ISSN: 1343-4292
Volume 140, Issue 01, April, 2022

Levels of some vitamins and minerals in children with autism spectrum disorder in Baghdad city and their relationship to gender

Hajir Muayad Yaseen[1], Areej Abbas Zabbon[2], Adil A.S. Al-Salihy[3]

Department of Biology, College of Science, Mustansiriyah University, Bagdad, Iraq[1,2]
Consultant Cognitive Neurologist, department, Psychological Research Centre, Ministry of Higher Education and Scientific Research. Bagdad, Iraq[3]

ABSTRACT— The objective of this research was to compare numerous vitamins and minerals levels in children with autism spectrum disorders (ASD) compared to those in age-matched normally developing (TD) children, as well as to evaluate their relationship to gender. The study involved the ASD children attending Iraqi association for psychotherapy in Baghdad .The current study was carried out on 63 child with both genders (33 test group with17males and 16 females and 30 control group with16males and 14 females) The diagnosis of autism spectrum disorder (ASD) was determined based on information acquired from history collection, clinical symptoms, and observations, The controls for the Childhood Autism Rating Scale (CARS) and There were no abnormal history of motor, verbal, or social developmental deficits in the checklists, as evidenced by the parents' remarks with age range (4-11) years, during the period extended between November (2020) and March (2021). The samples of children divided into test - control and male - female group. The present study, showed the level of zinc (mg/dl) decreased significantly ($P \leq 0.05$) in test when compared to control and significantly increase ($P \leq 0.05$) in male than female group, while level of calcium (mg/dl) decreased significantly ($P \leq 0.05$) in control group and its no different between male and female group.VitaminsD3 and B12 (ng/ml) levels decreased significantly ($P \leq 0.05$) in test group when compared to control group but in male -female group the level of vitamin D3 decreased significantly ($P \leq 0.05$) in male group while vitamin B12 deceased in female group. We discovered that children with ASD had higher vitamins and minerals deficits than typically developing children, and that the level of these vitamins and minerals correlated with ASD symptoms and the child's gender.

KEYWORDS: Autism spectrum disorder, calcium, zinc, D3, B12

1. INTRODUCTION

Autism spectrum disorder (ASD) is a complicated neurodevelopmental disorder with an estimated prevalence of one in 54, 52 million cases worldwide and 7.7 million disability adjusted life years [38], [27]. Boys are three to eight times more likely to be diagnosed with autism spectrum disorder (ASD) than girls. [24], [8]. Recent research suggests that repetitive and restricted behaviors and interests (RRBI) may be a strategy for understanding and explaining gender variations in ASD diagnosis rates [6], [14], [13]. Stereotypical behavior, confined behavior, similarity behavior, concentration on routines, repeated play or usage of materials, unusual preoccupations, restricted interests, self-injurious behaviors, and sensory avoidance/seeking behaviors are all symptoms of RRBI [39].

Calcium is crucial for neurodevelopment and may offer preventative and treatment options for ASD, [30] Calcium, for example, plays an important role in controlling synapse growth and function; hence, elevated

calcium levels may be advantageous for correct brain development, as aberrant synaptic development has been documented in ASD [37]. Zinc is one of the most common metal ions in the brain, where it governs neurogenesis, neuronal migration, and differentiation, impacting cognitive development and assisting in brain function maintenance [18], [40]. Synaptic transmission in ASD may be affected by immune system and zinc homeostasis disruptions [18]. It has been found that the dietary condition of children with ASD is affected [32]. Two consecutive hydroxylation mechanisms in the liver and kidneys convert vitamin D to its active form, 1,25(OH)D (Calcitriol) [16]. Calcitriol is a neuroactive hormone that regulates several aspects of brain growth including early cognitive development [33]. Vitamin D promotes brain cell growth and neurotransmission activities, potentially influencing neurodevelopmental processes [31].

Vitamin B12 (vitB12) is necessary for DNA synthesis as well as the production of cellular energy [10], [29]. A number of symptoms linked with gastrointestinal, hematological, neurological, and psychiatric diseases can be noticed as a result of the deficiency [11], [3]. Motor problems are among the known signs of cobalamin insufficiency, abnormal balance and reflexes, sensory and memory impairment, cognitive impairment, irritability and brain shrinkage are all symptoms of this condition [21], [19]. Furthermore, Vitamin B12 plays a role in both methylation and redox state, There have been reports of alterations in plasma methionine, homocysteine, cysteine, and glutathione levels as risk factors for ASD. (GSH) levels after vitamin intake, in addition to the nitrous oxide effect [26], [9].

2. Materials and methods

This study involved the autistic children attending Iraqi association for psychotherapy in Baghdad .The current study was carried out on 63 child with both genders (33 test group and 30 control group) child with autism spectrum disorder The Autism Spectrum Disorder (ASD) diagnosis was obtained based on information acquired through history gathering, clinical symptoms and observations, the Childhood Autism Rating Scale (CARS), and checklists According to the parents' reports, the controls had no atypical histories of motor, linguistic, or social developmental impairments with range age (4-11) years, during the period extended between November (2020) and March (2021) then special tests were conducted at the International Center for Research and Development. The current study include 30 controls volunteers healthy had negative family history of any chronic or systemic disease. After their parents' permission, a blood sample was extracted from them. to calculate biomarker concentrations, Approximately (4ml) of human blood was collected intravenous from patient and control groups by using a sterile syringe. The blood sample were transferred into gel activator tube and, then centrifuged at 3000 rpm for 10 minutes to separate serum, Every individual's serum was collected and stored in several sterilized eppendorf Tubes at -20C^0 until it was time to determine different parameters. All samples marked with a serial number and the name of the individual. vitamins D3 was measured by (ELISA) kit (My BioSource, USA, Cat No. MBS264661 and also B12 (Bio Vision, U.S.A) from serum samples by enzyme-linked immunosorbent assay (ELISA) using This experiment employs the double-sandwich ELISA technique .and minerals zinc ,calcium were measured by Automatic biochemistry analyzer (flexor EL80).

3. Results

Table (1) showing the differences in the level of calcium (Ca^{+2}) and zinc (Zn^{+2}) indicators by variables (test-control), the t-test value of Ca^{+2} was its significant ($P\leq0.05$) for the benefit of test group, but it's not significant ($P>0.05$) between males and females, and the effect size was 0.645. Its an medium effect according to Cohen's standard of effect size. The Zn^{+2} values where significant ($P\leq0.05$) in favor of control and significant for male groups, while the effect size was 0.295, indicating a slight effect according to Cohen's standard.

ISSN: 1343-4292
Volume 140, Issue 01, April, 2022

Table (1): Shows the moderation distribution of both Ca^{+2} and Zn^{+2} levels in test group compared with control group and between female and male groups.

Parameter	Group	Mean	Std. Deviation	T-test	F-Test	Sig.	Size effect Partial Eta Squared
Calcium mg/dl	Test	9.2091	0.27994	10.166	35.726	0.001	0.645
	Control	7.3267	1.02316				
	Male	8.3682	1.11388	0.384			
	Female	8.2517	1.29500				
Zinc mg/dl	Test	60.2061	20.34610	-3.423	8.230	0.001	0.295
	Control	79.4333	24.21268				
	Male	76.7212	21.16045	2.662			
	Female	61.2667	24.89694				

The table (1) showing the differences in both D3 and B12 indicators by variables (test-control). The value of B12 There is significant ($P \leq 0.05$) and there is differences between test and control, but there is no significant difference between males and females while the value of F was significant ($P \leq 0.05$), while the effect size was 0.300 indicating a slight effect according to Cohen's standard. For D3, there are differences in favor of the control group, and there are differences in favor of females, while the f value showed and it was a significant ($P \leq 0.05$). There were significant differences in vitamin B12 and D3 levels between groups and also the effect size was 0.16 its slight effect according to Cohen's standard. Both vitamins were significantly lower in test group compared to control.

Table (2) Shows the moderation distribution of both vitamin D3 and B12 in test group compared with control group and between female and male groups.

Parameter	**Group**	**Mean**	**Std. Deviation**	**T-test**	**F-Test**	**Sig.**	**Size effect Partial Eta Squared**
B12 ng/ml	Test	6.7196	3.88440	-3.665	8.421	0.001	0.300
	Control	10.9642	5.26275				
	Male	9.1421	5.80122	0.661			
	Female	8.2995	4.07290				
D3 ng/ml	Test	25.7104	14.18404	-2.610	3.734	0.016	0.160
	Control	35.6229	15.96074				
	Male	29.8198	16.31685	-0.321			
	Female	31.1026	15.33996				

4. Discussions

Calcium is an essential element for neurodevelopment and may offer preventative and treatment options for autism spectrum disease [30] Calcium, for example, is important for regulating synapse growth and regeneration, As aberrant synapse formation was found in ASD, higher calcium levels may be useful for regular brain development [37]. Magnesium insufficiency can result in an increase in intracellular calcium, [25]. It was believed that greater intracellular calcium levels in ASD children compared to controls were

produced by the inactivation of ionized Ca2+-activated K+ channels, resulting in neuronal hyperexcitability [25]. However, no significant differences in serum calcium levels were seen between ASD and the control group [1].

Zinc deficiency was discovered to be common in ASD children [42]. Other studies have found links between low or deficient zinc levels and neurological function in autistic patients [17]. While [7] concluded in study There is no statistically significant difference between boy and girl trace element levels [7]. Researchers discovered that men have higher zinc levels than women because Many variables influence zinc levels, the most important of which are body mass index (BMI) and triglycerides (TG), Metabolic Syndrome, and because autistic people have absorption and metabolism issues, It is further hampered by differences in zinc levels between men and women [15].

Previous study showed low level of 25-hydroxyvitamin D (25(OH)D) when ASD children were compared to their healthy peers [5]. Furthermore, Serum 25(OH)D levels in autistic individuals were found to be considerably lower than in the control group. Autism has been connected to biological and lifestyle variables like as birth, kinship, BMI, physical activity, and vitamin D level. Serum 25(OH)D levels in ASD children were fewer than in a control group of children of same ethnicity, age, and gender. Furthermore, a recent meta-analysis demonstrated an inverse connection between blood levels of the primary circulating form of vitamin D and the risk of ASD [41]. It is unknown if ASD children are born with low vitamin D levels or whether limited sunlight exposure leads to lower vitamin D levels in ASD patients [12]. According to some research Low serum vitamin D levels in ASD children may have a hereditary basis [34]. Kocovska et al. discovered that children with ASD have significantly lower levels of vitamin D than their siblings, who all live in a low-sun environment [22]. The connection between vitamin D and autism was discovered after researchers discovered that children who grew up in areas with low levels of ultraviolet-B rays had a greater ASD frequency than those who lived in sunny places [12].

Our results showed that serum vitamin B12 was lower in children ASD compared to controls in our study. This finding backs up previous research that found ASD individuals had low blood vitamin B12 levels. Autism has been connected to reduced vitamin B12 food intake, which has been linked to poor vitamin B12 absorption, Symbiosis in the gut lining results in poor B12 absorption, autoimmune antibodies, neurotoxic and heavy metal poisoning, and neurons that are insensitive to conventional B12 dosages [2]. Previous research has found that vitamin and mineral deficiencies are common in children with ASD [35], [4]. Megaloblastic anemia and nervous system diseases are caused by vitamin B12 deficiency, In ASD digestive difficulties prevent the absorption of certain nutrients, such as Vitamin B12, resulting in Vitamin B12 insufficiency [28]. Children with ASD had lower levels of VB12 than typically developing children [36]. Vitamin B12 in the brain may play an important function in methylation-dependent actions, Serum vitamin B12 deficiency in ASD patients was thought to imply increased oxidative stress and impaired DNA methylation, Both of which may have a role in the pathophysiology of ASD [20], [23].

5. Conclusions

We discovered that children with ASD had greater vitamins and minerals deficits than typically developing children, found that the levels of these vitamins and minerals were connected to ASD symptoms and the gender of the child.

6. References

[1] Adams, J. B., Audhya, T., McDonough-Means, S., Rubin, R. A., Quig, D., Geis, E., Gehn, E., Loresto, M., Mitchell, J., & Atwood, S. (2011). Nutritional and metabolic status of children with autism vs.

neurotypical children, and the association with autism severity. Nutrition & Metabolism, 8(1), 1–32.

[2] Al-Farsi, Y. M., Waly, M. I., Deth, R. C., Al-Sharbati, M. M., Al-Shafaee, M., Al-Farsi, O., Al-Khaduri, M. M., Gupta, I., Ali, A., & Al-Khalili, M. (2013). Low folate and vitamin B12 nourishment is common in Omani children with newly diagnosed autism. Nutrition, 29(3), 537–541.

[3] Allen, L. H., Miller, J. W., De Groot, L., Rosenberg, I. H., Smith, A. D., Refsum, H., & Raiten, D. J. (2018). Biomarkers of Nutrition for Development (BOND): vitamin B-12 review. The Journal of Nutrition, 148(suppl_4), 1995S-2027S.

[4] Altun, H., Kurutaş, E. B., Şahin, N., Güngör, O., & Fındıklı, E. (2018). The levels of vitamin D, vitamin D receptor, homocysteine and complex B vitamin in children with autism spectrum disorders. Clinical Psychopharmacology and Neuroscience, 16(4), 383.

[5] Arastoo, A. A., Khojastehkia, H., Rahimi, Z., Khafaie, M. A., Hosseini, S. A., Mansouri, M. T., Yosefyshad, S., Abshirini, M., Karimimalekabadi, N., & Cheraghi, M. (2018). Evaluation of serum 25-Hydroxy vitamin D levels in children with autism Spectrum disorder. Italian Journal of Pediatrics, 44(1), 1–5.

[6] Attwood, T., & Grandin, T. (2006). Asperger's and Girls: World-renowned Experts Join Those with Asperger's Syndrome to Resolve Issues that Girls and Women Face Every Day! Future Horizons.

[7] Auda, F. M., Ali, A. M., & Dhyaa, S. (2021). Levels of Heavy Metal and Trace Element Among Children with Autism Spectrum Disorders. Journal of Physics: Conference Series, 1879(2), 22069.

[8] Baio, J., Wiggins, L., Christensen, D. L., Maenner, M. J., Daniels, J., Warren, Z., Kurzius-Spencer, M., Zahorodny, W., Rosenberg, C. R., & White, T. (2018). Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2014. MMWR Surveillance Summaries, 67(6), 1.

[9] Belardo, A., Gevi, F., & Zolla, L. (2019). The concomitant lower concentrations of vitamins B6, B9 and B12 may cause methylation deficiency in autistic children. The Journal of Nutritional Biochemistry, 70, 38–46.

[10] Bjørklund, G., Waly, M. I., Al-Farsi, Y., Saad, K., Dadar, M., Rahman, M. M., Elhoufey, A., Chirumbolo, S., Jóźwik-Pruska, J., & Kałużna-Czaplińska, J. (2019). The role of vitamins in autism spectrum disorder: what do we know? Journal of Molecular Neuroscience, 67(3), 373–387.

[11] Briani, C., Dalla Torre, C., Citton, V., Manara, R., Pompanin, S., Binotto, G., & Adami, F. (2013). Cobalamin deficiency: clinical picture and radiological findings. Nutrients, 5(11), 4521–4539.

[12] Cannell, J. J. (2008). Autism and vitamin D. Medical Hypotheses, 70(4), 750–759.

[13] Duvekot, J., van der Ende, J., Verhulst, F. C., Slappendel, G., van Daalen, E., Maras, A., & Greaves-Lord, K. (2017). Factors influencing the probability of a diagnosis of autism spectrum disorder in girls versus boys. Autism, 21(6), 646–658.

[14] Frazier, T. W., Georgiades, S., Bishop, S. L., & Hardan, A. Y. (2014). Behavioral and cognitive characteristics of females and males with autism in the Simons Simplex Collection. Journal of the American Academy of Child & Adolescent Psychiatry, 53(3), 329–340.

[15] Ghasemi, A., Zahediasl, S., Hosseini-Esfahani, F., & Azizi, F. (2014). Gender differences in the relationship between serum zinc concentration and metabolic syndrome. Annals of Human Biology, 41(5), 436–442.

[16] Gil, Á., Plaza-Diaz, J., & Mesa, M. D. (2018). Vitamin D: classic and novel actions. Annals of Nutrition and Metabolism, 72(2), 87–95.

[17] Goyal, D. K., Neil, J., Simmons, S. D., Mansab, F., Benjamin, S., & Pitfield, V. (2019). Zinc deficiency in autism: a controlled study. Insights Biomed, 4, 12.

[18] Grabrucker, A. M. (2013). Environmental factors in autism. Frontiers in Psychiatry, 3, 118.

[19] Green, R., Allen, L. H., Bjørke-Monsen, A.-L., Brito, A., Guéant, J.-L., Miller, J. W., Molloy, A. M., Nexo, E., Stabler, S., & Toh, B.-H. (2017). Vitamin B 12 deficiency. Nature Reviews Disease Primers, 3(1), 1–20.

[20] Guy, J., Cheval, H., Selfridge, J., & Bird, A. (2011). The role of MeCP2 in the brain. Annual Review of Cell and Developmental Biology, 27, 631–652.

[21] Hunt, A., Harrington, D., & Robinson, S. (2014). Vitamin B12 deficiency. Bmj, 349.

[22] Kočovská, E., Andorsdóttir, G., Weihe, P., Halling, J., Fernell, E., Stóra, T., Biskupstø, R., Gillberg, I. C., Shea, R., & Billstedt, E. (2014). Vitamin D in the general population of young adults with autism in the Faroe Islands. Journal of Autism and Developmental Disorders, 44(12), 2996–3005.

[23] Ladd-Acosta, C., Hansen, K. D., Briem, E., Fallin, M. D., Kaufmann, W. E., & Feinberg, A. P. (2014). Common DNA methylation alterations in multiple brain regions in autism. Molecular Psychiatry, 19(8), 862–871.

[24] Lai, M.-C., Lombardo, M. V, Auyeung, B., Chakrabarti, B., & Baron-Cohen, S. (2015). Sex/gender differences and autism: setting the scene for future research. Journal of the American Academy of Child & Adolescent Psychiatry, 54(1), 11–24.

[25] Laumonnier, F., Roger, S., Guérin, P., Molinari, F., M'rad, R., Cahard, D., Belhadj, A., Halayem, M., Persico, A. M., & Elia, M. (2006). Association of a functional deficit of the BK Ca channel, a synaptic regulator of neuronal excitability, with autism and mental retardation. American Journal of Psychiatry, 163(9), 1622–1629.

[26] Li, Y.-J., Ou, J.-J., Li, Y.-M., & Xiang, D.-X. (2017). Dietary supplement for core symptoms of autism spectrum disorder: Where are we now and where should we go? Frontiers in Psychiatry, 8, 155.

[27] Maenner, M. J., Shaw, K. A., & Baio, J. (2020). Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States,

2016. MMWR Surveillance Summaries, 69(4), 1.

[28] Metyas, M. M., Abdelhakim, A. S., & Ghandour, H. H. (2020). Screening of vitamin B12 in children diagnosed as Autism Spectrum Disorder. QJM: An International Journal of Medicine, 113(Supplement_1), hcaa063-006.

[29] O'Leary, F., & Samman, S. (2010). Vitamin B12 in health and disease. Nutrients, 2(3), 299–316.

[30] Palmieri, L., Papaleo, V., Porcelli, V., Scarcia, P., Gaita, L., Sacco, R., Hager, J., Rousseau, F., Curatolo, P., & Manzi, B. (2010). Altered calcium homeostasis in autism-spectrum disorders: evidence from biochemical and genetic studies of the mitochondrial aspartate/glutamate carrier AGC1. Molecular Psychiatry, 15(1), 38–52.

[31] Şengenç, E., Kıykım, E., & Saltik, S. (2020). Vitamin D levels in children and adolescents with autism. Journal of International Medical Research, 48(7), 0300060520934638.

[32] Sharp, W. G., Postorino, V., McCracken, C. E., Berry, R. C., Criado, K. K., Burrell, T. L., & Scahill, L. (2018). Dietary intake, nutrient status, and growth parameters in children with autism spectrum disorder and severe food selectivity: an electronic medical record review. Journal of the Academy of Nutrition and Dietetics, 118(10), 1943–1950.

[33] Siracusano, M., Riccioni, A., Abate, R., Benvenuto, A., Curatolo, P., & Mazzone, L. (2020). Vitamin D deficiency and autism spectrum disorder. Current Pharmaceutical Design, 26(21), 2460–2474.

[34] Stubbs, G., Henley, K., & Green, J. (2016). Autism: Will vitamin D supplementation during pregnancy and early childhood reduce the recurrence rate of autism in newborn siblings? Medical Hypotheses, 88, 74–78.

[35] Sweetman, D. U., O'Donnell, S. M., Lalor, A., Grant, T., & Greaney, H. (2019). Zinc and vitamin A deficiency in a cohort of children with autism spectrum disorder. Child: Care, Health and Development, 45(3), 380–386.

[36] Tan, M., Yang, T., Zhu, J., Li, Q., Lai, X., Li, Y., Tang, T., Chen, J., & Li, T. (2020). Maternal folic acid and micronutrient supplementation is associated with vitamin levels and symptoms in children with autism spectrum disorders. Reproductive Toxicology, 91, 109–115.

[37] Tang, G., Gudsnuk, K., Kuo, S.-H., Cotrina, M. L., Rosoklija, G., Sosunov, A., Sonders, M. S., Kanter, E., Castagna, C., & Yamamoto, A. (2014). Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. Neuron, 83(5), 1131–1143.

[38] The epidemiology and global burden of autism spectrum disorders. (2015). Psychological Medicine, 45(3), 601–613.

[39] Vahia, V. N. (2013). Diagnostic and statistical manual of mental disorders 5: A quick glance. Indian Journal of Psychiatry, 55(3), 220.

[40] Vela, G., Stark, P., Socha, M., Sauer, A. K., Hagmeyer, S., & Grabrucker, A. M. (2015). Zinc in gut-

brain interaction in autism and neurological disorders. Neural Plasticity.

[41] Wang, T., Shan, L., Du, L., Feng, J., Xu, Z., Staal, W. G., & Jia, F. (2016). Serum concentration of 25-hydroxyvitamin D in autism spectrum disorder: a systematic review and meta-analysis. European Child & Adolescent Psychiatry, 25(4), 341–350.

[42] Yasuda, H., Yoshida, K., Yasuda, Y., & Tsutsui, T. (2011). Infantile zinc deficiency: association with autism spectrum disorders. Scientific Reports, 1(1), 1–5.