all subjects was assessed by a questionnaire conducted by researchers for parents of all cases and controls. All subjects were in a good nutritional status; children with feeding problems or malnutrition were excluded from the study. Subjects who had associated gastrointestinal problems, autoimmune disorders, anemia, neurological diseases (such as cerebral palsy and tuberous sclerosis) and metabolic disorders (e.g. phenylketonuria) as well as any subjects receiving vitamin D containing preparations and drugs that may affect vitamin D (e.g. steroid and antiepileptics) were excluded. All control cases were screened by DSM-IV-TR diagnostic criteria and CARS, none of them had any clinical findings suggestive of any mental or autistic manifestations.

Of the ASD group, 106 patients with low-serum 25-OHD levels (<30 ng/ml) participated in the open label trial. They received vitamin D3; 300 IU/kg/day not to exceed 5000 IU/day (cholecalciferol; MUP Laboratory, Egypt; quality certified by the Egyptian Ministry of Health) for 3 months. This group was again evaluated with 25-OHD serum levels. The measures for the outcome of vitamin D therapy were the CARS and aberrant behavior checklist (ABC).[27] The 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 zero (not at all a problem) to three (the problem is severe in degree)), with higher scores indicating more severe problems.[27]

Biochemical measurements

Serum level of 25-OHD was estimated using the 25-OH Vit D ELISA Kit; Immundiagnostik AG, Germany. All subjects were studied during 2 months (May and June) to avoid the seasonal variation of vitamin D levels. Normal level of vitamin D is defined as a 25-OHD concentration >30 ng/ml, vitamin D insufficiency (20–30 ng/ml), and vitamin D deficiency (<20 ng/ml).[28]

Statistical analysis

Statistical Package for Social Sciences (SPSS) program version 11 was used for data analysis. Descriptive statistics as minimum, maximum, and mean ± SD and independent sample *t*-test, correlation, and linear regression. *P* value of ≤0.05 denoted the presence of statistically significant difference.

Results

The age, vitamin D levels of the ASD individuals, and controls are summarized in Table 1. Only 13% (16/122) of the patients in the present study had normal serum 25-OHD concentration (>30 ng/ml), while 57% (70/122) had vitamin D deficiency, and the rest 30% (36/122) had vitamin D insufficiency. The mean 25-OHD levels in patients with severe autism were significantly lower ($P < 0.0001$) than those in patients with mild/moderate autism (Table 2). Serum 25-OHD levels had significant negative correlations with CARS scores ($r = -0.502$, $P < 0.0001$) (Fig. 1). No significant correlation was found between 25-OHD level and age and sex.

One-hundred and six ASD individuals begun vitamin D3 treatments by oral route, and all patients' parents were advised as to good nutrition with foods containing vitamin D and sun exposure for their children. Twenty-three families were not compliant on daily vitamin D treatment and excluded from the second part of the study. Eighty-three subjects completed 3 months of daily vitamin D treatment. The side effects that noted by the research team during the 3-month study period included; skin rashes, itching, and diarrhea. All were mild, transient, and all patients continued the treatment. The patients

Table 1 Age and baseline 25-OHD levels of ASD subjects and controls

	Autism patients (122)				Control cases (100)			
Variable	Mean	SD	Min	Max	Mean	SD	Min	Max
Age (years)	5.09	1.42	3.00	9.00	4.88	1.30	3.00	9.00
25-OHD (ng/ml)	18.02*	8.75	5.00	46.00	42.51*	9.48	16.00	59.00

*($t = 7.91$, *P* value ≤0.0001).

Table 2 Baseline 25 (OH) D levels and severity of autism on CARS

	Severity of autism	Number	%	Mean	SD	Min	Max
25-OHD (ng/ml)	Mild and moderate	80	65.60	21.10*	8.47	11	46
	Severe	42	34.40	12.16*	5.83	5	32

*($t = 7.81$, *P* value ≤0.0001).

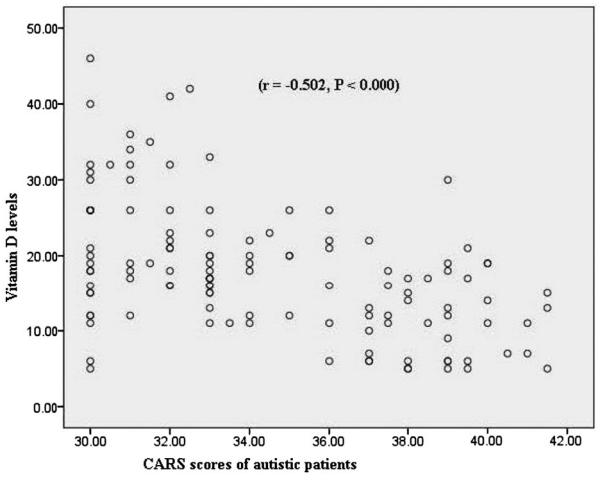

Figure 1 Correlation autism scores and vitamin D levels.

with vitamin D treatment were classified into three groups. The first group consisted of 16 patients with final serum 25-OHD levels >40 ng/ml; all had decreased CARS scores between 3.5 and 6.5 points. The second group consisted of 49 patients with final serum 25-OHD levels between 30 and 39 ng/ml. Most of them (31/49 patients) had decreased CARS scores by 1.5–4.5 points, while the rest of this group (18 patients) had no improvements. Collectively, 80.72% (67/83) of subjects who received vitamin D3 treatment (5000 IU/day) had significantly improved ($P \leq 0.05$) outcome. Table 3 and Fig. 2 showed the details of CARS scores before and after vitamin D therapy. Regarding ABC subscales before and after vitamin D therapy, there were statistically significant improvement in irritability ($P = 0.021$), lethargy/social withdrawal ($P = 0.028$), hyperactivity ($P = 0.01$), and stereotypic behavior ($P = 0.04$), with no significant difference in inappropriate speech (Table 4).

Discussion

Many studies have investigated the plasma levels of vitamin D directly in individuals with ASD. In agreement of the present study, Mostafa and Al-Ayadhi[29] reported that autistic children had significantly lower serum levels of 25-OHD than healthy children and 25-OHD was inversely associated with severity on autism rating scales ($R = 0.84$). In addition, children with ASD had significantly higher serum levels of anti-MAG autoantibodies ($R = 0.86$). Gong *et al.*[30] reported that mean serum 25-OHD levels were significantly lower in autistic children as compared with normal cases. There was a significant negative relationship between circulating serum 25-OHD levels and the severity of ASD. Meguid *et al.*[4] reported a cohort of autistic children having significantly lower levels of calcifediol and calcitriol values compared to healthy controls. The season of birth in relation to vitamin D and ASD was also taken into account, but no significant difference was found for the month or season of birth in either group. Molloy *et al.*[23] compared the plasma calcifediol levels in a cohort of autistic boys (4–8 years old) and a group of boys having intravenous catheters placed for outpatient tonsillectomies. There were no differences observed in the levels of vitamin D between ASD and controls. The drawbacks of this study were that the patient group included only males, and the control groups were all suffering from acute inflammation, which could affect the levels of plasma vitamin D.[18] Humble *et al.*[22] tested vitamin D levels in adult patients with psychiatric disorders and found that those with a diagnosis of ASD had significantly lower levels of vitamin D than any of the other groups. However, the study did not have a control group and examined only adults with a range of disorders, not only ASD.[18] Another study examined

Table 3 CARS scores in ASD patients before and after vitamin D therapy

Outcome measure	Mean ± SD scores before therapy	Mean ± SD scores after therapy	*t*	*P*-value
Relating to people	3.05 ± 1.7	2.09 ± 1.8	5.58	<0.001*
Emotional response	2.30 ± 1.6	1.02 ± 0.7	4.89	<0.001*
Imitation	3.01 ± 1.0	1.76 ± 0.3	5.02	<0.001*
Body use	1.9 ± 1.1	1.47 ± 1.7	2.77	0.01*
Object use	1.42 ± 1.1	1.03 ± 0.8	2.82	0.01*
Adaptation to change	2.18 ± 1.3	1.89 ± 1.0	3.21	0.004*
Listening response	1.92 ± 1.0	1.57 ± 1.7	2.71	0.01*
Taste, smell, touch	2.00 ± 1.3	2.02 ± 1.1	1.93	0.1
Visual response	2.89 ± 1.0	2.29 ± 1.2	3.34	0.003*
Fear	2.61 ± 0.8	2.46 ± 0.7	1.71	0.13
Verbal communication	2.66 ± 1.2	2.35 ± 1.1	1.04	0.3
Activity level	2.00 ± 0.8	1.93 ± 0.9	1.01	0.32
Non-verbal communication	2.04 ± 2.2	2.19 ± 1.8	1.04	0.2
Level and consistency of intellectual response	2.77 ± 1.3	2.67 ± 1.2	1.6	0.1
General impression	3.31 ± 1.2	2.41 ± 0.8	6.65	<0.001*
Total CARS score	37.7 ± 2.4	30.7 ± 2.8	5.77	<0.001*

*Significant values.

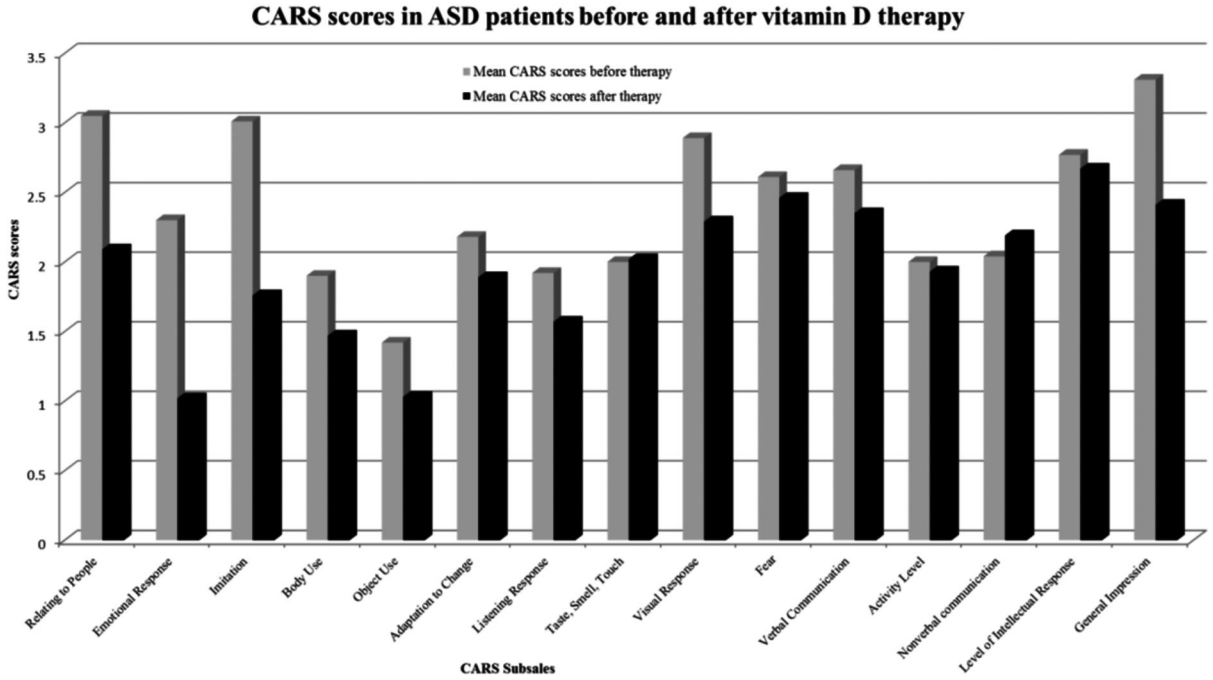

Figure 2 CARS scores in ASD patients before and after vitamin D therapy.

Table 4 ABC subscales in ASD patients before and after vitamin D therapy

ABC measure	Mean ± SD scores before therapy	Mean ± SD scores after therapy	*t*	*P*-value
Irritability	21.0 ± 7.61	16.7 ± 10.35	3.45	0.021*
Lethargy/social withdrawal	17.3 ± 8.18	11.6 ± 89.30	2.52	0.028*
Hyperactivity	28.7 ± 12.14	21.1 ± 12.01	2.67	0.01*
Inappropriate speech	5.8 ± 3.53	5.48 ± 4.04	0.73	0.52
Stereotypic behavior	18.2 ± 5.61	12.5 ± 3.79	2.21	0.04*

*Significant values.

mothers of Somali origin with children with ASD in Sweden.[21] In this small study, Somali mothers with autistic children had average vitamin D levels of 6.7 ng/ml, about 30% lower than Somali mothers without autistic children.

The present study confirms that 25-OHD levels in ASD children are lower than controls, that 25-OHD levels are inversely correlated with autism rating scales, and, for the first time, that the open label administration of high doses of vitamin D appears to improve ASD rating scales. We noted a more substantial improvement of ASD rating scales in patients whose final 25-OHD was >40 ng/ml compared to subjects with lower levels ($P \leq 0.05$). All children with final 25-OHD levels >40 ng/ml had improved CARS scores.

Vitamin D has multiple anti-inflammatory effects. It inhibits the synthesis of proinflammatory prostaglandins, which are elevated in ASD. In addition, it inhibits NF-κB, which is involved in aberrant signaling in autistic brains.[17,31] The antiautoimmune effects of vitamin D may explain the reported epidemiological associations between vitamin D status and many autoimmune disorders. Thus, vitamin D may have a potential role in treating diseases with autoimmune involvement, such as ASD.[17,32,33] Vitamin D might also reduce the severity of autism through an increasing seizure threshold, increasing T-regulatory cells, protecting the mitochondria, and upregulating glutathione, which scavenges oxidative byproducts and chelates heavy metals.[17]

Conclusion

Vitamin D deficiency is a frequent finding among children with ASD. Like two other groups, we found the severity of the deficiency is significantly inversely associated with autism rating scales.

Vitamin D is inexpensive, readily available and safe. It may have beneficial effects in ASD subjects, especially when the final serum level is more than 40 ng/ml. Vitamin D insufficiency is common in autistic children. A baseline measurement of 25-OHD level should be obtained to determine the dosage that will result in a final 25-OHD of at least 40 ng/ml.

There are an increasing number of studies suggesting an important link between low vitamin D and ASD. Randomized controlled trials using adequate doses of vitamin D3 are urgently needed.

Disclaimer statements

Contributors K.S., J.J.C., G.B. and M.K.A.-R. conceptualized and designed the study protocol development, assessment, and writing manuscript. A.A.A.-R., Y.M.E., A.A.Al-A., N.H.R. A.El-A., and A.M.A. performed all clinical and neuropsychiatric assessments of all cases and reviewed and revised the manuscript. H.A.K.O. performed all lab investigations. A.A.El-H., K.A.A.El-B., and A.E.A. designed the data collection instruments, and coordinated and supervised data collection. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Funding None.

Conflicts of interest J.J.C. is president of the nonprofit Vitamin D Council and receives remuneration from Purity Products. The other authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics approval The Ethical Committee of Assiut University, Assiut, Egypt, approved the study. All methods and procedures used in this study were approved by the Institutional Review Board at Assiut University, Egypt. Informed consent followed the guidelines set forth by the Institutional Review Board at Assiut University and included a brief description of study procedures, potential benefits and risks, a discussion of the voluntary nature of the study, right to withdraw without consequences, and confidentiality of information. Informed consents were written in Arabic language that was age appropriate for all participants in the study. Prior to participation in the study, children were required to give their assent and parents were required to give consent for participation. Finally, participants were given a Participant's Bill of Rights.

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