

Study Correlation between Vitamins E and D3 with Autism Spectrum Disorder (ASD) in Baghdad City

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Abstract

Background: Autism spectrum disorder (ASD) is a wide spectrum of neurodevelopmental disorders involving social cognitive deficiency, communicative disorders, repetitive behaviors, and confined interests. The specific mechanism of ASD has yet to be identified; however, multiple genetic, environmental, and immunologic factors play non-negligible roles in its etiology. **Objectives:** This study aimed to identify the possible correlation between vitamin E (VE), vitamin D3, and autism disorder, as well as the effects of gender and their levels. **Materials and Methods:** This study consists of 150 participants, divided into two groups: 100 ASD children and a control group (50), their age range is 4–10 years. **Results:** This study showed decreased levels of VE in ASD children when compared with controls ($P < 0.01–0.05$). Significant variances were seen between VE and ASD ($P < 0.001$). No influence of gender on VE levels; also, there were no significant variations in levels of Vit. D3 levels compared with the Control, also no influence of gender on the level of D3. There is a highly significant difference between children with ASD and controls, and there is no influence of gender on VE levels, so we can utilize VE concentration as an indicator of ASD. **Conclusion:** According to the results of this study, vitamin D3 levels in healthy individuals are generally lower compared with the normal range, and as a result, they are close to the values for ASD children. As a result of the low concentration of vitamin D3, we are incapable of using it as a marker for ASD or other ailments.

Keywords: Autism, vitamin D3, vitamin E

INTRODUCTION

Autism spectrum disorders (ASDs) are a group of neurodevelopmental disorders categorized by compromising verbal and nonverbal communication and also social interactions associated with stereotyped patterns of behavior and mannerisms. ASDs continue to increase in prevalence about 1% of the populace affecting with disorder, which is generally identified in early childhood. Its etiology is unknown, but genetic and wide-ranging environmental factors have been implicated in the disorder. The history of the patient and the behavioral criteria observation are the basis of the diagnosis, rather than physical examination findings or laboratory tests.^[1]

The neurological condition known as the disease spectrum disorder (AUTISM) is defined by the following indications, primary categories: difficulties in social

communication and social interaction; unnatural and repetitive behaviors, activities, or hobbies.^[2] This progressive disorder could be detected at any age; however, in general, its indications occur before the age of 3 years. The disease is a lifelong disorder; however, appropriate treatments could improve a person's quality of life in each life stage. AUTISM is characterized by wide indications, variance, and severity and might occur in every ethnic, racial, or economic population. Due to its relatively high dominance, suggesting each child should be screened for the disease.^[3-5] The Centers for Disease Control and

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Prevention's latest data demonstrated that the frequency of disease detections is escalating. In 2000, it reached 1 in 154 children, and the data from 2016 showed dominance of around 1 in 54 children.^[6] In addition, the increasing incidence and often far-reaching effect on affected people and their families' operative present substantial challenge for public health and the educational system.^[7]

Autism has no definitive biological markers, which makes the diagnosis depend on the patient's behavioral signs, although endogenous factors need to be evaluated. One of the essential nutrients is vitamin D3, which is necessary to sustain human health. The classic role of vitamin D3, since it is one of the steroid hormone family, is regulating metabolism, affecting cell proliferation,^[8] and differentiation.

Vitamin E (VE) stabilizes glutamate receptors is stabilized by VE, which also decreases the excitotoxicity linked to high levels of glutamate. This vitamin can be considered a fat-soluble vitamin, and an antioxidant that stops free radical damage in the body. An important role in the central nervous system is controlled by the bio-elements. A reliable biomarker might significantly add to an early and more specific autism diagnosis, a crucial requirement for an early behavior-changing therapeutic intervention.^[9]

Biomarkers have been defined as specific measurements evaluating the responses of the pharmacological agents to a therapeutic intervention, pathogenic processes, or standard biological processes. In other words, a biomarker is any neurophysiological, neuropsychological, neuro-anatomical, endocrinological, biochemical, cognitive, or genetic marker that is indicative of the presence of disease.

MATERIALS AND METHODS

Study design

Blood samples were collected from 150 children attending the Welfare Teaching Hospital, Medical City, Baghdad, Iraq, for the period from December, 2022 to March, 2023 were enrolled in this study. The samples were divided into two groups: a control group (50) and 100 children with ASD, aged from 4 to 10 years. The research questions included descriptive information for each (gender and age).

Sample collection and procedures

About 5 mL of blood from the venous was collected from ASD children and healthy controls, and kept in a gel tube and left to clot. Then, the serum was separated by centrifugation for 5 min. Serum is divided into separate Eppendorf tubes (500 μ L), and all were labeled with the name and number, then put into the freezer until work time. The samples were thawed before starting to work at room temperature.

VE was determined according to information of manufactured company using the (general VE vitamin/E Elisa) kit from SunLong Biotech Company in China using the Reader Automated Elisa. Vitamin D3 was determined

according to information of manufactured company using the [25-OH-vitamin D total (CLIA) kit] kit from Mindray Company in Germany using Mindray CL-900i Chemiluminescence Immunotest Analyzer.

Statistical analysis

The statistical analysis was performed utilizing GraphPad Software Inc., La Jolla, CA, USA (GraphPad Prism version 9.2). One-way analysis of variance (Tukey's test) and *t* test were utilized to determine whether group variance was different or not. Pearson's coefficient *r* was employed to assess correlation. Data were expressed as mean \pm standard deviation, and statistical differences were defined as **P* < 0.05 and ***P* < 0.01.

Ethical approval

The study was conducted in accordance with the ethical principles of the management of the Children Welfare Teaching Hospital, and it was carried out with the community and their families. The study information was approved by document number (3/6619) on November 21, 2022.

RESULTS

ASD children with control

It is obvious from Figure 1 and Table 1 that there are significant variances in vitamin E means between control and ASD children *P* value of <0.0001.

Vitamin E and gender

Obviously, from Tables 2 and 3, and Figure 2, there is no significant variance comparing vitamin E means in ASD children, and the gender *P* value of 0.8799 means there is no gender influence on vitamin E concentration in ASD.

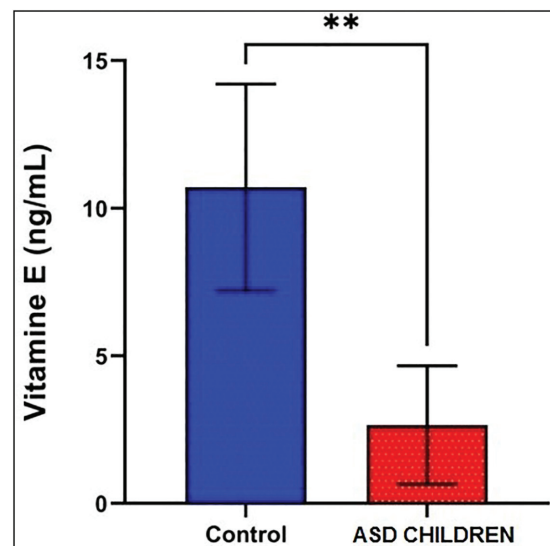


Figure 1: Comparison of variances in vitamin E means between control and children with autism spectrum disorder

Table 1: Comparison of variances in vitamin E means between control and autism spectrum disorder (ASD) children

Unpaired <i>t</i> test	Number of samples	Mean	Standard deviation	<i>P</i> value	Significance
Control	50	10.71	3.502	<0.0001*	Yes
ASD children	100	2.657	2.007		

*Refer to the statistically significant value

Table 2: Comparison of variances in vitamin E means between control and autism spectrum disorder children by gender

Test of Šídák's multiple comparisons	Adjusted <i>P</i> value
Male-female	
Control	0.9756
Patients	0.8799

*Refer to the statistically significant value

Table 3: The means of vitamin E concentrations in control and autism spectrum disorder (ASD) children

	Control female	Control male	ASD children female	ASD children male
Number of values	25	25	50	50
Mean	10.63	10.79	3.110	2.205
Standard deviation	3.423	3.648	2.545	1.149
Standard error of the mean	0.6846	0.7295	0.5089	0.2299

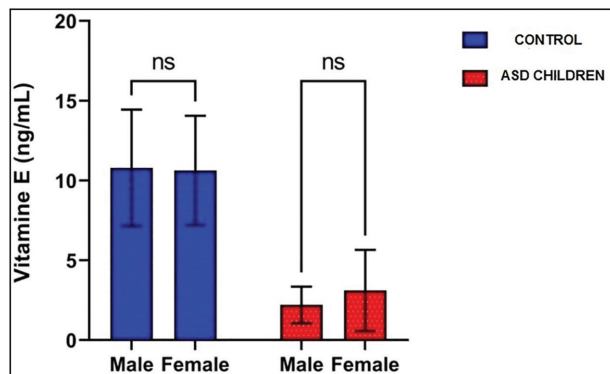


Figure 2: Comparison of variances in vitamin E means between males and females in control and autism spectrum disorder children

Vitamin D3

ASD children with Control

It is obvious from Table 4 and Figure 3 that there is no significant variance in vitamin D₃ means between control and ASD children *P* value of 0.0957. This result is different from previous studies.

Vitamin D3 with gender

It is obvious from Tables 5 and 6, and Figure 4 given below, that there are no significant variances when comparing the means of vitamin D₃ in ASD children among

Table 4: Comparison of variances in vitamin D₃ means between control and autism spectrum disorder (ASD) children

Unpaired <i>t</i> test	Number of samples	Mean	Standard deviation	<i>P</i> value	Significant
Control	50	15.36	4.899	0.0957*	No
ASD children	100	17.45	7.295		

*Not significant at *P* ≤ 0.05

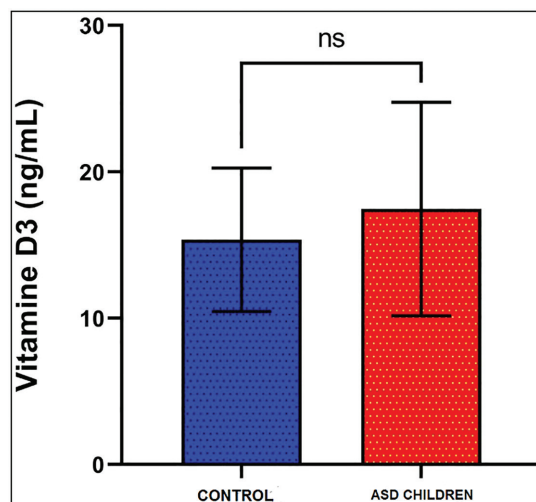


Figure 3: Comparison of variances in vitamin D3 means between control and autism spectrum disorder children

Table 5: Comparison of variances in vitamin D3 means between control and autism spectrum disorder (ASD) children among gender

Test of Šídák's multiple comparisons	Adjusted <i>P</i> value
Male-female	
Control	0.9997*
ASD children	0.9951*

*Not significant at *P* ≤ 0.05

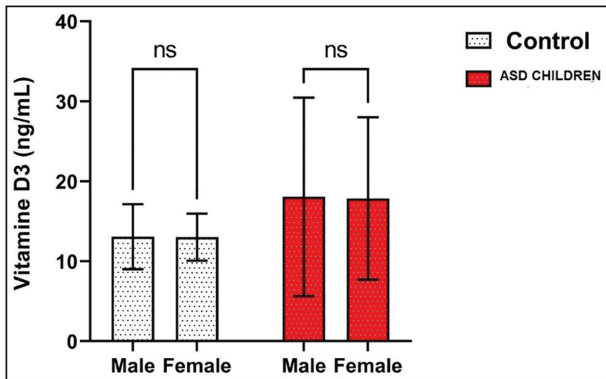
gender, with a *P* value of 0.9951, which means there is no influence of gender on vitamin D concentration in autism disorder.

DISCUSSION

A fat-soluble antioxidant, VE protects the body from the harm caused by free radicals. Only foods made from

Table 6: The means of vitamin D3 concentrations in control and autism spectrum disorder children

	Control female	Control male	Patients female	Patients male
Number of values	25	25	50	50
Mean	13.02	13.07	17.85	18.06
Standard deviation	2.946	4.071	10.16	12.40
Standard error of the mean	0.5893	0.8141	3.033	2.481

**Figure 4:** Comparison of variances in vitamin D3 means between males and females in control and children with autism spectrum disorder

plants, such as grains, nuts, seeds, and oils, contain it. VE has been discovered to alleviate the speech disease, verbal apraxia, when paired with omega-3 fatty acids. VE reduces excitotoxicity brought on by high glutamate levels by stabilizing glutamate receptors.^[10]

Numerous studies have demonstrated that ASD impairs every player in the VE/vitamin C/glutathione network. Both ASD rodent models and autistic people exhibit changes in the expression of the key antioxidant enzymes of the reactive oxygen species scavenging system, both in the brain and in the peripheral blood.^[11,12] This suggests that oxidative stress levels might be higher in those with ASD. It has been noted that lower blood levels of VE were linked to ASD-related behaviors in autistic individuals.^[13,14]

There is a safe upper limit for VE because it is a fat-soluble vitamin. It has a minor tendency to thin the blood and can make bleeding and bruising easy.^[15]

Omega-3 fatty acids and VE might help autistic youngsters with linguistic difficulties speak more clearly. The 187 verbal apraxic children who were given VE and polyunsaturated fatty acid supplements were gathered by the American researchers. The researchers found that 181 families (97%) that received supplements reported “dramatic improvements in some areas.” These included problems with eye contact, communication, conduct, and other senses.^[16]

Verbal apraxia is a complex syndrome that impacts a person’s neurologic function rather than being only a speech impairment. Autism, sensory disorders, poor muscle tone, food allergies, challenges with coordination, and reduced gastrointestinal function are characteristics of a minority of children. VE, omega-3, and carnitine deficits are linked to the occurrence of numerous allergies and gastrointestinal issues.^[17]

The clinical study and protocol are intended to continue understanding the effects of omega-3 and VE on autism and verbal apraxia and to start identifying the underlying processes in verbal apraxia. Work in this area is ongoing.^[18]

No variance in terms of gender; however, variances in autism syndrome severity and symptoms between males and females. Improvement is conducted in this field in different aspects, though the mechanism underlying this gender disparity is still unidentified.

According to recent studies,^[19] children with autism have lower vitamin D3 levels. This vitamin is crucial for bone growth and development, and children with autism have thinner cortices in their brains when they are deficient in it.^[20] On the other hand, vitamin D blocks the brain’s main enzyme for the metabolism of glutamate, -glutamyl transpeptidase. Accordingly, vitamin D3 boosts glutamate levels, which increases the brain’s antioxidant defense. Adults with schizophrenia and autism had their vitamin D3 levels examined by Jung *et al.*^[21] They discovered that treatment with vitamin D3 decreased their vitamin D3 levels, reduced their depression, and improved their symptoms. According to research, all vitamin supplements are probably going to help autistic individuals’ nutritional and metabolic state, as well as reduce oxidative stress. Vitamin therapy can certainly keep the antioxidant capacity against oxidative stress strong.^[21]

According to the results of this study, vitamin D3 levels in healthy individuals are generally lower compared with the normal range, and as a result, they are close to their value for patients. As a result of the low concentration of vitamin D3 in the majority of individuals, we are incapable of using it as a marker for ASD or other ailments.

Studies proved that the female gender was independently associated with severe vitamin D3 deficiency (41.9% vs. 30.4%, $P = 0.001$; adjusted odds ratio; 95% confidence interval = 1.42 (1.08–1.87), $P = 0.01$) and was associated with lower vitamin D levels (14.5 vs. 15.9, $P = 0.007$). Therefore, it has been proposed that there are gender differences in vitamin D3 status, with post-menopausal women having a higher rate of deficiency, which raises the risk of bone fractures and osteoporosis.^[22] The hydroxylized activated form of vitamin D (25(OH)D) is a steroid and a precursor hormone because it alters the expression of several

matrix metalloproteinases, growth factors, and cytokines that are involved in the inflammatory response as well as coagulative and thrombotic pathways, which in turn affects endothelial function, platelet aggregation, and the development of atherosclerosis.^[23,24] In fact, lower levels of 25(OH)D have been linked to coronary artery disease^[25]; in addition, serum vitamin D3 levels are negatively correlated with vascular stiffness and calcifications^[26] as well as with major cardiovascular risk factors, such as hypertension, diabetes, obesity, and metabolic syndrome.^[27]

Actually, recent research shown that 25(OH)D status is affected by genetic regulation, sex, and age,^[28] and numerous studies have demonstrated the advantages of vitamin D3 supplementation on skeletal and muscle health as well as overall mortality in post-menopausal women.^[29-31]

CONCLUSION

According to these study results, there is a highly significant difference between children with ASD and the control group, and there is no influence of gender on VE levels, so we can utilize VE concentration as an indicator for ASD. According to the results of the current study, vitamin D levels in healthy individuals are generally lower compared with the normal range, and as a result, they are close to the value for ASD children. As a result of the low concentration of vitamin D, we are incapable of using it as a marker for ASD or other ailments.

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Conflicts of interest

There are no conflicts of interest.

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