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25-hydroxyvitamin D Level in Autism Spectrum Disorders and the Efficacy of Vitamin D Supplementation in Saudi Autistic Children

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Background: Autism spectrum disorder (ASD) is a frequent developmental disease characterized by defects in nonverbal and verbal communication, pervasive deficits in social interaction, and stereotyped patterns of activities and interests. Previous researches reported vitamin D deficiency in autistic children; however, there is a deficiency of vitamin D supplementation trials in ASD children. This study aimed to assess vitamin D levels in ASD children and the effects of vitamin D supplementation on behavior and ASD score severity.
Methods: Eleven Saudi autistic children (9 males and 2 females) with ages ranged from 4 to 12 years were included in this prospective study during the period from January to July 2022. Children with ASD obtained from Will Be center for Special Needs and The Leading Academy for Autism. The study was carried out in two stages. In the first stage, 25-hydroxyvitamin D serum levels and autism severity via Gilliam Autism Rating Scale (GARS) were estimated. Then participants who fulfilled the inclusion criteria (9 out of 11) were included and given vitamin D supplements for three months. During treatment, ASD child behavior was observed. For the second stage, after treatment, changes in vitamin D level and autism score severity were evaluated.

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Results: A total of 11 autistic children participated; two of them had vitamin D levels that were > 80 nmol/L excluded. Out of 9 ASD included 5 cases had deficiency and 4 cases had severe deficiency of vitamin D (55.56% and 44.44%). Before treatment, ASD severities were above average (n=1, 11.11%), average (n=6, 66.67%), and low severity (n=3, 33.33%). After 3 months of vitamin D treatment, vitamin D serum levels were sufficient in 2 patients (22.22%) and insufficient in 7 cases (77.78%). Meanwhile, ASD severity had no clear improvement following vitamin D supplementation with 7 (77.78%) cases showed average severity and 2 (22.22%) cases with low severity. Nevertheless, the specialist clarified that there was an enhancement in some behaviors and responses for most patients. After treatment, there was a significance increased in 25-hydroxyvitamin D serum levels and a decrease in ASD severity compared to its level before treatment.

Conclusions: Vitamin D levels are insufficient in 82% of ASD children (9 out of 11). Of 9 ASD patients; vitamin D levels were deficiency and severe deficiency and ASD severities were average and low severity. After treatment, vitamin D level was sufficient only in 2 patients (22.22%) and only one ASD case had improved in ASD score (Average to low). Nevertheless, specialist reported enhancement in some behaviors and responses for most patients. Nutritional interventions with vitamin D make a little difference in autistic children's life by reducing their symptoms.

Keywords: Autism spectrum disorder; children; vitamin D; Gilliam autism rating scale.

1. INTRODUCTION

Autism spectrum disorders (ASD) refers to a complex childhood neurodevelopmental disorder that presents either from birth or in an early stage of development [1]. ASD is marked by impairments or deficits in communication and social interaction, where autistic patients have problems responding to social signals, including failure to understand facial expressions and recognize emotions [2]. In addition, ASD is connected with repetitive and stereotypical actions, and delayed response to environmental stimuli [3,4]. The prevalence of autism has risen considerably over the years. In the Arabian Gulf countries, ASD prevalence ranges from 1.4 to 29/10,000 [5]. In 2017, King Salman Center for Disability Research estimated that ASD cases were 50,714 in Saudi Arabia. Additionally, gender is risk factor for ASD with males four times more likely to exhibit it than girls [6].

The etiology of ASD is complex, it is assumed that the combination of genetic, epigenetic and environmental factors has a significant influence [7]. Approximately 30% of ASD cases were linked to genetic causes, in which more than 440 identified gene variants linked to ASD [8]. Epidemiological studies indicated that there are potential links between multiple nutritional factors and ASD risks. Vitamin D is one of the remarkable nutritional factors that have potential relevance to ASD risk [9].

Vitamin D is a neuroactive hormone [9] that plays an important roles in many health benefits including neuroprotective effects and influence on several neurotrophic factors [10]. To activate vitamin D in the body, it must bind to the vitamin D receptor (VDR). VDR is a nuclear receptor, ligand activated transcription factor, and member of the superfamily of nuclear hormone receptors. VDR is mainly located in the brain and peripheral neurons [3,11]. Vitamin D deficiency associated with a wide range of neuropsychiatric and neurodegenerative diseases [9]. Autistic children had considerably lower vitamin D levels than healthy children [1,6,12-14]. The recommended daily allowance of vitamin D for children (1–18 years) was 200–600 IU [15,16]. Lack of vitamin D may be an environmental risk factor for ASD [17]. Children with autism have previously been found to have vitamin-D deficiency [17-19]. Cannell and Grant suggested that large doses of vitamin D would treat ASD [20]. Saad et al. found that 80% of 86 ASD children who administered vitamin D3 (5000 IU/day) for three months showed improvement of symptoms [21]. Several factors, including latitude, seasons, skin colour, and age, might alter vitamin D levels in the body. Recent cases reported that in different societies, latitude and sunlight exposure affect the prevalence of autism. These reports demonstrate a higher prevalence of ASD in those with less sunlight exposure, higher latitudes or cloud covering [3,22].

The current study purposes to assess vitamin D levels in autistic children and to investigate the effects of vitamin D supplementation on the core symptoms of autism in children.

2. MATERIALS AND METHODS

2.1 Participants

Eleven Saudi autistic children (9 males and 2 females), aged from 4 to 12 years were included in this prospective study. Children with ASD were selected randomly from Will Be center for Special Needs and The Leading Academy for Autism to participate in the current study during period from January to July 2022. Children were previously diagnosed with ASD by an experienced psychologist based upon Gilliam Autism Rating Scale (GARS). The inclusion criteria for this research were proven diagnosis of autism, inadequate serum level of vitamin D, and good nutrition status. Participants with an adequate serum level of vitamin D, genetic diseases, metabolic disorders, neurological disorders, malnutrition, feeding problems, and individuals taking vitamin D supplements or drugs affecting vitamin D status were excluded.

2.2 Gilliam Autism Diagnostic Inventory-Second Editions (GARS-2)

Gilliam Autism Rating Scale (GARS) is an instrument designed to help psychologists and autism specialists in identify and diagnose behavioral problems as ASD and contains 42 items describing the characteristics behaviors of autistic children. The items categorized into three subscales (Stereotyped Behaviors, Communication, and Social Interaction). Each subscale contains 14 items. The score comprises Standard scores, percentiles, severity, and probability of autism. In the present study, GARS was done by autism specialists and concentrated on autism severity score.

2.3 Blood Sample

Eleven venous blood samples were collected from autistic children for measuring 25-hydroxyvitamin D levels. The blood samples were collected in serum separator SST tubes. After sampling the blood from ASD children, serum levels of 25-hydroxyvitamin D were determined by immunoassay technique using the ARCITECT ci8200 device. The results were determined automatically by the device software. Serum 25-hydroxyvitamin D was considered to be severe deficient (< 24nmol/L), deficient (25-49 nmol/L), insufficient (50-79 nmol/L), and sufficient (> 80 nmol/L) [23]. Serum levels of 25-hydroxyvitamin D were measured before and after three months of taking vitamin D supplements.

2.4 Methods

Participants had been previously diagnosed with ASD by autism specialists according to GARS. This study was carried out in two stages. In the first stage, we measured serum levels of 25-hydroxyvitamin D and assessed the severity of autism via GARS. Then participants who fulfilled the inclusion criteria were taking vitamin D supplements for three months under the follow-up of the parents and researcher. During the treatment, we asked parents, caregivers, and teachers about the behavior of autistic children. For the second stage, after three months of treatment, the rate of changes in vitamin D level and the severity of autism were evaluated once again.

2.5 Vitamin D_3 Supplementation

Vitamin D supplements were used for autistic children with low levels of 25-hydroxyvitamin D. Vitamin D doses were given as drops depending on age, gender, and body mass index. ASD children received vitamin D drops, 500 IU/kg/day not to exceed 5000 IU/day for three months under the supervision of a pediatrician and endocrinologist.

2.6 Statistics

Statistical analysis was performed using statistical package for social sciences computer software (SPSS for Windows, version 23.0, SPSS Inc 2015). The Categorical variables were presented as frequency and percentages (%) and parametric data as mean +/- standard deviation. Paired sample *t*-test used to compare the differences in vitamin D levels and ASD disorders severity before and after treatment. *P* values <0.05 was considered statistical significance.

3. RESULTS

A total of 11 autistic children participated in the study; two of them whose vitamin D levels were > 80 nmol/L excluded from entering the study. In 11 samples of ASD children, 46% had vitamin D deficiency, 36% of them had a severe deficiency, and 18% of them had sufficient vitamin D levels. Included in this study, 9 ASD patients of them 5 cases had deficiency vitamin D and 4 cases had severe deficiency (55.56% and 44.44%) and ASD severities were above average (n=1, 11.11%), average (n=6, 66.67%) and low severity (n=3, 33.33%). After 3 months of daily vitamin D

Table 1. Vitamin D serum levels and severity of autism before and after taking vitamin D supplementation

Autistic sample	Vitamin D serum levels		Severity of ASD score	
	Before Treatment	After Treatment	Before Treatment	After Treatment
1	Deficient	Sufficient	Average	Average
2	Deficient	Insufficient	Average	Average
3	Sever deficient	Insufficient	Low	Average
4	Sever deficient	Sufficient	above average	Average
5	Deficient	Insufficient	Average	Average
6	Deficient	Insufficient	Average	Average
7	Sever deficient	Insufficient	Average	Average
8	Sever deficient	Insufficient	Low	Low
9	Deficient	Insufficient	Low	Low

Table 2. Vitamin D serum levels and ASD severity before and after vitamin D supplementation

Treatment	25-OHD levels (nmol/L)	ASD severity
Before	*29.43±7.82*	104.22±12.1
After	*71.86±15.68*	88.22±9.71
P-value	*0.001*	0.06

treatment, vitamin D serum levels were sufficient in only 2 patients (22.22%) and insufficient in 7 cases (77.78%). Also, ASD severity had no clear improvement following 3 months of vitamin D supplementation with 7 (77.78%) cases showed average severity and 2 (22.22%) cases low severity (Table 1).

3.1 Vitamin D Levels and Severity of ASD before and after Vitamin D Supplementation

Table 2 revealed significance increased in 25-hydroxyvitamin D serum levels after supplement of vitamin D for 3 months compared to its level before treatment (71.86±15.68 versus 29.43±7.82, p <0.001). Additionally, mean of ASD severity was significance decreased after supplement of vitamin D for 3 months compared to its level before treatment (88.22±9.71 versus 104.22±12.10, *p*= 0.06).

4. DISCUSSION

ASD is a common neuro developmental disease that can cause impaired behavior and a lack of social communication. The frequency of ASD has gradually increased over the last two decades, according to a WHO analysis from 2019, 1 in 160 youngsters globally are thought to have ASD [24]. Along with the universal increase in the cases of ASD, including in Middle East and Saudi Arabia, there is an increasing concern over finding autism etiology and therapy. Lately, developing evidence suggested that vitamin D deficiency may be an autism risk factor [25].

The present findings in this study showed that serum vitamin D levels were significantly low in ASD children. In 11 samples of ASD children, 46% had a deficiency, 36% of them had a severe deficiency, and 18% of them had sufficient vitamin D levels. Of 9 ASD included patients; vitamin D levels were deficiency in 55.56% and severe deficiency 44.44%. Previous studies showed that vitamin D serum level ASD children was significantly lower than healthy ones [14,26-28]. In researches, it was found that ASD children had a significant frequency of vitamin D deficiency, ranging from 13% to 100% [21,27,29,30]. Saad et al. reported that 57% of ASD patients involved in their study had vitamin D deficiency, and 30% had vitamin D insufficiency [21]. Mostafa and Al-Ayadhi reported that 25-OHD was inversely correlated with autism severity on rating scales (R = 0.84) and autistic children had considerably lower 25-OHD serum levels than healthy children [31]. Gong et al. reported that autistic children had lower mean serum 25-OHD levels than normal control [32].

The likely cause of this phenomenon is that children with ASD have different living behaviors than children without ASD. ASD children are pickier about food than other children; thus, they consume less vitamin D. Furthermore, another explanation of this result might be related to that

ASD children spend less time outside than typical children. As a result, autistic children were less exposed to light, implying that they received less vitamin D. Concerning vitamin D supplementation, the result of present study showed that after three months of treatment with vitamin D supplementation, serum levels of vitamin D were sufficient in only 2 patients (22.22%) and insufficient in 7 cases (77.78%).

The autistic children were examined by the applied behavior analyst (ABA) to see if there was an improvement in their autistic symptoms and evaluate their conditions before and after vitamin D supplementation. The results of this study revealed that before receiving vitamin D treatment, ASD severities were above average (11.11%), average (66.67%) and low severity (33.33%). Based on GARS, ASD severity had no clear improvement following 3 months of vitamin D supplementation with 77.78% cases showed average severity and 22.22% cases low severity. However, based on the specialist's testimony, it was found that there was an improvement in some behaviors and responses for most patients.

Vitamin D prevents the production of proinflammatory prostaglandins that increased in ASD. Additionally, it prevents NF-κB which is included in abnormal signaling in autistic brains [33,34]. In addition, vitamin D may lessen the severity of autism by protecting mitochondria, lowering the threshold for seizures, elevating T-regulatory cells, and upregulating glutathione, which scavenges oxidative free radicals and chelates heavy metals [33].

Recent experimental studies revealed that vitamin D supplementation significantly improved autism symptoms based on multiple ASD severity scales [4,21,29]. Saad et al. reported that 80.72% (67/83) of patients' administration vitamin D3 had significantly improved outcome, which was mainly in sections of Childhood Autism Rating Scale (CARS) scores and aberrant behavior checklist subscales that measure eye contact, behavior, stereotypy, and attention span [21].

With vitamin D3 treatment, a tendency of significantly better outcomes was seen across various ASD severity measures, pointing to a potential function for vitamin D, particularly in a population where vitamin D insufficiency is quite prevalent. With the exception of two studies [35,36], a consistent improvement was seen across trials for Childhood Autism Rating Scale (CARS) [8,21,29,37-39] and Autism Treatment Evaluation Criteria (ATEC) [37], as well as for some subscales of the Autism Behavior Checklist (ABC*) and ABC [27,29-31,40]. Given limitations of small sample size of children with ASD and potential impact of additional confounding factors, such as ethnicity, sunlight exposure, and skin color, parathyroid disorders, dietary practices, and nutritional status, these findings call for a careful interpretation of the data [41,42]. A considerable improvement in the children's autism severity index was anticipated if the study's sample size was larger and the duration of the children's therapy and monitoring was prolonged.

5. CONCLUSIONS

The finding of this study showed that vitamin D levels insufficient in 9 out of 11 cases ASD children (82%). Of the 9 ASD included patients; vitamin D levels were deficiency and severe deficiency and ASD severities were average and low severity. After three months of treatment with vitamin D supplementation, vitamin D level was sufficient in only 2 cases (22.22%) and one out of 9 ASD cases had improved in test scores (Average to low). Nevertheless, the specialist clarified that there was an enhancement in some behaviors and responses for most patients. The present study identifies nutritional interventions with vitamin D make a little difference in autistic children's life by reducing their symptoms.

6. FUTURE WORK

This work's findings open the path for more extensive studies of the Saudi population. To confirm the results of this study, it is recommended to conduct further studies with the same subject on a larger sample size and a more extended period of treatment and observation.

CONSENT AND ETHICS APPROVAL

The King Abdul Aziz University Center of Excellence in Genomic Medicine Research's ethics approval number was followed in the design of this project, which adhered to its regulations and procedures (10-CEGMR-Bioeth-2018). The study was carried out in conformity with the Declaration of Helsinki and the standards set by King Fahd Center for Medical Research at King Abdullah University in Jeddah, Saudi Arabia.

A brief explanation of the study's methods, any potential advantages and dangers, a discussion of the study's voluntary nature, the option to withdraw from it without penalty, and information confidentiality were all included in the informed consent process. Written in Arabic were informed consents. Children and parents had to agree to take part in the study before taking part.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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