



Clinical Study of Vitamin D3 Deficiency and some Trace Elements in Autism

Atteqa M. Sultan¹, Husam M. Kredy^{1*}, Naama J. Gazar²

¹ Chemistry Department, College of Science, University of Thi -Qar, Al-Nasiriya, Iraq.

² The Autism Center in Nasiriyah, Thi-Qar Governorate, Mohammed Al- Mousawi.

*Corresponding Author: Husam M. Kredy

Abstract

Autism is a disorder of nerve growth characterized by weak social interaction and verbal and nonverbal communication, as well as restricted behavior and stereotypes. The subjects of the study were 90 autistic children with varying degrees of severity (mild, moderate and severe) who were diagnosed with DSM-IV, thirty children in each group and 30 healthy children (age and sex were identical). The boys and girl ratio involved in this study was 4:1, and they were 4-12 years of age. The study showed a significant elevation in the levels of Cu and Pb in the blood sample of autistic children when compared to a healthy control group. The elevation was much pronounced in severe group subjects when compared among autistic groups mild and moderate. The levels of Zn and Vitamin D3 were significantly decreased in autistic children when compared to control. The Zn and Vitamin D3 showed significant variation in blood of severe group children when compared to control group and other study groups. The correlation between Zn and Vitamin D3 in these patient groups were positive. Whereas the correlation between Zn and each of (Cu and Pb) in these patient groups were negative.

Keywords: Autism, Children, Blood, Trace Elements, Vitamin D3.

Introduction

Autism Spectrum Disorder (ASD) is a neurodegenerative disorder characterized by frequent stereotypical behavior and disruption of social interaction with the onset of the first [1]. ASD currently affects 1 of sixty-eight young people [2]. ASD is a childhood psychiatric disorder that includes a wide range of clinical symptoms associated primarily with disorders of perception, perception, communication and social sensitivity [3].

Boys are more susceptible to ASD than girls, About 4: 1 ratio [4,5]. The main cause of ASD is unclear, and perhaps there are many factors that are involved in its evolution. These factors may include genetic and environmental factors. Other risks include the history of the ASD family, larger than the parents, single-gene disorders, and family history of immunological conditions, vasodilations and pregnancy complications [6]. Treatment of these children is multi-faceted and includes many interventions such as behavioral analysis, speech therapy,

occupations, sensory integration therapy, counseling, medication management, food modification and special educational resources [7]. Bioelements play an important role in the central nervous system. Lack or increase of trace minerals can cause a variety of health problems and can contribute to ASD damage. Autistic children appear in some cases naturally until age 1-3. Then, sudden changes occur that indicate the presence of the ASD.

Possible causes include exposure to toxic metals with inadequate nutritional status [8]. Vitamin D products that were slightly different in their molecular regulation. The two most dominant metabolites at the time were 13 Vitamin D2 and Vitamin D3. These metabolites have become clinically and nutritious to humans [9]. In our study, the significant elevation in the concentration of Cu and Pb and significant decrease in the concentration of Zn and D3 observed in the blood sample of autistic subjects could be well correlated with their degrees of severity.

Materials and Methods

This study has been conducted a group of patients at the Autism Center in Thi -Qar Governorate, Iraq. Including 120 children (90 autistic children and 30 healthy children) were screened for both sexes from 2 to 10 years from December 2018 to June 2019. The ratio of boys to girls was (4:1).

Determination of Copper (Cu) and Zinc (Zn) in Serum

Dilute 1ml the standers, samples (serum) and quality control specimens 9 fold with distilled water (HPLC) then mix well. A sample was taken for the measurement by a flame atomic absorption spectrophotometer was used after drawing the calibration curve at a wavelength of 324.7nm to Copper while Zinc 213.9nm.

Determination of Lead (Pb) in Whole Blood

2 ml of sample (blood) is taken and placed on the vibrator for one hour after which added 5 ml tri-chloro acetic acid with continuous shaking for 15 minutes and then centrifuge and 3000 (rpm) where the separation is then transferred net filtration for the purpose of measurement of the flame atomic absorption of the where they read at a wavelength of 283.3 nm.

Determination of Vitamin D3

Vitamin D3 is an automated quantitative test for use on the cobas e411 analyzer. For immunoassay determination of D3 in human serum or plasma using electrochemiluminescence (ECL) technique.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows version 24.0 software. The descriptive data were expressed in mean and standard deviation with minimum and correlation coefficient values normally distributed continuous variables were analyzed using one-way analysis of variance (ANOVA) among the groups. A p-value of 0.05 or less 0.001 was considered statistically significant.

Results & Discussion

Table (1) shows that the levels of copper and Lead severely autistic patients were elevated

significantly compared with those of the controls. In the current study, copper is statistically higher in patients than controls. Similarly [10], Unlike that [11]. High copper level is common in autistic children Sequential element is necessary For a number of systems, including the central nervous system, Excessive accumulation of copper leads to oxidative stress [12].

It may lead to a defect in the secretion of the minerals, digestive system, in turn, leads to abnormal accumulation of these elements in children In addition, the physiological conditions of ASD children can be affected by a mixture of mixtures Nutrition factors and clinical treatments, which can also stimulate the metal metabolites [13].

Previous study, have confirmed that blood sample is reliable for the detection of alteration in the metal lead in patients with autism found to be abnormally changed in blood of lead [14], but at no different concentration Pb in [15]. Lead poisoning is suggested as a potential risk factor Autism was reported at a high level of childhood, this is much higher than usual [16].

Addition, shows that the levels of zinc and D3 severely, moderately and mildly autistic patients were significantly lower compared with the normal case. In a study by [17]a significant variation was found for Zn in whole blood of autism group children when compared to a control group.

This result matched with the results of a study of [18] a no significant difference was found for Zn in serum and hair of autistic patients compared with the control. Children with severe zinc deficiency It can create emotional changes such as self-instability, irritability and depression [19]. In a previous study [20], vitamin D3 measured levels of dried blood spots in autistic patients were significantly lower than those in controls.

Another study in serum [21], which is similar to the current study. It may be speculated that the chances of normal vitamin D levels decrease when the child suffers from a psychiatric illness such as ASD. In other words, low levels of vitamin D may negatively affect brain growth and play a role in the development of mental illness [22].

Table 1 characteristic Parameter data for all studied groups

Parameters Groups	S.Cu(μmol/L) Mean ± S.D	S.Zn(μmol/L) Mean ± S.D	B.Pb(μmol/L) Mean ± S.D	S.D3 (nmol/L) Mean ± S.D
<i>Mild autism</i>	15.55 ± 2.43 ^c	14.91 ± 1.94 ^b	0.44 ± 0.15 ^c	45.62 ± 12.87 ^b
<i>Moderate autism</i>	18.97 ± 2.81 ^b	10.21 ± 1.11 ^c	0.50 ± 0.16 ^b	39.02 ± 14.52 ^c
<i>Severe autism</i>	26.50 ± 2.89 ^a	9.71 ± 1.30 ^c	0.66 ± 0.12 ^a	34.07 ± 13.96 ^c
<i>Control</i>	18.52 ± 3.02 ^b	15.92 ± 2.26 ^a	0.36 ± 0.05 ^d	90.87 ± 14.55 ^a
<i>L.S.D</i>	1.18	0.73	0.05	5.94

Correlation analysis of serum zinc level in Table (2) shows that there is an inverse relationship with (copper and lead). On the other hand, there is a positive Correlation with vitamin D3. In this study there is a relationship between the level of zinc and the biochemical parameters included in this study for autistic patients.

It is important to monitor and follow up the values both zinc and copper during zinc treatment, because these two trace elements are the responsibilities of the job, but at the same time essential for living cells [23]. In addition. It is known that this adaptive response to zinc deficiency increases the risk of high toxic metal absorption such as lead.

Thus, infants with zinc deficiency are at increased risk of absorbing a high amount of toxic metals and keeping them in the body. These findings suggest that an increased risk of toxic metal burdens associated with infantile zinc deficiency may also contribute to autism spectrum disorders.

The mechanisms of zinc deficiency in autistic infants and children with ADHD can be explained by their unbalanced food intake and low intestinal absorption [24]. Vitamin D deficiency may alter the immune responses in individuals with ASD [25]. Note: - Each value represents mean ± S.D values with non-identical Superscript (a, b or c... etc.) was considered significant differences (0.05). ≤ (P-LSD: - Least Significant Difference.

Table 2: Correlation analysis of 90 children with autism spectrum disorder shows positive and negative zinc relationships with measured parameters

Zn	R	Zn	Cu	Pb	D3
		1	-0.545 **	-0.374 **	0.224*
	Sig. (2-tailed)		0.001	0.001	0.03

** . Correlation is significant at the 0.01 level

* . Correlation is significant at the 0.05 level

R-correlation coefficient

References

1. Strati F, Cavaliere D, Albanese D, De Felice C, Donati C, Hayek J, Jousson O, Leoncini S, Renzi D, Calabro A, De Filippo C (2017) New evidences on the altered gut microbiota in autism spectrum disorders. *Microbiome*, 5: 24. doi: 10.1186/s40168-017-0242-1.
2. Christensen DL, Baio J, Van NB, Bilder D, Charles J, Constantino JN, Daniels J, Durkin MS, Fitzgerald RT, Kurzius-Spencer M, Lee L, Pettygrove S, Robinson C, Schulz E, Wells C, Wingate MS, Zahorodny W, Yeargin-Allsopp M (2016) Prevalence and characteristics of autism spectrum disorder among children aged 8 years-autism and developmental disabilities monitoring network, 11 sites, United States, 2012. *MMWR Surveill. Summ.*, 65: 1-23.
3. Robertson CE, Baron-Cohen S (2017) Sensory perception in autism. *Nat. Rev. Neurosci.*, 18: 671-684.
4. Hertz-Picciotto I, Schmidt RJ, Krakowiak P (2018) Understanding environmental contributions to autism: causal concepts and the state of science. *Autism Res.*, 11: 554-586.
5. Kim YS, Leventhal BL, Koh YJ, Fombonne E, Laska E, Lim EC, et al (2011) Prevalence of autism spectrum disorders in a total population sample. *Am. J. Psychiatry*, 168: 904-912.
6. Centers for Disease Control and prevention (2016) Community report on autism. Retrieved from
7. Centers for Disease Control and Prevention (2015c) Autism spectrum disorder (ASD): 57 treatment. Retrieved from <http://www.cdc.gov/ncbddd/autism/treatment.html>.
8. Blaurock- Busch E, Amin OR, Dessoki HH, Rabah T (2012) Toxic metals and essential elements in hair and severity of symptoms among children with autism. *Maedica (Buchar)*, 7: 38-48.

9. Bikle D (2000) Vitamin D: Production, Metabolism, and Mechanisms of Action. 2014 Jan 1. In: De Groot LJ, Beck-Peccoz P, Chrousos G, et al., editors. Endotext (Internet). South Dartmouth, MA. MDText.com, Inc.
10. El-Baz F, Mowafy ME, Lotfy A (2018) Study of serum copper and ceruloplasmin levels in Egyptian autistic children. *Egyptian Journal of Medical Human Genetics*, 19(2): 113-116.
11. Skalny AV, Simashkova NV, Klyushnik TP, Grabeklis AR, Bjørklund G, Skalnaya MG, Tinkov AA (2017) Hair toxic and essential trace elements in children with autism spectrum disorder. *Metabolic brain disease*, 32(1)L 195-202.
12. Scheiber IF, Mercer JF, Dringen R (2014) Metabolism and functions of copper in brain. *Progress in neurobiology*, 116: 33-57.
13. Kang DW, Ilhan ZE, Isern NG, Hoyt DW, Howsmon DP, Shaffer M, Krajmalnik-Brown R (2018) Differences in fecal microbial metabolites and microbiota of children with autism spectrum disorders. *Anaerobe*, 49: 121-131.
14. Zhai Q, Cen S, Jiang J, Zhao J, Zhang H, Chen W (2019) Disturbance of trace element and gut microbiota profiles as indicators of autism spectrum disorder: A pilot study of Chinese children. *Environmental research*, 171: 501-509.
15. Baz FE, El-Setouhy MA, Mouharam WA, Abdel-Raouf AM, Youssef AM (2016) Autism and Lead: Is There a Possible Connection?. *Pediat Therapeut* 6: 292. doi: 10.4172/2161-0665.1000292 Page 2 of 6 *Pediat Therapeut* ISSN: 2161-0665 Pediatrics, an open access journal Volume 6• Issue 2• 1000292. R P Age-0.04 0.83 IQ 0.28 0.13 CARS-0.11 0.55 Table, 3, 3.
16. Zafeiriou DI, Ververi A, Vargiami E (2007) Childhood autism and associated co morbidities. *Brain and development*, 29(5): 257-272.
17. Crăciun EC, Bjørklund G, Tinkov AA, Urbina MA, Skalny AV, Rad F, Dronca E (2016) Evaluation of whole blood zinc and copper levels in children with autism spectrum disorder. *Metabolic brain disease*, 31(4): 887-890.
18. Skalny AV, Simashkova NV, Klyushnik TP, Grabeklis AR, Radysh IV, Skalnaya MG, Tinkov AA (2017) Assessment of serum trace elements and electrolytes in children with childhood and atypical autism. *Journal of Trace Elements in Medicine and Biology*, 43: 9-14.
19. Russo AJ, deVito R (2011) Analysis of copper and zinc plasma concentration the efficacy of zinc therapy in individuals with Asperger's syndrome, pervasive developmental disorder not otherwise specified (PDD-NOS) and autism. *Biomark Insights*, 6: 127-133.
20. Fernell E, Bejerot S, Westerlund J, Miniscalco C, Simila H, Eyles D, Humble MB (2015) Autism spectrum disorder and low vitamin D at birth: a sibling control study. *Molecular autism*, 6(1): 3.
21. Feng J, Shan L, Du L, Wang B, Li H, Wang W, Staal WG (2017) Clinical improvement following vitamin D3 supplementation in autism spectrum disorder. *Nutritional neuroscience*, 20 (5): 284-290.
22. Föcker M, Antel J, Ring S, Hahn D, Kanal Ö, Öztürk D, Libuda L (2017) Vitamin D and mental health in children and adolescents. *European child & adolescent psychiatry*, 26 (9): 1043-1066.
23. Bjørklund G (2013) The role of zinc and copper in autism spectrum disorders. *Acta Neurobiol Exp (Wars)*, 73(2): 225-236.
24. Adams JB, Johansen LJ, Powell LD, Quig D, Rubin RA Gastrointestinal flora and gastrointestinal status in children with autism-comparisons to typical children and correlation with autism severity. *BMC Gastroenterology*, 11: 22-34.
25. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J (2011) Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. *Brain, behavior, and immunity*, 25 (1): 40-45.