ENVIRONMENTAL CO-EXPOSURE TO LEAD AND MANGANESE AND NEURODEVELOPMENTAL DISORDERS IN EGYPTIAN CHILDREN A CROSS-SECTIONAL STUDY

By

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ABSTRACT

Background: *Environmental pollutants are byproducts of our industrialized society. Exposure to Lead and Manganese resulting in elevated levels in the human body is becoming increasingly highlighted as findings, in Attention Deficit Hyperactivity Disorder (ADHD) and Autism which are of complex aetiology and showing increasing prevalence.*

Objective: *This study aimed to investigate the association between ADHD, Autism and environmental exposure to Lead and Manganese.*

Methods: *This case-control study included 100 children aged (2 – 13) years, 70 children previously diagnosed with psychiatric disorders (37 ADHD and 33Autism), diagnosed according to DSM-V criteria coming for follow-up in the Child Psychiatry clinic, Children's hospital Ain Shams University, and 30 apparently healthy children comparable for age, sex, education and socioeconomic levels as control during the period from July 2018 to August 2020, they were selected by simple random method. Venous blood samples were taken from the studied population to determine the level of Lead and Manganese in the blood along with an interview questionnaire including personal, environmental, medical, psychiatric and social factors which was completed by parents.*

Results: *Blood Lead levels ranged between 2-24 µg/dl among cases and 2-9 µg/dl among controls while Manganese levels were between 5-36 µg/dl among cases and 4-15 µg/dl among controls. Statistical analysis revealed a significantly elevated mean blood Lead level and mean blood Manganese level in children with ADHD and Autism versus the control group ($P<0.01$).*

Conclusion: *It was found that there is a statistically significant higher blood level of Lead and Manganese In patients with ADHD and Autism versus the control group.*

Environmental pollution is an important factor to be considered when handling psychiatric illnesses.

Keywords: *ASD; ADHD; Children; Neurodevelopmental; Heavy Metals; Lead and Manganese.*

INTRODUCTION

Natural elements known as "heavy metals" are distinguished by their relatively high atomic mass and high density. Unfortunately, toxic heavy metals are a result of our industrialized society and are found in our air, water, soil, and food supply. Pollution in our environment is so prevalent that it is no more a matter of whether someone has been exposed to poisons, but rather, how much exposure they have had **(Koller et al., 2018)**. Exposure to heavy metals has grown due to anthropogenic and industrial activities, as well as modern industrialization, which has negative impacts on human health **(Luo et al., 2020)**.

Neurodevelopmental disorders have been linked to environmental pollutants such as toluene, lead, mercury, arsenic, and polychlorinated biphenyls (PCBs). Children are especially susceptible to the harmful health effects of environmental exposures due to their rapid neurodevelopment. The first 1000 days of life are when the brain is most plastic and goes through a complicated series of changes, including neurogenesis, myelination, and synaptic pruning. These experiences accumulate over time to affect critical cognitive functions like language and speech production, attention, behavior, and thinking **(Villar et al., 2019)**. This means that alterations to their biological environments, such as exposure to toxins, may upset the ordered progression of events, resulting in irreversible side effects like learning challenges, behavioral problems, and delays in neurodevelopment. Children are more susceptible to harmful environmental exposures than adults due to extra-biological and social factors in addition to this age of rapid brain development. Children tend to eat more non-food things, spend more time on the ground, and consume more food and drinks in relation to their body weight **(Davis et al., 2019)**.

Manganese is naturally present in surface and groundwater, but it is also discharged into the environment as a result of human activity. Manganese is often regarded as one of the least dangerous metals when supplied orally because homeostasis limits gastrointestinal absorption.

However, there is mounting evidence that oral neurotoxicity exists, especially in neonates. Compared to adults, they have a more sensitive nervous system and less developed homeostasis. Breast milk normally contains little manganese, whereas infant formula has significant amounts. The amount of manganese in the water used to mix the formula with the newborn may also have a significant impact on the infant's daily exposure to manganese because infant formula is often sold in powder form **(Ljung; and Vahter; 2007)**. It is usual for excess manganese in the body to accumulate in the basal ganglia, especially in the globus pallidus, and it has also been observed in the frontal cortex **(Ma et al., 2018)**.

It can be difficult for children with ADHD, a neurological condition characterised by impulsivity, distractibility, and restlessness, to develop normally and integrate into society **(Milano, 2018)**. According to estimates, 9.8% of children and adolescents worldwide suffer from ADHD, making it the third most common mental health disorder after depression and anxiety. However, this prevalence is rising in Egypt, where it has reached 20% **(Aboul Ata et al., 2018)**. The prevalence of ADHD has increased over the past few years, which has led many people to look at environmental factors as likely reasons **(Bitko RH et al., 2022)**.

The evidence indicates that disruption of the dopaminergic system has a substantial impact on the pathophysiology of attention-deficit/hyperactivity disorder (ADHD) in children. Through the presynaptic Dopamine transporter, manganese builds up in dopaminergic neurons and exerts its damaging effects **(Shih et al., 2018)**. Additionally, it has been established that elevated manganese levels affect the Dopamine system, where the majority of pharmaceutical ADHD therapies work **(Farias et al., 2010)**.

Current research suggests that environmental variables, particularly environmental contaminants, also play a role in autism spectrum disorders (ASD), despite the consensus that genetic flaws play a role in these diseases. However, a complete analysis of these toxicant-related investigations in ASD has not yet been conducted **(Rossignol, 2014)**. Although little is understood about the possible relationship between lead and manganese exposure and children's intelligence, research suggests that lead and manganese may interact and modify each other's effects on school-aged children's intelligence. As a result, more care should be taken to avoid exposing disadvantaged children to different combinations of toxic substances **(Kim et al., 2009)**.

AIMS OF THE WORK

This work aimed to determine the blood level of Lead and Manganese in Egyptian children with ADHD and Autism compared to normal controls and their correlation with some environmental pollution.

SUBJECTS AND METHODS

Ethical considerations:

- A written informed consent was obtained from patients or their legal guardians.
- Approval by the local ethical committee of the pediatric department and faculty of medicine, at Ain Shams University was obtained before the study.
- The authors declared no potential conflicts of interest concerning research, authorship, and/or publication of the article.
- All the data of the patients are confidential and the patients have the right to keep it.
- The patient has the right to withdraw from the study at any time.
- The researcher explained the aim of the study to the patients and their guardians.

Sample size:

Using EPI INFO sample size calculator; with 0.05 alpha error, confidence interval of 0.95 and power of the study of 0.80. The minimum sample size calculated to detect the blood level of Lead and Manganese in Egyptian children with ADHD and Autism compared to normal controls was 100 children.

Inclusion criteria: children who were diagnosed with ADHD or ASD using DSM–V criteria.

Exclusion criteria: Patients with any psychiatric disease, intellectual handicap, or chronic significant medical or neurological disorders were excluded.

Study design:

This case-control study was conducted on a total of 100 children recruited from The child Psychiatry clinic Pediatric Hospital/Ain Shams University, during the period from July 2018 to August 2020.

The studied children were classified into three groups as follows:

Group I: Thirty-seven children whose ages ranged from 6-14 years old, previously diagnosed with ADHD by the Diagnostic and Statistical Manual of Mental Disorder V(DSM-V,2013), coming regularly to follow up on their treatment and behavioral therapy.

Group II: thirty-three children, age ranges 2-10 years old, diagnosed with an Autism spectrum disorder (ASD) by the Diagnostic and Statistical Manual of Mental Disorder V(DSM-V,2013), coming to the same departments for follow-up.

Group III: included thirty age and sex-comparable apparently normal children as a control group.

All studied groups underwent:

- Full medical history taking laying stress on the onset of illness, course of the disease, neurological manifestations, learning disabilities and behavioural disturbances.
- Thorough clinical examination, laying stress on signs of toxicity and neurological manifestations.
- Questionnaire asking about environmental risk factors including residence, housing, dietary habits, history of breast/artificial feeding, parental occupation, sibling's health, etc.(filled by parents).
- Psychiatric evaluation and diagnosis by a Psychiatric consultant DSM -V Classification **(APA, 2013)**.

Laboratory investigations for Lead and Manganese:

Three (3) ml of venous blood (heparinized) was withdrawn from each child and immediately frozen until analyzed to determine blood levels of Lead and Manganese. Analysis was done using graphite furnace atomic absorption Perkin Elmer Analyst 600 spectrophotometer **(D'Ulivo, 2004)**, in the Unit of Medical Analysis for Prevention and Treatment of Occupational diseases, Community medicine department, Ain Shams University.

Statistical analysis: Data were analyzed using SPSS (Statistical Package for Social Sciences) version 12. A Chi-square test was performed to compare individual characteristics. Results were expressed as the mean ± standard deviation (SD) and compared using a T-test. Significant values of P at less than 0.05 and 0.01

were considered highly significant.

RESULTS

Our results will be demonstrated in the following tables and figures:

Table (1): Demographic data of the studied groups

		Group I (no.37)		Group II (no.33)		Group III (no.30)			
Age	**Range (yrs)**	6-14		2-10		2-14			
	Mean ± SD	7.9± 2.4		5.3± 2.3		2.9± 1.2			
		no.	**%**	**no.**	**%**	**no.**	**%**	**X^2**	**P**
Gender	**Female**	31	84	25	76	17	56.6	1.8	0.4
	Male	6	16	8	24	13	43.4		
Residence	**Rural**	12	32.4	4	12.12	8	26.6	0.4	0.7
	Urban	25	67.6	29	87.87	22	73.3		
Exposure to industrial zones		21	56.7	18	54.54				
Non-exposure to industrial zones		16	43.2	15	45.45				

Group I: ADHD, Group II: Autism, Group III: Control, P> 0.05
i.e. not significant, SD: standard deviation

Table (1): There was no statistically significant difference between the three studied groups as regards gender, residence and presence of industrial zones. The mean age was higher among patients in group I compared to other groups.

Table (2): Comparison between the three studied groups as regards the Lead and Manganese levels in the blood of the three studied groups

		Group I (no.37)	Group II (no.33)	Group III (no.30)	F	P
Blood Lead levels(µg/dl)	**Range (µg/dl)**	3-21	1-24	2-9	6.7	**0.002****
	Mean ± SD	8.7± 3.6	7.4± 4.2	5.5± 2.0		
Blood Manganese levels(µg/dl)	**Range(µg/dl)**	5-33	5-36	4-15	5.2	**0.007****
	Mean± SD	13.3± 6.6	10.7± 6.3	8.8± 2.8		

Group I: ADHD, Group II: Autism, Group III: Control, P> 0.05
i.e. not significant, Blood Lead levels of more than 10 µg/dl are considered high. A blood Manganese level of more than 15 µg/dl is considered high

Table (2): There was a higher mean level of Lead and Manganese in blood in group I and group II compared to group III and the difference is statistically highly significant amongst the studied samples (P=0.002 and P=0.007, respectively).

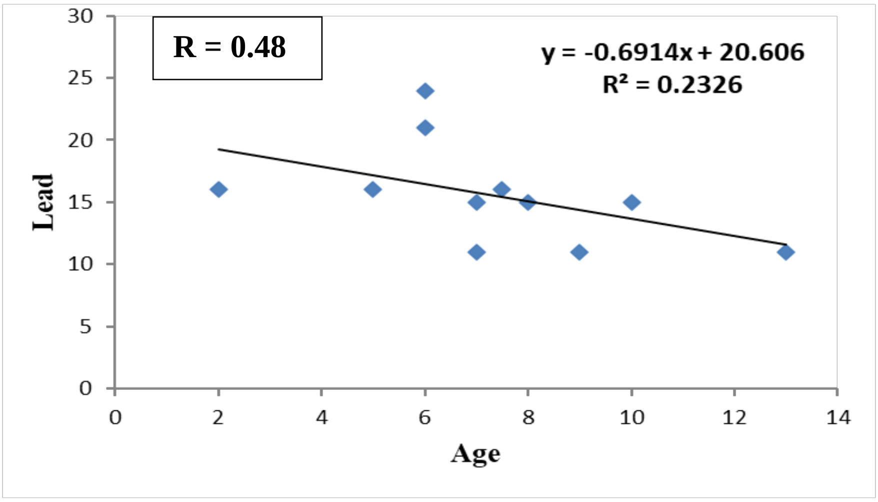

Figure (1): Relation between age and Lead level > 10 µg/dl

Figure (1): The ages of all patients with lead levels greater than 10 g/dl were correlated. The age of a child and blood lead level seems to be inversely correlated (higher blood lead in younger age).

Table (3): Comparison between residence and exposure to industrial zones of studied groups I and II in relation to blood Lead and Manganese levels

			Normal		High		X^2	P
			No.	%	No.	%		
Group I (no.37)	Blood Lead Levels	Rural (no.12)	10	83.3	2	16.7	0.5	0.4
		Urban (no.25)	18	72.0	7	28.0		
		Non-industrial zone (no.16)	11	68.8	5	31.3	0.7	0.3
		Industrial zone (no.21)	17	81.0	4	19.0		
	Blood Manganese Levels	Rural (no.12)	10	83.3	2	16.7	0.9	0.3
		Urban (no.25)	17	68.0	8	32.0		
		Non-industrial zone (no.16)	13	81.3	3	18.8	0.9	0.3
		Industrial zone (no.21)	14	66.7	7	33.3		
Group II (no.33)	Blood Lead Level	Rural (no.4)	2	50.0	2	50	3.1	0.07
		Urban (no.29)	25	86.2	4	13.8		
		Non-industrial zone (no.15)	11	73.3	4	26.7	1.3	0.2
		Industrial zone (no.18)	16	88.9	2	11.1		
	Blood Manganese Level	Rural(no.4)	3	75.0	1	25	0.3	0.5
		Urban (no.29)	25	86.2	4	13.8		
		Non-industrial zone (no.15)	12	80.0	3	20.0	0.5	0.4
		Industrial zone (no.18)	16	88.9	2	11.1		

Group I: ADHD, Group II: Autism, Group III: Control, P> 0.05 i.e. not significant, High blood Lead > 10µg/dl, High blood Manganese > 15 µg/dl.

Table 3: In groups, I and II, cases residing in urban areas had a higher percentage of high Lead and high Manganese levels than cases living in rural areas, although the difference is not statistically significant. In terms of high Lead and Manganese levels, there is no statistically significant difference between subjects who were exposed to an industrial source and subjects who were not.

DISCUSSION

Our results showed higher mean age among patients in group I (ADHD) compared to other groups. This can be attributed to the nature of ADHD and its time of diagnosis which is more common nearer to the age of start of school, as compared to Autism and the control group.

Evaluation of Lead level in the present work showed statistically significantly higher levels in ADHD patients (group I) and ASD patients (group II) compared to control subjects (group III) (P<0.01).

Our findings support those of **(Awaga et al., 2020)**, who conducted a study to check the potential link between lead exposure and ADHD in children and found that lead level was extremely significant in the ADHD group compared to the control group. Additionally, **(Hawari et al., 2020)** supported our findings by stating that Lead levels were greater in male ASD patients compared to female ASD patients and in children with ASD aged 5 or younger when compared to controls (P = 0.001). In the same perspective, **(Rossignol, 2014)** demonstrated a significant link between perinatal exposure to air pollutants (including mercury, lead, nickel, manganese, diesel particulate matter, and methylene chloride) and an elevated risk of ASD. However, **(Hawari et al., 2020)** reported that blood manganese levels were lower in the groups of ADHD (9.95%, P = 0.026), and ASD (9.64%, P = 0.046), which went against our findings regarding manganese levels. Lead and manganese, however, had a positive correlation with one another (P = 0.01). The incidence of ASD, ADHD, or its co-occurrence may be associated with the lead rise and manganese decrease. Meanwhile, there is a link between elevated lead levels and the emergence of ADHD in youngsters, according to a review study by **(Thach et al., 2017)**. However, there was no link between higher blood lead levels and the onset of ASD. It was also noted that the majority of studies had weak points like small sample sizes, trouble matching control pairs, and failure to account for confounding factors.

(Gorini et al., 2014) outlined the part lead plays in neurotoxicity, The disruption of calcium homeostasis, mitochondrial damage, stimulation of calcium release from the mitochondria, modification of lipid metabolism, and accumulation in astrocytes are examples of direct pathways. Lead also can replace zinc, which is necessary for the regulation of gene transcription via proteins with zinc fingers or receptor channels with zinc-binding sites. It's possible that modifications to the systems that regulate gene expression during early neurodevelopment would result in a loss of grey matter, a change in myelin, and adult neurological disorders. Disruption of thyroid hormone transport into the brain is another indirect consequence of lead on the brain.

Residence effects on Lead and Manganese overexposure in our community were evaluated in the present study. Regarding patients with Autism (group II), the results showed a higher percentage of blood Lead levels among cases living in rural areas compared to those in urban areas (yet not statistically significant). We have to mention that within the group of Autism only 4 cases lived in rural while 29 lived in urban so the small sample size is an important element in this finding although it agrees with the results provided. This result can be attributed to housing factors and the possible fact that children are allowed to play outdoors thus getting more exposed to dust and soil **(Carrel et al., 2017)** measured the blood lead levels of neonates born to urban and rural mothers. Their findings support the idea that both urban and rural people are equally at risk of having elevated blood lead levels (BLLs). Additionally, **(Aelion and Davis, 2019)** carried out a study in which BLLs were gathered from roughly 140,000 children ages 1 to 6 years, both in urban and rural settings. BLLs were more prevalent in urban children than in rural children, although BLL increased relatively in rural children from age 1 year to age 2 years.

On the other hand, in our study, patients with ADHD who lived in urban regions had higher blood Lead levels than those who did in rural areas. According to a study by **(Abdelrasul et al., 2012)** on 190 children in urban and rural areas, children in urban areas had higher blood levels of lead than children in rural areas, which were attributed to the high levels of lead in the air in those places. Therefore, specific exposure sites, traffic, and vehicle emissions have a significant impact on such findings.

In this study, approximately 16% of the 100 children had blood Manganese levels of more than 15 μg/dl which is the cutoff point according to the Agency for Toxic Substances and Disease Registry **(Williams et al., 2012)**. Comparison of Manganese levels between studied groups showed significantly higher levels in patients with ADHD (group I) compared to control subjects (group III) (P<0.01). Autism patients (group II) had a higher mean Manganese level compared to controls but the difference was statistically non-significant. This finding is consistent with that of a study by **(Zoni and Lucchi, 2013)** who reported a link between manganese exposure and hyperactive behavior. The effects of manganese on the dopaminergic and gamma butyric acidergic system, which contributes to childhood hyperactivity and is susceptible to manganese, were cited as the cause of this link.

Additionally, **(Hong et al., 2014)** hypothesised that children with ADHD are particularly sensitive to the negative consequences of high manganese levels due to the striatal dopamine transmission associated with the pathophysiology of ADHD being involved in the neurotoxic mechanism of manganese. According to **(Rossignol, 2014)**, prenatal exposure to manganese among other substances was strongly linked to a higher chance of developing autism spectrum disorder.

A higher percentage of instances with elevated manganese levels were found in cases living in urban areas compared to cases living in rural areas in the ADHD (group I) and Autism (group II) patients, although the difference was not statistically significant. The small sample size of patients in the current study is responsible for the non-significant difference. Individuals who were exposed to an industrial source did not significantly differ in their blood lead and manganese levels. This can be related to the direction of the wind and air currents about residential localization, the kinds of surrounding companies, and those industries' relationships with various pollutants. The correlation cannot be used as a reliable statistical benchmark due to the small sample size, although younger patients had higher blood Lead levels. This is explained by greater hand-to-mouth activity and gastrointestinal Lead absorption in younger age groups. This conclusion is in line with that of **(Boseilla et al., 2004)**, who made the same claim in a thorough investigation.

CONCLUSION

Patients with ADHD and autism have significantly higher blood lead and manganese levels than non-affected individuals.

RECOMMENDATIONS

Additional extensive research is required to determine the precise pathophysiology of such an environmental risk, and environmental biomarkers are required to verify exposure areas.

LIMITATIONS OF THE STUDY

Our study limitations may be attributed to the small sample size the short period of data collection and the no availability of funding sources.

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