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Assessment of Serum Calcium and Vitamin D in Autism Spectrum Disorders

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ABSTRACT

Background & Objective: Autism is a neurodevelopmental disorder that affects social interaction, communication, and behavior. It is believed that biological and genetic factors play a key role in the development of autism. Children with autism spectrum disorder (ASD) often present with nutritional, metabolic, and gastrointestinal abnormalities that can affect their calcium and vitamin D_3 states. The reason for assessing these parameters is based on biological, behavioral, and pharmacological factors that may increase the risk of disease abnormalities. This study aims to compare the autism patient group with a control group in terms of gender and age distribution, as well as assess the levels of calcium and Vitamin D3 in both groups.

Materials & Methods: The study was conducted on 50 autism patients and 50 individuals in the control group. Data related to gender and age group were collected, along with measurements of calcium and Vitamin D3 levels in the blood. So, it was approved to conduct the study by the Thi-Qar Health Department, Research and Department of the College of Pharmacy / University of Thi-Qar. This study was conducted from 11/2024 to 4/2025, at Thi-Qar Rehabilitation Center for autistic disorders.

Results: It showed significant differences between the two groups. The proportion of males in the autism group was higher (74%) compared to the control group (42%). This could be due to the normal variation of the selected samples in this study. The most represented age group in the autism patients was 7-10 years (42%), while the most represented age group in the control group was 11 years and older (50%). Additionally, the study found a significant decrease in both calcium and Vitamin D3 levels in autism patients compared to the control group.

Conclusion: The findings suggest a significant relationship between calcium and Vitamin D3 deficiencies in autism patients, which may contribute to the severity of the symptoms.

Keywords: Serum Calcium, Vitamin D3, Children, Autism Spectrum Disorders

1. Introduction

1.1 Autism spectrum disorders (ASDs)

Autism spectrum disorders are complex neurodevelopmental conditions that typically emerge within the first few years of life and persist into adolescence and adulthood. They are characterized by impairments in social communication and interaction, along with restricted and repetitive behaviors, interests, and activities. The etiology of ASDs is multifactorial (1). The term "autism spectrum disorders" refers to a group of early-onset conditions marked by deficits in social communication and repetitive sensory-motor behaviors. These disorders are strongly influenced by genetic factors, although other contributing causes have also been identified (2). While the exact etiology of ASD remains unknown, it is believed to result from a combination of genetic, immunological, and environmental influences (3).

1.2 Risk factors of ASDs

Research suggests that several factors during pregnancy can affect fetal brain development and increase the likelihood of ASD. One key factor is poor maternal nutrition, as deficiencies in folic acid, omega-3 fatty acids,

and vitamin D may contribute to neurodevelopmental issues. Additionally, exposure to environmental pollutants, such as air pollution, pesticides, heavy metals, and industrial chemicals found in plastics, may negatively impact brain development. Maternal health conditions, including obesity, gestational diabetes, hypertension, and immune disorders, have also been linked to a higher risk of ASD. Moreover, certain medications taken during pregnancy, such as antidepressants, anti-epileptic drugs, and frequent use of acetaminophen (paracetamol), have been associated with an increased risk. Lastly, psychological and social factors, including chronic stress, advanced maternal age, and birth complications, may further influence fetal neurodevelopment. Maintaining a healthy lifestyle during pregnancy-through proper nutrition, minimizing exposure to harmful substances, and managing health conditions can help reduce potential risks to the baby's development (4).

1.3 ASD Symptoms

The biological basis of autism spectrum disorders (ASDs) remains unknown, making diagnosis dependent on behavioral manifestations and developmental history. Significant progress has been made in identifying early ASD signs through retrospective video analysis, parental reports, screening studies, and high-risk sibling research. Although behavioral markers can be detected within the first year of life, the average age of diagnosis is around three years, leading to delays in early intervention. Research on ASD identification in community-based samples before 18 months remains limited. Future studies should incorporate repeated behavioral assessments to enhance early detection and track subtle developmental changes in infants and toddlers with ASD (5). Studies indicate that ASD symptoms typically manifest within the first two years of life, affecting social communication, behavior, motor skills, attention, and emotion regulation. In pediatric settings, parents frequently express concerns regarding potential gastrointestinal (GI) symptoms in children with ASD; however, the specificity of these has not been thoroughly investigated (6, 7).

1.4 Vitamin D

Vitamin D, often called the "sunshine vitamin," is essential not only for maintaining bone health in both children and adults but also for providing other health benefits, such as lowering the risk of chronic conditions like autoimmune diseases, certain cancers, and cardiovascular disease. However, the vitamin is biologically inactive in its natural form, whether produced in the skin or obtained through diet (8).

Vitamin D plays a crucial role in brain development and function, influencing neuronal differentiation, proliferation, and apoptosis. It also regulates synaptic plasticity and the dopaminergic system while helping to reduce oxidative stress. Research indicates that vitamin D3 supports the development of regulatory T-cells, which help modulate the immune system and prevent excessive immune responses and autoimmune reactions. Additionally, vitamin D is essential for regulating gene expression (9).

Research suggests that low serum vitamin D levels may be associated with certain symptoms in children with ASD. Genetic factors, including variations in the vitamin D receptor (VDR) gene, can influence vitamin D levels in the body. VDRs are widely present in brain regions involved in cognitive functions such as learning, memory, and executive functioning, indicating a potential link between vitamin D and dopamine transmission in the brain (10). Some studies indicate that vitamin D may help reduce autism symptoms by promoting synaptic plasticity, regulating the dopaminergic system, and reducing oxidative stress (11-13). However, research findings are mixed, while some studies report no significant correlation between serum vitamin D levels and factors such as IQ, language skills, or autism severity based on DSM-5 criteria, others suggest a negative correlation between vitamin D deficiency and autism severity, as measured by CARS scores (14, 15).

1.5 Calcium

Calcium is a vital mineral best recognized for its role in maintaining healthy bones and teeth, yet it also contributes significantly to several physiological processes. It is indispensable for blood coagulation, muscle contraction, and the regulation of heart rhythm and nerve signaling. Approximately 99% of the body's calcium is stored in the skeletal system, with the remaining 1% distributed throughout the blood, muscles, and other tissues (16).

Genetic studies have implicated voltage-gated calcium channels as potential contributors to autism spectrum disorder (ASD). Mutations have been identified in nearly all pore-forming and several auxiliary subunits of these channels among individuals and populations affected by ASD. Collectively, both genetic and functional evidence indicate a possible involvement of voltage-gated calcium channels in the pathophysiology of ASD. Further investigations are needed to refine clinical and systems biology models of ASD and to adopt a comprehensive molecular approach that considers the diverse roles of calcium channel function (17).

2. Materials and Methods

2.1 Study design

This study was conducted at the Thi-Qar Rehabilitation Center for Autistic Disorders. A structured questionnaire was developed to collect essential information about patients and control subjects. The questionnaire was reviewed and evaluated by experts. The study involved samples from 50 autistic children at the center and 50 control children, aged 2–16 years. Data were collected from the laboratory and analyzed using various statistical techniques.

2.2 The study population

The study was conducted on children with ASDs, involving the collection of samples and measurement of vitamin D and calcium levels following scientific protocols and ethical standards. The participants included both male and female children under the age of 16, with their medical records being reviewed and compared. Additionally, samples were collected from neurotypical individuals (healthy individuals) of both genders for comparison. There were exclusion criteria including over 16 years.

2.3 Sample Collection

Blood samples were collected from 50 patients with autism spectrum disorder, ranging in age from 2 to 16 years (males and females). Additionally, blood samples were obtained from 50 control children within the same age range (males and females).

2.4 Principle of biochemical test for vitamin D

The biochemical estimation of vitamin D (Vitamin D kit Biolabo) is based on the competitive binding immunoassay, followed by spectrophotometric measurement (Cobas e411). In this method, 25-hydroxyvitamin D in the sample competes with a labeled analog for specific antibody binding sites. The resulting complex produces a color change, which is measured using a UV-visible spectrophotometer (Apel) at a specific wavelength. The absorbance is inversely proportional to the vitamin D concentration in the serum **(18, 19)**.

2.5 Principle of biochemical test for calcium

The estimation of calcium is based on the colorimetric reaction with Arsenazo III, where calcium ions form a blue-violet complex with the dye in an acidic medium. The intensity of the formed color is directly proportional to the calcium concentration and is measured at 650 nm using a UV-visible spectrophotometer **(20-22)**.

2.6 Procedure for measuring vitamin D

Venous blood samples were collected from children diagnosed with autism and a control group using sterile collection tools to ensure accuracy and avoid contamination. The Apel Atomic Absorption Spectrophotometer (AAS) was calibrated using standard solutions with known concentrations of vitamin D. The concentration of 25-hydroxyvitamin D was determined by measuring the absorbance at a specific wavelength. The obtained absorbance was compared with a calibration curve to calculate the actual concentration in the samples. The vitamin D levels were compared between the autism and control groups. Statistical analysis was performed to determine if any significant differences existed between them **(20)**.

2.7 Procedure for measuring calcium

Blood samples were drawn from both autistic and control children using sterile tools to maintain sample quality. Samples were centrifuged to isolate serum or plasma, which were preserved in appropriate storage conditions until testing.

The Apel AAS instrument was calibrated using calcium standard solutions. Serum calcium levels were measured by analyzing the absorbance at 422 nm, which corresponds to the calcium absorption line. The absorbance values were compared to a standard curve to quantify the calcium concentration. Calcium concentrations were statistically analyzed and compared between the autism and control groups to identify any significant deficiencies **(23)**.

2.8 Statistical Analysis

The data obtained in the present study were statistically analyzed using the SPSS version 26. The analyses were performed using independent sample t-tests, one-way ANOVA, and Chi-square tests, with a significance threshold set at $p < 0.05$. Statistical notes: A p-value marked with two asterisks indicates a highly significant difference at $p < 0.01$, whereas a p-value marked with one asterisk denotes a significant difference at $p < 0.05$. A p-value without an asterisk represents a non-significant difference. Means sharing the same lowercase letter indicate no significant difference, whereas those labeled with different letters indicate statistically significant differences.

3. Result

3.1 Distribution of Autism Patient and Control Group According to Sex and Age Groups

The current study recorded a significant difference at p-value <0.05 between the patient and control group according to sex and age group. The study noted that the high ASD patient group was male group 74.0% compared with the male control group, 42.0%. Regarding age groups, the study showed the most ASD patients in the second age group, 42.0%, while the most control group was in the third age group, 50.0%, as shown in Table 1.

3.2 Serum Levels of Calcium and Vitamin D3 in ASD Patient and Control Groups

The data recorded a significant difference at p-value <0.05, between the patient and control group, showing recorded the levels of both calcium and vitamin D3 decreased significantly in ASD patients compared with the control group, as in Table 2.

3.3 Serum levels of calcium and vitamin D3 in ASD patients according to sex

The present study recorded a non-significant difference at p. value <0.05, in the ASD patient according to sex was. The levels of both calcium and vitamin D3 decreased non-significantly in the male autism group compared with the female group, as shown in Table 3.

3.4 Serum levels of calcium and vitamin D3 in ASD patients according to age

The present study recorded a non-significant difference at p. value <0.05, in the level of calcium in the autism patient according to age group. On the other hand, the study recorded that the levels of both vitamin D3

increased significantly in autism with increasing age, as in Table 4.

Table 1. Distribution of ASD patients and control group according to sex and age groups.

Variables		Patients		Control		P value
		No.	%	No.	%	
Sex	Male	37	74.0	21	42.0	**<0.01****
	Female	13	26.0	29	58.0	
Age (years)	3-6	20	40.0	11	22.0	**<0.01****
	7-10	21	42.0	14	28.0	
	≥ 11	**9**	**18.0**	**25**	**50.0**	

Data are expressed as mean ± S.D.

P<0.05 is considered significant, P<0.01 is considered highly significant.

* Represents significant values

** Represents statistically significant values

Table 2. Serum levels of Calcium and Vitamin D_3 in ASD patient and control groups.

Biochemical parameters	Patients	Control	P value
	Mean ± SD		
Calcium (mg/dl)	9.389 ± 0.49	9.876 ± 0.17	**<0.01****
Vit D_3 (ng/ml)	**19.54 ± 6.19**	**29.83 ± 8.96**	**<0.01****

Data are expressed as mean ± S.D.

P<0.05 is considered significant, P<0.01 is considered highly significant.

* Represents significant values

** Represents statistically significant values

Table 3. Serum levels of Calcium and Vitamin D3 in ASD patients according to sex.

	ASD Patients		
Biochemical parameters	**Male**	**Female**	**P value**
	Mean ± SD		
Calcium (mg/dl)	9.329 ± 0.47	9.558 ± 0.52	**0.182**
Vitamin D_3 (ng/ml)	**18.72 ± 6.29**	**21.89 ± 5.44**	**0.096**

Table 4. Serum levels of calcium and vitamin D_3 in ASD patients according to age.

	ASD Patients	
	Ca^{++}	$VitD_3$
Age (years)	**Mean ± SD**	
3-6	9.365 ± 0.46	**15.92 ± 5.28^{c}**
7-10	9.337 ± 0.52	**20.75 ± 6.15^{b}**
≥ 11	9.563 ± 0.51	**24.76 ± 2.58^{a}**
P value	**0.690**	**<0.01****

The superscript letters a, b, and c indicate significant differences between the age groups.

4. Discussions

This study aimed to assess the differences between children diagnosed with ASD and a control group in terms of serum levels of calcium and vitamin D. The analysis included 100 children, divided equally into 50 patients and 50 controls. A statistically significant difference was observed in the sex distribution between the two groups ($p < 0.01$). The proportion of males among ASD patients was notably higher (74.0%) compared to the control group (42.0%). This strongly suggests a male predominance among autism patients, which is consistent with epidemiological data. A comprehensive review, reported that ASD is 4 to 5 times more prevalent in males than females. This disparity has been attributed to biological and genetic factors, including sex-linked genetic mutations and neurodevelopmental differences (18). Regarding age distribution, the results also revealed a significant difference ($p < 0.01$). The highest proportion of autism cases was observed in the 7–10 years age group (42.0%), followed by the 3–6 years group (40.0%), while only 18.0% of ASD patients were aged ≥11 years. In contrast, the control group had the highest proportion in the ≥11 year's category (50.0%). These findings highlight that ASD is more frequently diagnosed during early and middle childhood, likely due to increased awareness and developmental concerns becoming more evident at these stages. Early detection is critical, as timely intervention is associated with improved developmental and behavioral outcomes. These findings suggest that autism is more likely to be diagnosed in younger age groups, especially during early childhood when symptoms such as language delay, social withdrawal, or repetitive behavior become more apparent. Early diagnosis is crucial for timely intervention and improved developmental outcomes. This study highlights key factors associated with autism, including male gender, younger age groups, and deficiencies in calcium and vitamin D3. These results emphasize the importance of early screening, especially in males and younger children, and suggest that nutritional assessment should be part of autism management. Addressing deficiencies in essential nutrients such as calcium and vitamin D3 might offer potential benefits in supporting the overall health and neurological function of children with ASD.

The results of this study indicate an inverse relationship between calcium and vitamin D3 levels and autism spectrum disorder. Specifically, it was observed that both calcium and vitamin D3 levels were significantly lower in children with autism compared to healthy children. This result is highly significant, with a p-value of less than 0.01, reflecting a clear difference between the two groups, which is consistent with findings from previous studies. Control Group (Healthy individuals): 29.5 / Autism Group (Patients): 19.5. This shows a significant difference in vitamin D3 levels between the two groups, indicating a substantial deficiency in vitamin D3 levels in autism patients compared to healthy individuals. Since the vitamin D3 level in the patients is 19.54, which is below the minimum level considered sufficient for general health (typically ranging between 20-50), this suggests that these patients may require supplements or dietary interventions to improve their levels.

It is important to note that vitamin D3 plays a vital role in regulating the immune system and is involved in many neurological and developmental processes. It is essential for brain growth and neurological functions. Its deficiency could impact the mental and neurological health of children with autism and may exacerbate behavioral symptoms associated with the disorder. This is consistent with the study's findings, which showed that vitamin D3 levels were significantly lower in children with autism. This result is supported by several studies, including a study that highlighted an association between low vitamin D levels during pregnancy or early childhood and an increased risk of developing autism. Moreover, vitamin D3 plays a crucial role in brain development and the regulation of gene expression related to behavior (24). The results of the current study showed a significant decrease in calcium and vitamin D3 levels in autism patients compared to individuals in the control group, with

a p-value less than 0.01, indicating statistically significant differences. These results align with previous studies that have linked the deficiency of certain nutrients with behavioral and neurological changes in ASD.

Calcium plays a crucial role in several neurological processes, such as neurotransmission release, muscle contraction, and neurotransmitter suggests that calcium deficiency may affect neuronal function and increase the likelihood of developing autism-like behavioral symptoms. Additionally, there are scientific hypotheses that propose mineral metabolism disorders may contribute to the neurological changes associated with autism.

The serum levels of calcium and vitamin D3 in autism patients according to sex showed a non-significant difference between males and females with p-values of 0.182 for calcium and 0.096 for vitamin D3, respectively ($P \geq 0.05$). While calcium levels were slightly lower in males (9.329 ± 0.47) compared to females (9.558 ± 0.52), non-significant difference was observed. Similarly, vitamin D3 levels were lower in males (18.72 ± 6.29) compared to females (21.89 ± 5.44), but again, no significant difference was noted. These results suggest that sex does not have a notable effect on calcium and vitamin D3 levels in autism patients. This is consistent with some previous studies that reported mixed results regarding the influence of sex on nutrient levels in individuals with autism spectrum disorder. Further studies with more precise measurements of factors affecting nutrient levels are needed to draw more definitive conclusions.

These results can be interpreted to suggest that blood levels of calcium and vitamin D3 may be influenced by several factors other than sex, such as diet, sunlight exposure, and biological interactions within the body. It is important to note that autism is a complex condition involving both genetic and environmental factors, which makes it difficult to identify consistent biological markers that clearly distinguish between male and female patients. The findings of this study revealed no statistically significant difference in calcium levels among different age groups of autism patients ($p = 0.690$). This suggests that age may not be a determining factor in calcium levels within this population. Calcium levels across age groups may reflect the body's regulatory mechanisms maintaining homeostasis regardless of age. On the other hand, the study showed a statistically significant increase in vitamin D3 levels with age among autism patients ($p < 0.01$). Younger children (ages 3–6) had significantly lower levels of vitamin D3 (15.92 ± 5.28 ng/mL) compared to those aged 7–10 (20.75 ± 6.15 ng/mL) and those aged 11 and above (24.76 ± 2.58 ng/mL). This finding could be attributed to increased outdoor activities and sun exposure as children grow older, which directly enhances endogenous vitamin D synthesis. The progressive increase in vitamin D3 with age might also reflect improved dietary intake, supplementation, or better health awareness in older children and their families. These results emphasize the importance of early nutritional assessment and intervention in younger children with autism, as vitamin D3 plays a vital role in brain development, immune function, and behavior regulation.

This study also aimed to assess the goal of identifying potential age-related biochemical variations that may inform clinical management. The findings revealed no statistically significant differences in calcium levels among the age groups ($p = 0.690$), indicating that calcium homeostasis appears to be maintained regardless of age in autistic individuals. This is consistent with previous studies that have reported relatively stable serum calcium concentrations in both neurotypical and neurodivergent pediatric populations (25). Calcium plays a critical role in neuromuscular function and intracellular signaling, and while essential, its regulation may not be as vulnerable to age-related changes as other nutrients in this population. In contrast, vitamin D_3 levels demonstrated a significant increase with age ($p < 0.01$), rising from 15.92 ± 5.28 ng/mL in the youngest age group to 24.76 ± 2.58 ng/mL in the oldest. These findings align with literature suggesting that younger children with autism are more susceptible to vitamin D deficiency (26), possibly due to limited sun exposure, dietary restrictions, or sensory sensitivities that affect food intake. As children grow older, improved dietary diversity, increased outdoor activities, or proactive supplementation may contribute to better vitamin D_3 status (27). The observed trend highlights the need for early screening and targeted nutritional interventions, particularly during early childhood, which is a critical period for neurodevelopment. Vitamin D_3 has been implicated in a variety of neurodevelopmental processes, including brain differentiation, neurotransmission, and immune modulation. Deficiency in this vitamin has been associated with increased risk and severity of autism symptoms (28). Therefore, ensuring adequate levels during the early developmental window may offer therapeutic benefits and support cognitive and behavioral outcomes in autistic children. The findings of this study contribute to the growing body of evidence emphasizing the importance of nutritional monitoring in children with ASD.

5. Conclusion

Clinicians are encouraged to consider routine assessments of serum calcium and vitamin D_3 levels, especially in younger patients, and to provide supplementation when necessary. Future research should focus on longitudinal studies to examine how changes in vitamin D_3 status over time may correlate with symptom progression or therapeutic response. Additionally, investigating other micronutrients and their interaction with age, dietary patterns, and autism severity could provide a more comprehensive understanding of nutritional needs in this population.

6. Declarations

6.1 Acknowledgments

The author would like to express his sincere gratitude to all children and their parents who agreed to participate in this study.

6.2 Ethical Considerations

The study protocol was approved by the Research Committee of the Thi-Qar Health Directorate (Approval No. 236/2024). Written informed consent was obtained from all participants prior to enrollment. Patient confidentiality was ensured through anonymization of identifiers during data extraction and analysis.

6.3 Authors' Contributions

The author reviewed, edited, and approved the final version of the manuscript.

6.4 Conflict of Interest

The author has no financial conflicts of interest and no funding sources to disclose.

6.5 Fund or Financial Support

The author received no funding from any government or private organizations for this work.

6.6 Using Artificial Intelligence Tools (AI Tools)

The author was not utilized AI Tools.

7. Publisher's Note

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