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Evaluation of Serum Vitamin D and Ionized Calcium Levels in Sudanese Child with Autistic Spectrum Disorder

Abuagla M. Dafalla[1, 2*], Leena A. Dafalla[1, 2], Yousif A. Mohammed[1], Albadawi A. Talha[3], and GadAllah Modawe[4]

[1] *Department of Clinical Chemistry, Faculty of Medical Laboratory Sciences, University of Gezira, Wad Madani, Sudan.*

[2] *Kampala International University (KIU), School of Allied Health Sciences, Uganda*

[4] *Department of Clinical Laboratory Sciences, Jouf University, Saudi Arabia.*

[5] *Department of Biochemistry, Faculty of Medicine and Health Sciences, Omdurman Islamic University, Omdurman, Sudan.*

E-mail: agla4321@yahoo.com, Leenaelsidig33@gmail.com, aYousifna2015@gmail.com , aaabdelbagi@ju.edu.sa, gadobio77@hotmail.com

ABSTRACT

Background: *Autism is a neurodevelopmental condition that affects people of all ages; occur due to the genetic exchange or mutation within the fragile X gene or other environmental factors. Autism Spectrum Disorder (ASD) child has frequently experience behavioral and physiological symptoms.* **Objective:** *The goal of this study was to measure serum vitamin D and ionized calcium level among children with ASD.* **Methods:** *This is case control study conducted in Al-Sharif Comprehensive Center for Special Needs in Wad Medani, Gezira State, Sudan from May to August 2017. Eighty individuals were included forty were ASD as cases group and forty healthy-matched children as control group. Blood sample were collected and serum ionized calcium level was measured by using (Cobas 9180)electrolyte analyzer also serum vitamin D was measured, as 25-Hydroxycholecalciferol by Electrochemiluminescence immunoassay using Cobas e411 Analyzer both automated analyzer were from (Roche Diagnostics Roche Diagnostics Corporation 9115 Hague RdIndianapolis, IN 46256USA) that utilizes Elecsys reagent kits. Data were collected through designed questionnaire and performed using Statistical Package for Social Sciences (SPSS) version 20.P value less than 0.05 was considered as statistically significant.* **Results:** *The average of serum vitamin D level (ng/ml) among ASD children was decreased when compared with healthy control (29.4 ±11.3) via (36.8 ± 5.9) with significant differences (p value = 0.001).On the other hand, result showed significant decreased in serum ionized calcium level (mg/dl) of ADS patients (4.06 ± 0.46) when compared with control group (4.56 ± 0.53) with (P. Value = 0.000). Measuring ionized calcium and vitamin D routinely in ASD children, in additional to giving vitamin D and calcium as nutritional supplement for ASD and pregnant women highly recommended.*

Conclusions: *Autism Spectrum Disorder children had a decreased serum ionized calcium and vitamin D.*

Keywords: Vitamin D, Serum Calcium, Sudanese, Autism spectrum disorder (ASD)..

Article Information

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INTRODUCTION

Autism is a neurodevelopmental condition characterized by significant impairments in communication; cognition, attention regulation, motor, sensory, and affective processes (1). Many of them have repetitive, limited interests and stereotypic behaviors (2). Concerning to widespread signs and symptoms of child's, ASD is subdivided into extraordinary subgroups, which includes Asperger syndrome, autistic disorder, and. pervasive. developmental disorder not otherwise specified (3). ASD usually takes place through the first three years, due to an increase in social demands, it may be appear late in some cases (4). Autism develops as a result of a confluence of genetic and environmental factors(5).

Genetic factors consist of genetic alternate or mutation in the fragile X gene. ASD children have special fragile X syndrome phenotype associated with deficits in complex social interaction (6). Parents determined that one of the most characteristic behaviors is the absence of interest in other people (7). Children have alterations in inflammatory markers, including autoantibodies to glutathione and brain and abnormalities in inflammatory, these results suggest that ASD is a systemic disorder (8). Approximately a third to a half of ASD don't enhance sufficient natural speech to meet their every day wishes (9) less probably to make requests and greater tolerable to really repeat others' words, despite the fact that a few children have gastrointestinal symptoms (10).

Most professionals understand that there's no cure; therapies that promise a treatment either exaggerate some effectiveness or lack scientific background (11). Prevalence of ASD was increased in last few years. Furthermore, it reported less than 10 in 10,000 individuals in previous studies, According to a recent study, rates range from 90 to 250 per 10,000 people, regarding to awareness increased and ASD cases reporting. ASD is thought to affect 24.8 million people worldwide (12), globally about 1–2 per 1,000 are affected; it exists 4 to 5 times greater in boys than girls. The prevalence of autism among adults aged 18 and above is 1.1 percent in the United Kingdom. Since the 1980s, the number of persons diagnosed with autism spectrum disorder has increased due to changes in diagnostic practice and improved diagnostic criteria. It is widely assumed that autism spectrum disorder is genetically driven and due to risk elements in the environment (13). Children with the AA genotype of the GC gene (D binding protein), the GG genotype of the CYP2R1 gene (a catalytic enzyme involved in the conversion of vitamin D to 25(OH) D), the Bsml genotypes of the VDR gene, and paternal Taql have a higher risk of ASD (14). Vitamin D is a fat-soluble vitamin, have different forms including ergocalciferol or D2 and cholecalciferol or D3 (15), it is essential for mineral homeostasis and adequate skeleton formation,1, 25(OH) 2D3 is the most active form of vitamin D, which stimulates absorption of calcium and phosphate from gut for metabolism and bone growth (16), and in addition with parathyroid hormone, which stimulates bone to increase calcium and phosphate mobilization (17). Vitamin D assist cells growth rate regulation and preventing DNA damage by reducing oxidative damage which vitamin D supplementation decreased 8-hydroxy-2'-deoxyguanosine, a marker of oxidative harm and through regulation of the poly-ADP-ribose polymerase activity within the DNA damage response pathway. It's also able to regulating the cell cycle to prevent the propagation of damaged DNA and to regulate apoptosis (18). When both genetic and environmental factors are taken into account, studies show that calcium dys-regulation is the most major biological factor in ASD risk (19). The prevalence of ASD has risen in recent years, with the number of people diagnosed having risen considerably since the 1980s, owing to advances in diagnostic techniques,

increased public awareness, and reports of disorder (20). The goal of this study was to assess blood ionized calcium and vitamin D levels in ASD children.

METHODS

A laboratory-based analytical case-control study was conducted to assess the vitamin D concentration and ionized calcium in ASD children. The study was carried out in Al-Sharif comprehensive center for special needs in Wad Medani, Gezira state, Sudan. A random sample was collected from ASD children attending Al-Sharif comprehensive center for special needs from May to August 2017.

A total of 80 people took part in this study, 40 were ASD child cases and 40 were healthy individual (non ASD child) as a control group. ASD cases were included according to the final diagnoses by Al-Sharif comprehensive center for special needs physicians. ASD child on medication that may impact vitamin D and calcium levels, or with one of the following diseases: metabolic diseases, atherosclerosis, neoplastic, colon cancer, and immunological illness, Type 1 diabetes mellitus, and multiple sclerosis were all ruled out. Consanguinity, family history, treatments, duration of disease, degree of intelligence and communication, and exclusion criteria were among the demographic data obtained from each participant after final diagnosis using a questionnaire.

Ethical consideration

The study was approved by the College of Graduate Studies, Faculty of Medical Laboratory Sciences, University of Gezira, and the Sudanese Ministry of Health. Before collecting specimens and obtaining consents for participation, all enrolled patients' parents were told about the study's goal.

Data variable, Collection and Criteria

Sex, age (Year) - Vitamin D concentration (ng/ml) - Calcium ionized (mg/dlm, the duration of the disease (Year) - Clinical evaluation –Intelligence and communication level (Good, Moderate, Weak) - Autism severity (Severe, Moderate, Weak) - Congeniality (Yes, No) –The variables employed were family history (Yes, No) and data was acquired utilizing laboratory analysis and questionnaires. Limit sources of bias by rigorous criteria for case and control eligibility.

Measurement of Vitamin D and S. ionized Calcium

Each participant's blood was collected in three ml lithium heparin anticoagulant tubes for laboratory examination; plasma was separated into two new plain containers and examined within 2 hours or kept at -20 Cº until analysis. The laboratory work was done in National Cancer Institute Department of laboratory, University of Gezira. COBAS e411 automated machine (Japan) was used to measure vitamin D levels while COBAS9180 (China) was used to measure ionized calcium levels. Circulating levels of vitamin D <20 ng/ml was considered deficient, 21-29 ng/mL as insufficient, ≥30 ng/mL as a normal and >100 ng/mL was considered to be toxic [16].

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 20 was used to analyze the data. Data were expressed as (Mean ± SD). The independent t-test was employed to compare differences between the means of continuous variables. Also The Pearson correlation test was used to measure the strength and direction of the linear relationship between two continuous variables. P-values less than 0.05 were considered statistically significant.

RESULTS

There were 80 people in this study, 40 ASD children as case group and 40 non ASD child (healthy individuals) enrolled as a control group. About 52.5% of ASD children were less than 5 years in age and most of them were male (67.5%). The distribution of ASD children was 57.5% weak in communication, 52.5% was a moderate degree of Autism, 55% had a relationship (Consanguinity) between ASD child parents, and 60% of them had no family history of autism.

Table (1): Distribution of ASD children according to demographic and clinical data.

		Frequency	Percent	Valid Percent
Age	Less than 5 years	21	52.5	52.5
	More than 5 years	19	47.5	47.5
	Total	40	100.0	100.0
Gender	Male	27	67.5	67.5
	Female	13	32.5	32.5
	Total	40	100.0	100.0
Duration	Less than 2 years	20	50.0	50.0
	More than 2 years	20	50.0	50.0
	Total	40	100.0	100.0
Degree of Communication	Good	2	5.0	5.0
	Moderate	15	37.5	37.5
	Weak	23	57.5	57.5
	Total	40	100.0	100.0
Degree of Autism	Weak	16	40.0	40.0
	Moderate	21	52.5	52.5
	Severe	3	7.5	7.5
	Total	40	100.0	100.0
Consanguinity	Yes	22	55.0	55.0
	No	18	45.0	45.0
	Total	40	100.0	100.0
Family History	Yes	16	40.0	40.0
	No	24	60.0	60.0
	Total	40	100.0	100.0

Table (2): Mean of serum vitamin D and ionized calcium levels in ASD and control groups.

	N	Mean	STD	P. Value
Case	40	29.41	11.37	0.001
Control	40	36.77	5.90	
Case	40	4.06	0.46	0.000
Control	40	4.56	0.53	

Table (3): Mean of serum vitamin D in ASD children according to age, duration of disease, degree of communication, degree of autism, consanguinity, and family history.

		N	Mean	STD	P. Value
Age	Less than 5 years More than 5 years	21 19	30.44 31.89	9.94 15.16	0.720
Duration of disease	Less than 2 years More than 2 years	20 20	28.14 34.12	10.13 14.18	0.134
Degree of communication	Good Moderate Weak	2 15 23	4.50 4.06 4.03	0.85 0.38 0.48	0.329
Degree of autism	Weak Moderate Severe	16 21 3	4.14 3.99 4.10	0.46 0.48 0.26	0.088
Consanguinity	Yes No	22 18	29.34 33.32	12.34 12.78	0.327
Family history	Yes No	16 24	30.74 31.39	13.15 12.39	0.877

Table (4): Mean of serum ionized calcium level in ASD children according to age, duration of disease, degree of communication, degree of autism, consanguinity, and family history.

		N	Mean	STD	P. Value
Age	Less than 5 years More than 5 years	21 19	3.92 4.22	0.45 0.43	0.042
Duration of disease	Less than 2 years More than 2 years	20 20	3.89 4.24	0.43 0.43	0.716
Degree of communication	Good Moderate Weak	2 15 23	4.50 4.06 4.03	0.85 0.38 0.48	0.249
Degree of autism	Weak Moderate Severe	16 21 3	4.14 3.99 4.10	0.46 0.48 0.26	0.826
Consanguinity	Yes No	22 18	4.12 3.99	0.50 0.39	0.240
Family history	Yes No	16 24	4.22 3.96	0.52 0.39	0.053

Table (5): Association the vitamin D status (Deficiency < 20, Inadequate: 20 - 29, and Normal > 30) between ASD children and non ASD children.

	Case	Control	Total	P. value
Deficiency	7 (17%)	1(2%)	8	0.021
Inadequate	14(35%)	9(23%)	23	
Normal	19(48%)	30(75%)	49	
Total	40	40	80	

Table (6): Association the vitamin D status (Low < 30 and Normal > 30) between ASD children and non ASD children.

	Case	Control	Total	P. value
Low	21(53%)	10(25%)	31	0.011
Normal	19(47%)	30(75%)	49	
Total	40	40	80	

Table (7): The mean, STD, minimum, maximum, range and median of serum ionized calcium level and vitamin D in ASD children.

	N	Mean	STD	Minimum	Maximum	Range	Median
Vitamin D	40	31.13	12.54	3.00	54.76	51.76	30.91
Calcium	40	4.06	0.46	3.00	5.10	2.10	4.00

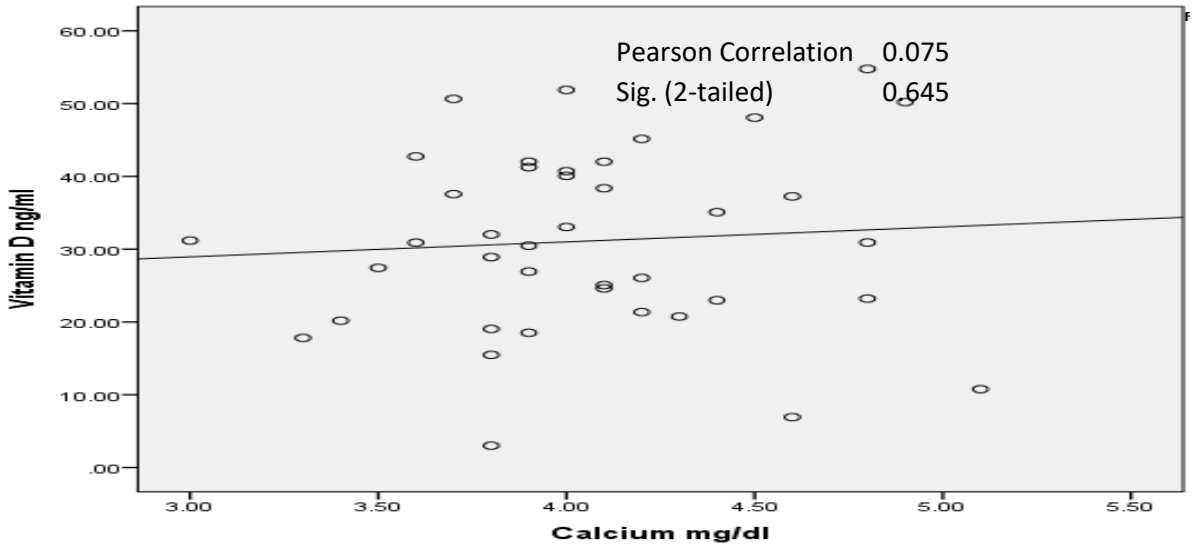

Figure (1): Correlation between 25(OH)D and calcium in children with autism (r ¼ 0.075, p =0.645).

DISCUSSION

Autism disorder is a group of lifelong developmental disabilities may relate to a various genetic or environmental risk factors or both. The incidence of children with autism spectrum disorders has risen over the past years. This is case control study conducted in Gezira State Sudan to evaluate serum ionized calcium level and vitamin D concentration in ASD children and comparing with control group. To our knowledge, this is one of few studies focusing mineral level among autism in Sudan. Vitamin D may play an essential role in etiology of Autism Spectrum disorders (ASD). Vitamin D is a neuroactive steroid affecting mental function and development. It plays an important role in myelination, which is essential for connectivity inside the brain. Studies have proven that decreased vitamin D levels, decreased maternal vitamin D levels during pregnancy, and decreased

exposure to sun UVB may increase the risk of ASD. Eighty individuals were participating in this study which 50% was ASD child (cases) and remain was non ASD child (control group). Regarding to Socio-demographic characteristics the male to female ratio was (2:1) 67% were male while 33% female, majority of ASD patient were less than five years according to disease degree 40% were weak, 52% moderate and 8% severe this low incident of severe disease which disagree with study conducted in Riyadh, Saudi Arabia this may be due to community variation between Sudan and Saudi population and their understanding this disease (R) according to consanguinity 55% had relative parents and 45% had not relative parents and according to family history 40% had a family history of ASD and 60% had no family history of ASD.

Our study find significant decrease in serum Vitamin D levels in ASD children mean (29.4 ±11.4) when compared with control mean (36.8 ± 5.9) with (p value =0.001), maybe this due to a combination of limited sunlight exposure, selective eating habits, gastrointestinal issues, reduced physical activity, use of sunscreen, possible genetic or metabolic differences, and medication-related effects on Vitamin D metabolism. On other hand, this finding in line with studies done in Qatar, Riyadh, Saudi Arabia, Egyptian, Ahvaz city - Iran and Italy was agreed with study of Abdulbari Bener*et al*,Gehan A Mostafa and Laila Y AL-Ayadhi, Nagwa A. Meguid, *et al* ,Ali Asghar Arastoo*et al* and Maria G. Petruzzelli.*et al* [21, 22, 23,24 and 25].

Serum Calcium was significant decrease in ASD patients when compared with control, this result is correlated with Vitamin D deficiency, selective eating, gastrointestinal malabsorption, hormonal imbalances, medication effects, and possible genetic or metabolic differences affecting calcium regulation. This result is agree with studies done in Qatar, Egypt and Istanbul, Turkey [26]. Our results demonstrated that age and gender failed to find significant differences between ASD patients this agree with several studies conducted in Riyadh, Saudi Arabia, Egypt, Ahvaz city - Iran, Italy and Istanbul, Turkey. Extensive results carried out show that vitamin D categories (Deficiency, Inadequate and Normal) has significant differences, a similar pattern of results was obtained from study conducted in Ahvaz, Iran, Riyadh, Saudi Arabia, Qatar and Istanbul, Turkey. We describe the results of ASD children family history, which show no significant differences in serum vitamin D level between ASD children which have a family history of A SD when compared with haven't Contrary to study done in Egypt our study show no significant correlation between vitamin D and calcium levels in children with autism. Our study assessment risk factors duration of disease, degree of communication, degree of autism and consanguinity and find no significant differences between serums ionized calcium level in this risk factors of ASD children

CONCLUSION

Our case control study concluded that Serum Vitamin D concentration and ionized calcium level were significantly decreased in children with ASD in comparison to controls. This finding confirms literature that ASD has associated with decrease in vitamin D level. Moreover; these findings point to a role for vitamin D deficiency in the genesis of autism. Vitamin D3 supplementation may be crucial in the treatment of children with autism. However, one of the study's weaknesses can be attributed to the limited sample size.

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Ethical considerations

This study has been approved by the Faculty of Medical Laboratory Sciences review Board, University of Gezira.

Competing interests

None declared.

Availability of Data and Materials

All data and materials associated with this paper were available through the corresponding author upon reasonable request.

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