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The Correlation Of Serum Copper, Zinc, Magnesium And Oxidative Stress With Autism Spectrum Disorder In South Tamil Nadu Population.

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ABSTRACT

The pathogenesis of autism spectrum disorder (ASD) remains a medical challenge even in the developed world. Although genetics and epigenetic factors have been variously indicted as major causes of the disorder, development of oxidative stress especially in the formative years of children has equally gained prominence as an etiological basis of the disorder. Oxidative stress is characterized by the production of excessive amounts of free radicals, decreased levels of antioxidants with the attendant imbalance in oxidant/antioxidant ratio. This study was designed to determine the levels of essential metals [magnesium (Mg), zinc (Zn), and copper (Cu)] and toxic metal, and generation of oxidative stress by their abnormal interaction. This study is aimed to find the correlation of serum copper, zinc and free radicals with autism spectrum disorder children less than six years of age, those who were registered and followed up in District early intervention center, GRH, Madurai IN the year between June 2020 to June 2021. Twenty-five children clinically diagnosed for ASD according to DSM—5 criteria and 25 neuro-typical (NT) children (controls), (aged 5.96 ± 1.40 years and 6.18 ± 2.59 years respectively) were recruited for this study. Essential and toxic metals were analyzed using induction-coupled plasma-mass spectrometry (ICP-MS); oxidative stress markers [malondialdehyde (MDA), total plasma peroxidase (TPP), and total antioxidant capacity (TAC)] were determined using appropriate biochemical methods. Oxidative stress index (OSI) was calculated. The levels of TPP and TAC were significantly reduced while MDA was higher in ASD compared to NT. Although OSI was higher in ASD, the difference was not significant. Mg, Zn, and Cu levels were reduced significantly in ASD compared to NT. A significant negative correlation between Mg and OSI (r = − 0.438; p = 0.029) was observed in NT. This study may be the basis of inadequate TAC manifesting as increased MDA and reduced TPP levels. The attendant imbalance in oxidant/antioxidant ratio may result in abnormality in neuronal transduction leading to the abnormal cognitive and speech functions characteristic of ASD.

Keywords: Essential and toxic metals, Oxidative stress, Autism spectrum disorder, Imbalance in oxidant/antioxidant ratio

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INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is becoming increasingly prevalent. ASD is a heterogeneous group of neurodevelopmental disorders characterized by impairments in verbal and non-verbal expressive speech, deficits in social interaction and hyper-focused repetitive behaviors [1, 2]. The causes and pathophysiology of ASD are not fully understood. There is a general agreement that ASD could result from interaction between genetic and environmental factors with oxidative stress as a potential link [3]. Oxidative stress has recently been linked to the etiology of this disorder along with multiple genetic and environmental factors [4, 5]. Oxidative stress is characterized by the production of excessive amounts of free radicals, de-creased levels of antioxidants, or both. Excessive production of free radicals or impaired antioxidant mechanism may cause oxidative stress which may induce several pathophysiological processes. Cellular antioxidant defense mechanism prevents the generation of free radicals and inactivates them after generation. Impaired antioxidant defense mechanism can result in cell membrane damage, alteration in membrane fluidity and permeability, and oxidative changes in proteins, lipids, and DNA [6]. That the pathophysiology of ASD involves oxidative stress resulting from exposure to heavy metals as environmental pro-oxidants has been reported [7].

MATERIALS AND METHODS

This Hospital based case control study was conducted during June 2020 to June 2021 at Institute of child health & Research centre, Government Rajaji hospital, Madurai medical college, Madurai. The study population include 50 children with ASD as cases, and 50 children without ASD as controls, from the Southern region of Tamil Nadu, India.

Inclusion Criteria: Children Of Less Than 6 Years Who Are Diagnosed To Have Autism Spectrum Disorder According to AIIMS Modified Incline Diagnostic Tool For Autism Spectrum Disorder Based On DSM 5 Criteria Will Be Included In The Study As Cases. Age And Sex Matched Healthy Children Will Be Included As Controls. Age and sex matched healthy children will be included as controls. An informed written consent will be obtained from parent / guardian of all children after fully explaining the study procedure. Basic demographic data were recorded for both cases and controls. 2 ml of blood sample was drawn from both cases and controls and were analysed for serum levels of copper, zinc and free radicals.
Exclusion Criteria: Participants that were suffering from liver or kidney disease, anemia, or current treatment for iron deficiency, progressive neurological disorders, or epilepsy were excluded from the study. None of the participants was on psychotropic drug.

Analytical Statistics

Student's *t* test was used to assess the statistical significance of the difference between two study groups means. ANOVA test was used to assess the statistical significance of the difference between more than two study groups means. Chi-square test was used to examine the relationship between two qualitative variables. Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells. *P* value of 0.05 was considered significant.

RESULTS

Table 1: Comparison Of Biodata And Biochemical Variables Between ASD And NT Groups Using Student T-Test

VARIABLES	ASD	NT	*P*-VALUE
	(*N* = 25)	(*N* = 25)	
Child's Age (yrs)	5.96 ± 1.45	6.18 ± 2.2.59	0.713
Mother's Age at Birth (yrs)	26.68 ± 2.69	27.96 ± 2.70	0.100
Father's Age at Birth (yrs)	31.72 ± 2.98	32.32 ± 3.86	0.541
Number of Siblings	1.72 ± 0.79	1.60 ± 0.91	0.622
Child's weight (kg)	19.64 ± 3.99	19.00 ± 5.18	0.627
Mg (mg/dl)	2.53 ± 0.46	3.13 ± 0.43	0.000*
Zn (µg/dl)	222.3 ± 63.8	438.5 ± 185.5	0.000*

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Cu (µg/dl)	4.32 ± 1.02	4.88 ± 0.94	0.049*
Zn/Cu	55.31 ± 22.04	92.29 ± 44.57	0.001*
Total Plasma Peroxidase (TPP)	105.9 ± 2.3	110.4 ± 7.9	0.010*
Total Antioxidant Capacity (TAC)	280.2 ± 34.4	303.8 ± 33.1	0.017*
Malondialdyde ($\times 10^{-5}$) (MDA)	2.27 ± 0.23	1.42 ± 0.13	0.000*
Oxidative Stress Index (OSI)	0.38 ± 0.05	0.37 ± 0.05	0.284
*Significant at $p < 0.05$			

Table 1 is a summary of the biodata variables between the autism spectrum disorders and neuro-typical children The results showed no significant differences in all the biodata variables except the birth weight that showed significantly increased birth weight for ASD compared to NT ($p = 0$). Although, there was a difference in birth weight of cases and controls, the two groups were com- parable based on another biodata obtained through structured questionnaire. Also, the summary of mean ± SEM of essential trace metals and toxic metal between ASD and NT showed significant differences in all the biochemical parameters. Mg, Zn and Cu were significantly reduced in ASD com- pared to NT ($p < 0.000$; $p < 0.000$ and $p < 0.049$) respectively. Zn/Cu ratio was also significantly reduced in ASD compared to NT ($p < 0.001$). Aside this, mean ± SEM levels of oxidative stress markers in ASD and NT showed that TPP and TAC were reduced ($p < 0.010$; $p < 0.017$) while MDA was higher in ASD compared to NT ($p < 0.000$). Although OSI was higher in ASD, the difference between the two groups was not significant ($p > 0$).

Table 2: Correlation Of Levels Of Essential And Toxic Metals Level With Oxidative Markers In NT

		TPP	TAC	MDA	OSI	Zn/Cu
Mg	r	−0.182	0.058	0.006	−0.092	0.589
	P	0.384	0.784	0.979	0.663	0.002*
Zn	r	−0.128	0.147	0.260	−0.172	0.839
	p	0.542	0.483	0.209	0.411	0.000*
Cu	r	0.384	0.202	0.095	−0.151	− 0.785
	p	0.058	0.334	0.653	0.472	0.000*
*Significant at $P < 0.05$						

Table :2 There were no significant correlations among the trace metals and oxidative markers in Neurotypical children. However, expressing concentration of Cu as a ratio of Zn showed that Mg positively correlated with Zn/Cu ratio ($r = 0.589$; $p = 0.002$). Cu had negative correlation with Zn/Cu ratio ($r = -0.785$; $p = 0.000$) while Zn positively correlated with the ratio (0.839; $p = 0.000$).

Table 3 Correlation Of Toxic And Essential Metals Levels With Oxidative Stress Markers In ASD

		TPP	TAC	MDA	OSI	Zn/Cu
Mg	r	− 0.337	0.291	0.284	−0.438	0.378
	p	0.099	0.158	0.169	0.029*	0.063
Zn	r	0.055	0.158	0.192	−0.106	0.907
	p	0.793	0.452	0.357	0.613	0.000*
Cu	r	0.222	−0.077	− 0.133	0.170	− 0.302
	p	0.287	0.715	0.527	0.417	0.142
*Significant at $p < 0.05$						

Table :3 There was a significant negative correlation between Mg and OSI ($r = -0.438$; $p = 0.029$) and a significant positive correlation between Zn and Zn/Cu ratio ($r = 0.907$; $p = 0.000$). also not significant. Like in ASD children, a highly sig- nificant negative correlation was obtained between OSI and TAC ($r = -0.832$, $p = 0.000$); however, contrary to those of ASD, a highly positive significant correlation was equally obtained between OSI and TPP in Neuroty- pical children ($r = 0.696$, $p =$

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0.000).\

Table 4: Correlation Of Zn/Cu Ratio With Oxidative Indices In Children With ASD

Correlations in ASD						
		TPP	TAC	MDA	OSI	Zn/Cu
TPP	Pearson Correlation	1	−.083	.041	.225	−.180
	Sig. (2-tailed)		.692	.846	.279	.389
	N	25	25	25	25	25
TAC	Pearson Correlation	−.083	1	.360	-.983[a]	−.093
	Sig. (2-tailed)	.692		.077	.000	.659
	N	25	25	25	25	25
MDA	Pearson Correlation	.041	.360	1	−.369	−.039
	Sig. (2-tailed)	.846	.077		.069	.852
	N	25	25	25	25	25
OSI	Pearson Correlation	.225	-.983[a]	−.369	1	.065
	Sig. (2-tailed)	.279	.000	.069		.759
	N	25	25	25	25	25
Zn/Cu	Pearson Correlation	−.180	−.093	−.039	.065	1
	Sig. (2-tailed)	.389	.659	.852	.759	
	N	25	25	25	25	25

Table :4 No significant correlations were observed among markers of oxidative stress including with Zn/Cu ratio in children with ASD except between OSI and TAC (r =-0.983, p = 0.000) where a highly significant negative correlation was obtained.

Table 5 Correlation of Zn/Cu ratio with Oxidative indices in NT children

Correlations IN NT						
		TPP	TAC	MDA	OSI	Zn/Cu
TPP	Pearson Correlation	1	−.198	−.067	.696[a]	−.031
	Sig. (2-tailed)		.344	.752	.000	.883
	N	25	25	25	25	25
TAC	Pearson Correlation	−.198	1	−.136	−.832[a]	.216
	Sig. (2-tailed)	.344		.518	.000	.300
	N	25	25	25	25	25
MDA	Pearson Correlation	−.067	−.136	1	.040	.241
	Sig. (2-tailed)	.752	.518		.849	.245
	N	25	25	25	25	25
OSI	Pearson Correlation	.696[a]	−.832[a]	.040	1	−.190
	Sig. (2-tailed)	.000	.000	.849		.364
	N	25	25	25	25	25
Zn/Cu	Pearson Correlation	−.031	.216	.241	−.190	1
	Sig. (2-tailed)	.883	.300	.245	.364	
	N	25	25	25	25	25

[a]Correlation is significant at the 0.01 level (2-tailed)

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DISCUSSION

Autism spectrum disorders is a complex, behaviourally defined neurodevelopmental disorder of early childhood characterized by social deficits, language impairments and repetitive behaviours. Autism spectrum disorders (ASD) should be diagnosed earlier for early intervention and better outcome. ASD is usually diagnosed clinically only after full manifestation of the disease. The etiology of this complex disease is highly heritable, but likely involves environmental factors [8]Trace elements play a critical role in the pathogenesis of ASD. Particularly copper and zinc are important trace elements that are involved in the metabolism of neuro transmitter and also important for development of the brain. Ratio of Cu and Zn is helpful for early diagnosis of the disease and start interventions earlier. Because of the potential association between ASD and Zn and Cu levels, patients with autism may be tested for plasma concentration of these elements and its clinical significance. In heavy metal toxicity, both Mg and Ca may changing the molecular configuration of the catalyzing enzyme with its attendant negative consequences. Hence, a reduction in level of these essential metals as seen in this study especially in the central nervous system may initiate a deficit in the neurological functions of the metals. Their deficiency may also elicit replacement in some of the enzymatic processes where they are involved or may exacerbate accumulation of other toxic metals leading to deleterious effect of the latter especially oxidation in sen- sitive organs like the brain. The observed reduced Mg level in children with ASD in this study may clearly corroborate this. Mg deficiency has been reported to in- crease NO accumulation and lipid peroxidation and lowers plasma antioxidants levels [9]. Its deficiency has also been reported to increase oxidative stress which was reversed by its supplementation in laboratory animals [10]. Hence, findings in this study were similar to earlier works reporting reduced Mg level in children with ASD as a possible basis of observed lipid peroxidation and increased NO accumulation in this disorder. Zn is also an essential trace metals involved in catalytic activities of over 300 enzymes and plays a crucial role in protein synthesis, immune system and cell division [11]. Zn deficiency has been reported to predispose individuals to development of neuropsychological changes like emotional imbalance, depression and irritability and cognitive disorders [12]. It has also been reported that reduced Zn level exacerbates Cu toxicity [13]. Because of the very crucial roles of this essential metal in many metabolic activities in the body, downregulation of its level as observed in children with ASD in this study in comparison to what obtained in NT children may be sig- nificant in the observed dysfunction and clinical symptoms associated with ASD. The expected role of Zn in protein synthesis and cell division may be easily com- promised in these children leading to abnormality in DNA synthesis and its attendant effect on genetic composition especially in the brain. One of the meta- bolic functions of Zn is its modulatory role on Cu ab- sorption at the intestinal level. The redox potential of Cu is also said to be closely linked with its rate of absorption in the intestine [14]. Aside from its role in redox reactions, Cu is also an important cofactor in many metalloenzymes. These antioxidative functions of Cu as a redox metal are largely dependent on the Zn/Cu combination in metallothionein. This molecule has been implicated as regulator molecules in gene expression, homeostatic control of cellular metabolism of metals, and detoxifying agent against toxic metals [15]. As observed in this study, there was a downregulation of Zn with the attendant imbalance in the Zn/Cu ratio. This might have precipitated a concurrent reduction in the metallothionein level and a deficit in the function of the latter. The consequence of this may be generation of oxidative stress which the imbalance in Zn/Cu ratio may have induced. The imbalance in the level of the two es- sential metals has been attributed to pathological changes in the intestinal mucosa in ASD leading to a downregulation of Zn and its consequent impairment of antioxidant defenses and impairment of DNA repair especially in sensitive organs like the brain [16]. It has also been previously reported that Zinc induces the intestinal synthesis of a copper-binding protein such as metallothionein. Metallothionein traps copper within intestinal cells and prevents its systemic absorption [17]. Thus, a reduction in the expected Zn level as seen from results of this work may have reduced the synthesis of metallothionein and its implication on the modulatory functions of this Zn/Cu molecule in attenuating toxicity of heavy metals. In this study, Zn/Cu ratio in NT children was 1:1; al- though some workers have postulated an inverse relationship in levels of the two metals in NT children [18]. Hence, however, the observed reduction in Zn level in children with ASD in this study resulting in a reduction in the ratio may precipitate a reduction in the concentration of metallothionein required for the necessary antioxidative activities of the essential metals [19].

Markers of oxidative stress determined in this study were TAC, TPP, MDA, and OSI. The presence of essential metals like Zn, Cu, and Mg in collaboration with other systemic antioxidants

was expected to moderate a balance in levels of oxidants and antioxidants to allow for a conducive milieu for normal metabolic activities. This is especially required in sensitive organs like the brain. The reduced TAC level seen in children with ASD in this study could be traced to increased MDA and reduced TPP levels which collectively would overwhelm the antioxidant pool of the body. TPP is an indication of the peroxidase capability in the membrane which with reference to the neuron will have a deleterious effect on its transmission capability. This effect may be accentuated by an increase in MDA level exacerbating the ROS and by implication the oxidation processes at the neural level. The increased MDA and reduced TPP which may explain the abnormal cognitive and speech functions characteristic of ASD. Previous studies have reported alteration in composition of fatty acids, oxidation of lipids and phospholipids of the membrane of cells in children with ASD [20-23] all these may have direct effect on the functions of proteins that are involved in signal transduction, an important process required in neural transmission. Elevation of MDA level is suggestive of damage to the lipid component of the cell which may lead to alteration in membrane lipid metabolism, such as composition of fatty acid content of the cell. The ultimate effect of this is its adverse effect on transduction and transmission processes involving these proteins [24]. Hence, an imbalance in oxidant/antioxidant system of the body especially in sensitive organs like the brain may lead to structural damage due to the deleterious effects of ROS and the attendant disruption in transduction and transmission of signals across neurons. This finding is similar to previous ones where reduced activity of antioxidant enzymes like glutathione peroxidase and superoxide dismutase as well as plasma glutathione concentration was reported in ASD [25].

CONCLUSION

This study showed that decreased levels of Zn and Mg is a possible etiological basis for oxidative stress with a reduction in TAC and the attendant abnormal neurological sequelae associated with children with ASD in this environment. It is therefore possible that enhanced MT induction by oxidative stress might have opposite net effects on functional Zn and Cu status, with Zn deficiency being combined with a tendency for Cu excess. In the brain, it is possible that MT over-induction caused by excess Cu may interfere with normal temporary storage in synaptic regions followed by mobilization of Zn for use as a cofactor of enzymes needed for long-term learning processes (because Cu might interfere with the second release phase of the storage-release process). This suggests that providing Zn to autistic children may be an important component of a treatment protocol, especially in children with Zn deficiency. 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 essential for living cell

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