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Role of Zinc Supplementation on Metallothionine System and Cognitive Motor Performance in Children with Autism

***[1]Ahmed Elnahry, [2]A.M. Eltohamy, [1]N.Abdel Meguid, [1]Ola H. Gebril, [1]A.F. Hashish and [1,3]M. Anwar**

[1]Department of Research on Children with Special Needs, National Research Center, Cairo, Egypt.
[2]Department of Physical Therapy for Disturbances of Growth and Development and its Surgery in Pediatrics, Faculty of Physical Therapy, Cairo University, Cairo, Egypt.
[3]Depatement of Pharmacology, College of Pharmacy, Al jouf University , Kingdom of Saudi Arabia

Abstract : The study was carried out on 30 children with autism, their ages ranged between 3-8 years. The aim of the study was to evaluate the effect of zinc supplement for 12 weeks according to their body weight (daily dose of zinc equal to weight (lbs) plus 15-20 mg.), on the level of plasma MT-1and on the severity of the disease symptoms specificallycognitive motor performance in addition to studying MT1ARNA expression, that might reflect the response to zinc supplement.
Our data revealed significant improvement in cognitive motor performance, increased plasma metallothione in addition to significant decrease in plasma level of copper after zinc supplement. The expression of MT-1 was high in autistic children before taking zinc supplement which would be related to decreased baseline zinc levels in those children, significant decrease was observed after zinc supplementation. We concluded that zinc supplement may be an important component ofa treatment protocol for children with ASD and that it requires attention to motivators and facilitators of exercise adherence.
Key words : Autism spectrum disorder, Cognitive motor performance, Zinc, Metallothionine.

Introduction

Increased number of autism spectrum disorders (ASD) children was reported recently. A prevelance rate of 1 in 45 children in the US with ASD was elucidated [1].

The main features of ASD include: social and communicative impairments as well as repetitive behaviors. Language, social, and cognitive differences are characteristics of ASD, but motor difficulties were significantly reported[2]. Significant delays in fine motor and gross motor skills were reported[3,4,5].

Toe walking, delayed walking, drool, clumsy, imitative movements and difficulty in using tools(dyspraxia) are the main features.

Other common motor symptoms include stereotyping, the purposeless repetition of hand flapping or twirling [6].Previous researches have identified deficits in coordination and general motor function [7], and the planning and execution of movement[8] in children with ASD.

Postural control impairment was hypothesized to delay the development of other motor skills[9].

The increased prevalence of ASD necessiated the focused study of ASD causes. It is hypothesized that heritable factors might contribute to increased ASD susceptibility, with increased risk in the presence of adverse environmental factors [10]**.**The variation in the interaction between inherited vulnerabilities and different environmental factors might account for the heterogeneity that is often associated with autism phenotype [11].

Bio-elements play crucial role in the central nervous system. The lack or excess of essential minerals and trace elements cause a variety of health problems, and an implication in autism etiology was suspected, as they disrupt enzyme functions and cell signaling pathways, with increased oxidative stress and disturbed immune functions[12,13].

Zinc is an essential trace element, with an important antioxidant role[14] and is required for the catalytic activity of many enzymes [15]**.**Prolonged Zn deficiency may therefore cause growth impairment[15]**.**

A significant role of zinc deficiency and severity of autism symptoms including expressive and receptive language, focus attention, hyperactivity, fine motor skills and gross motor skills was postulated[16]**.**The most common cause of Zn deficiency is dietary factors that reduce the availability of Zn, also inherited metabolic disturbances and intestinal diseases can result in reduced Zn levels[17]**.**

Another related element is Copper, an essential trace element for the development and maintenance of CNS functioning. Increased Cu concentrations cause oxidative damage to lipids, nucleic acids and proteins [18] and an association with infections, inflammation, Alzheimer's disease and autism was reported [18,14]**.**Russo and deVito**,**[14] reported that high serum copper disturbs Zn balance and interferes with adrenal hormone production in individuals with autism that may be associated with high norepinephrine, and low GABA with increased excitability and hyperactivity associated autistic symptoms.

Human metallothionein (MTs) genes represent a gene cluster on chromosome 16q13, with four isoforms. MT1 is the most important regulator of body heavy metals levels, with a high affinity for toxic metals and is induced in response to their increased levels.

It is up regulated in response to many metals mainly; Cadmium (Cd), copper (Cu), silver (Ag), mercury (Hg) and zinc (Zn) ions, and thus impacts metal-induced reactive oxygen species (ROS) production[19]**.**

Genetic variants in MTs genes in children could be related to the dysfunction of MT proteins and hence impact their detoxification functions and make them more vulnerable to environmental hazards of heavy metals[20]**.**

Aim of the study

1- Study the effect of Zinc supplementation on the level of plasma MT-1and on cognitive motor performance

2- Studying MT1A RNA expression , an isoform that is related to plasma zinc levels.

Plan of work:-

This study was carried out on 30 children with autism, their ages range between 3-8 years receiving care at the Out-patient clinic for "children with autism" at the center of excellence of Medical Research Centre, National Research Centre (NRC).Patient consent was obtained from all patients involved in this study and it was approved by ethical committee of NRC.

- **The inclusion criteria** Diagnosed cases using 3 psychometric assessments; Diagnostic and Statistical Manual of Mental Disorders, fifth Edition, Text Revision (DSM-IV), Childhood Autism Rating Scale (CARS) and Autism Diagnostic Interview- Revised (ADI-R).
- **Exclusion criteria** Cases with auditory, vestibular, visual and metabolic or dysmorphic defects.

All Participants will be subjected to the following:

Test of Gross Motor Development- Second edition (TGMD-2)

It is a normalized and criterion-referenced test that assesses the gross motor ability of children aged 3-10 years. TGMD-2 measures performance of 12 gross motor skills [21]**.** There are two subtests, locomotor subtest (run, gallop, hop, leap, jump, and slide) and object-control subtest (strike, dribble, catch, kick, throw, and roll). Raw scores from each subtest can be converted to standard scores from the standardized norms and overall

gross motor quotient scores (combination of all 12 gross motor skills)are converted from the sum of the standard scores. The standard scores and quotient scores are described as very superior, superior, above average, average, below average, poor, and very poor**.**

-The blood samples were collected in EDTA coated tubes; separated plasma was used for the following:

A) Determination of plasma copper, and zinc using atomic flame photometer [22]**.**

B) Determination of plasma metallothionein level using ELISA Kit according to the manufacturer's instructions.

RNA extraction (using qiagenRNeasy Mini Kit)for studying metallothionein RNA expression.

C) Preparation of cDNA was done using SensiFast cDNA synthesis kit, using an average volume of 10 µl from extracted RNA cases. The prepared cDNA from RNA samples was checked using ß- Actin PCR.

The cases were subjected to the following:-

-Assessment of severity of autistic features using CARS and ADIR scales.

- Re- assessment of plasma copper, zinc , metallothionein levels, severity of autistic features and motor skills after zinc supplemention for 12 weeks according to their body weight (daily dose of zinc equal to weight (lbs) plus 15-20 mg) [23]

Results

CARS Score of autistic children significantly decreased after zinc supplement P value 0.0002 which denotes improvement of severity of the symptoms.

Significant increased of plasma metallothionine levels after zinc supplemention on the other hand significant decreased of plasma copper levels after zinc supplemention as shown in table 1

Table (1): Effect of zinc before and after supplement on the parameter of the study for ASD .

Characteristics		**Before**	**After**	**P value**
CARS Score	Mean±SD	30.7±5.1	28.7±5.25	0.0002**
	95%CI	1.027-2.862		
	Correlation coefficient			>**0.0001
	Range	18.5-38.5	16.5- 40.5	
Metallothionine	Mean±SD	1.50±0.70	1.68±0.69	**0.0092
	95%CI	0.3070 - - to -0.04780		
	Correlation coefficient	0.8910		**0.0001>
	Range	3.61-0.57	3.21-0.63	
Zinc	Mean±SD	48.7±14.8	60.3±14.3	**0.0001>
	95%CI	-16.83 to -6.427		
	Correlation coefficient	0.5966		**0.0005
	Range	91-27	97-41	
Copper	Mean±SD	103.3±14.7	95.3±15.7	0.0001>
	95%CI	4.415 to 11.58		
	Correlation coefficient	0.8259		0.0001>
	Range	141-74	131-69	

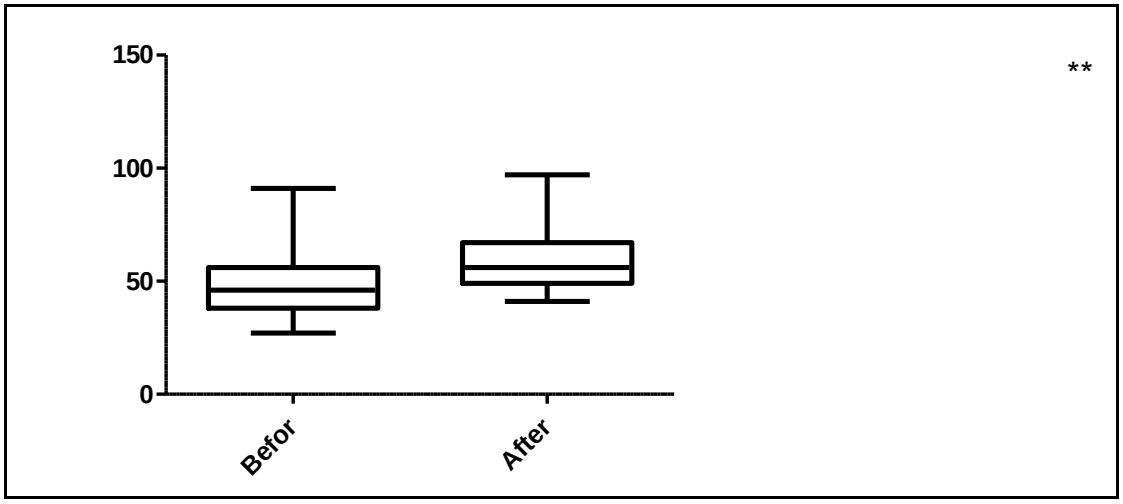


Fig 3: Zinc level (mean and SD) in ASD children before and after zinc supplement

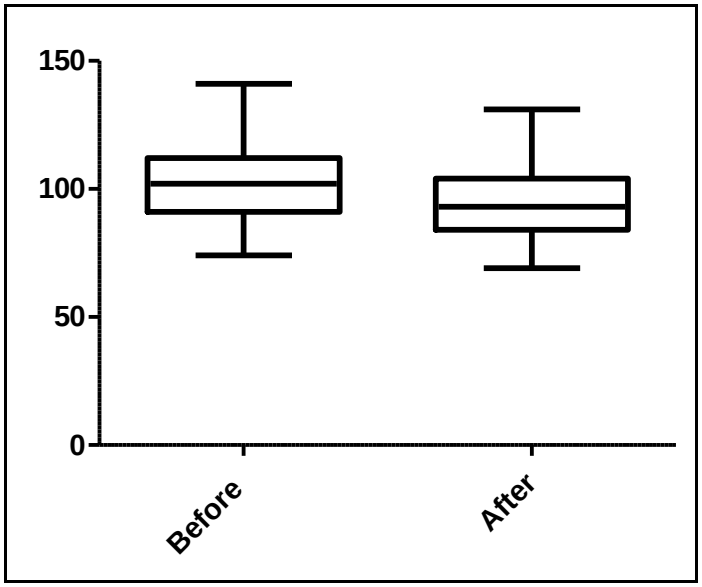


Fig 4: Copper level (mean and SD) in ASD children before and after Zinc supplement

Table (2):GMDS-2 before and after zinc supplementation

Characteristics		**Before**	**After**	**P value**
	Mean±SD	6.22±4,9	11.29±5.26	<0.0001
Locomotor score	95%CI	-6.306 to -3.843		
	Correlation coefficient	0.8154		<0.0001
	Range	18(19-1)	18(19-1)	
object control score	Mean±SD	8.33±4.59	13.29±4.8	0.0001>
	95%CI	1.819 to1.894		
	Correlation coefficient	0.876		0.0001 >
	Range	15	19	

Locomotor testing, object control showed significant increase after zinc supplementation($p< 0.0001$ and $p<0.0004$ respectively).

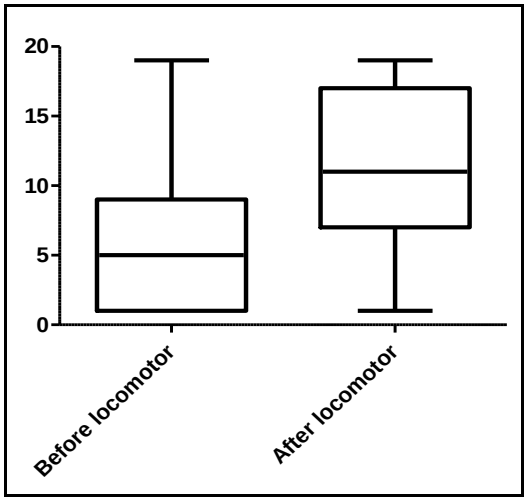


Fig 5: Locomotor testing in ASD children before and after zinc supplement

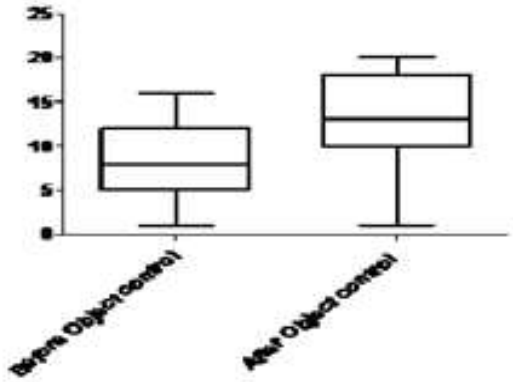


Fig 6:object control testing in ASD children before and after zinc supplement

No correlation was observed between zinc level and locomotor test score nor object controlafter zinc supplementation

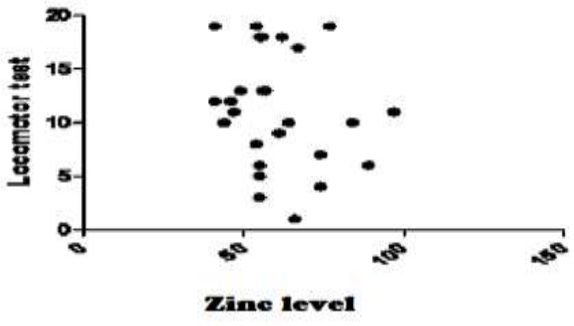


Fig (7): correlation between zinc level and locomotor test score after zinc supplementation

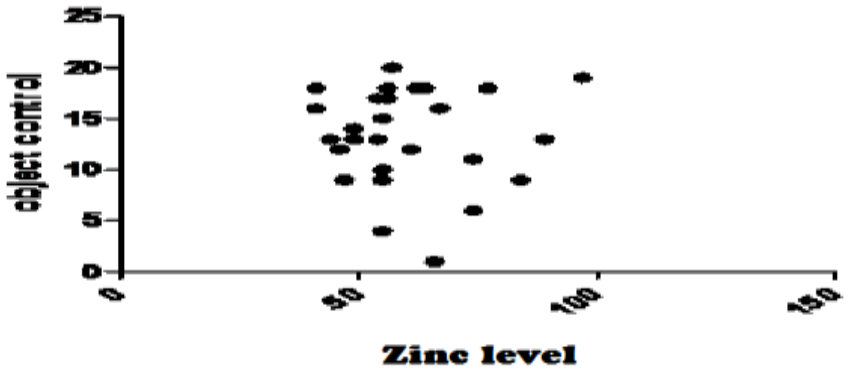
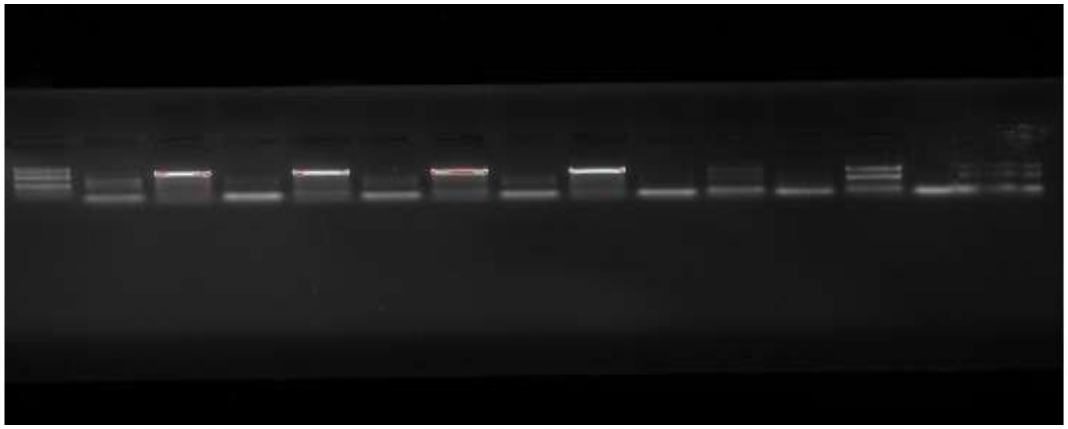


Fig (8): correlation between zinc level and object control test score after zinc supplementation MT-1A RNA expression

Relative gene expression presents the data of the gene of interest relative to some calibrator or internal control gene.

A conventional PCR for MT-1 and GAPDH was done to reveal an illustration of MT-1 and GAPDH expression as a house keeping gene at constant intervals of PCR. Relative MT1A expression was concluded (comparing both bands to DNA ladder intensity).

Fig 9 shows an example of PCR, each sample is represented by 2 lanes (one for GAPDH and the other for MT-1).



Fig(9) Sample of studied MT-1 and GAPDH PCR for comparative expression analysis(each case is represented by 2 lanes on 2.5% agarose gel):

Our results show increased MT-1A in autism children before taking zinc supplement compared to after zinc supplementation (p = 0.01), which would be related to decreased baseline zinc levels in those children.

Discussion

Children are at increased risk of zinc deficiency because of their increased needs for growth and development, and the poor quality of complementary foods [24].Previous studies showed that low Zn/Cu ratio decreased Zn, andelevated Cu levels, are common in ASD children [18,17,25].

In the present study we found a significant increased serum zinc level after zinc supplement for 12weeks (p>,0001) in individuals with autism, also our data revealed that there were significant decrease in serum copper after zinc supplement.

CARS score that is an indication of autism severity significantly decreased after zinc supplement (P value 0.0002) which denotes improvement of severity of the symptoms (Table 1). Zinc has been previously observed to be associated with GABA and glutamate regulation, particularly through anxiolytic activity, modulating GABAergic inhibition and seizure susceptibility.[26]

The low zinc and high copper was postulated to impact GABA levels in CNS, causing its decreased concentration. High copper may also be associated with high norepinephrine found in autistic children, and low GABA, both would be linked to increased excitability and hyperactivity associated autistic features[14].

Several randomized controlled trials have found benefits of zinc supplementation on child developmental parameters, particularly motor development[27,28,29]**,** including psychomotor and mental development scores [30], increased sitting time and decreased hyperactivity symptoms and signs [31,32]**.**

TGMD-2 was used previously to assess gross motor skills performance in children with ASD [33,34,5,35,36,37]**.** These studies have showed delayed motor skill performance in children with ASD, and criteria for motor delay were recorded in about 21% of those children using TGMD-2[38].

In the present study there was significant increase in TGMD-2 (locomotor and object control) in children with autism after zinc supplement for 12 weeks ($p<0.0001$), which indicates an improvement in the activity performance. It is worth noting that several other trials have not demonstrated a significant effect of zinc supplementation in infants and children on mental, psychomotor or behavioural domains [39]**.** Contradictory results were found in few studies, as in Bangladesh zinc supplements resulted in poorer developmental outcomes – possibly because of a micronutrient imbalance [40,41]**.**

Interestingly, a recent study in Peru[29]assessed the effect of zinc, iron and copper supplementation compared with iron and copper supplementation using a battery of outcomes including the Bayley Scales of Infant Development Second Edition (BSID-II).The addition of zinc improved development trajectories in attentional variables, they did not find an effect on the BSID-II or on inhibitory and memory processes.

Our data revealed significant increase of serum metallothionin after zinc supplement ($P> 0.009$) and the expression of MT-1 increased in autism children before taking zinc supplement compared to after zinc supplementation, which would be related to decreased baseline zinc levels in those children.

Bjørklund [17] suggested that Hg accumulation may occur as a consequence of metallothionein (MT) dysfunction in ASD children, which may be one of the effects of Zn deficiency. Zinc and Cu bind and control the synthesis of MT proteins, crucial in metal metabolism and regulation[42,17].

Zinc role inup-regulationof MT gene expression and reducing the toxicity of heavy metals, may suggest that the administration of Zn to ASD children (with diagnosed Zn deficiency) may offer some improvements as a part of other potential dietary interventions [43,17,44,45]**.** It is important to monitor and follow the values for both Cu and Zn together during Zn therapy, because these two trace elements are both antagonists in function, and crucial for living cells[17,25]**.**

Studies indicate that the GABAergic system may be involved in ASDs, and that Zn and Cu may play a role in this system and participate inmodification of synaptic transmission [46].

Conclusion

The present study showed improvement in motor parameters (locomotor and object control), CARS score and metllothionine level in ASD children after zinc supplement for 12 weeks. Confirming that Zn supplement to autistic children may be an important component of a treatment protocol, requiring attention to motivators and facilitators of exercise adherence especially in ASD children with Zn deficiency.

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