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Research Article

The Relationship between Cadmium and Tyrosine Hydroxylase Activity in Patients with Attention Deficit Hyperactivity Disorder (ADHD)

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Abstract

Background: Attention Deficit Hyperactivity Disorder (ADHD) is a common neurodevelopmental disease characterized by inattention, hyperactivity, and impulsiveness. Dopamine deficiency is considered one of the key neurochemical underpinnings of ADHD. Tyrosine Hydroxylase (TH), the rate-limiting enzyme in the production of dopamine and other catecholamines. It has been increasingly studied for its potential role in ADHD. Future insights into TH regulation and its modulation may offer novel therapeutic strategies for affected children and adolescents. This study aimed to assess TH activity in individuals with ADHD and explore its relationship with cadmium (Cd) levels, comparing findings with those of healthy controls.
Methods: The current study was designed as a case-control study, involving 90 healthy control subjects and ADHD patients, aged between 4 and 18 years. The patients were categorized into two groups: the first group included 60 patients with ADHD, and the second group represented the control group, which comprised 30 volunteers without any ADHD. The TH activity was measured using the ELISA technique. Additionally, the concentration of selected cadmium was determined using Atomic Absorption Spectroscopy (AAS).
Results: TH levels showed greater variability in ADHD patients but were not significantly different from controls. A significant negative correlation was found between TH and cadmium ($r = -0.230$, $p = 0.029$). Multiple linear regression confirmed cadmium levels and treatment type as significant predictors of TH activity.
Conclusions: Cadmium exposure had a negative influence on tyrosine hydroxylase activity in patients with ADHD, suggesting an environmental impact on dopamine synthesis.

Keywords: Attention Deficit Hyperactivity Disorder, Children, Adolescents, Cadmium

Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is one of the most common neurodevelopmental diseases in children and adolescents. It is typically diagnosed in childhood and often persists into maturity. It is one of the most prevalent reasons that children are taken to psychology and psychiatric clinics [1]. Timely and effective therapy to address core ADHD symptoms and co-occurring disorders is are high priority for healthcare and society as a whole. While significant research has established the advantages and disadvantages of numerous therapies for ADHD. [2]. It affects around 5% of children and adolescents, as well as 2.5% of adults. The illness has a complex etiology with a high heritability. Patients exhibit substantial interindividual and intraindividual diversity in symptoms, with executive impairments in numerous cognitive areas [3]. ADHD is increasingly linked to poor health consequences. Inattention, impulsivity, and poor planning are likely to contribute to bad health behaviors; yet, these same behaviors may also have an impact on ADHD [4]. A diagnosis of ADHD is determined by recognizing excessive inattention, hyperactivity, and impulsivity in a child under the age of 12 that impairs his or her functioning or development. The primary classification methods for diagnosing ADHD are the International Classification of Diseases and the Diagnostic and Statistical Manual of Mental Disorders (DSM) [5]. Children and adolescents with ADHD often struggle academically. The prevalence of issues among kids with ADHD varies from 50 to 80%, depending on the description of the problem [6].

The catecholaminergic neurotransmitters norepinephrine (NE) and dopamine (DA) play important roles in controlling prefrontal brain function. Given that disturbance of the excitation/inhibition balance in the prefrontal cortex is related to many of the aforementioned mental illnesses [7]. The norepinephrine transporter (NET) is an important target for treating ADHD. The NET gene has gained increasing interest as a possible regulator of ADHD pathogenesis [8]. The basic symptoms of ADHD are associated with anomalies in the corticostriatal-thalamo-cortical circuits. Dysregulation of the dopaminergic and noradrenergic systems disrupts the proper modulation of neurons in the prefrontal cortex (PFC) and basal ganglia, resulting in hyperactivity, impulsivity, and inattention [9]. Dopaminergic neuronal illnesses provide a significant challenge to millions of individuals globally. These disorders are characterized by dysregulation of the dopamine system [10].

Tyrosine hydroxylase (TH) is the enzyme responsible for synthesizing catecholamines, including dopamine (DA), noradrenaline (NA), and adrenaline. Multiple processes regulate TH activity, which is essential for proper physiology. It has been linked to several neuropathological disorders [11]. Catecholamines function as neurotransmitters and hormones in different bodily areas. Chemical messengers like dopamine, noradrenaline, and epinephrine (adrenaline) are formed from the amino acid tyrosine. Dopamine is predominantly produced in the brain, namely in the substantia nigra and ventral tegmental region, where it acts as a neurotransmitter [12]. The main source of TH activation is phosphorylation at Ser40, which is primarily mediated by cyclic AMP-dependent protein kinase A [13]. Tyrosine hydroxylase (TH; EC 1.14.16.2) is a homotetrameric enzyme that transforms L-tyrosine to L-dihydroxyphenylalanine (L-DOPA), using molecular oxygen as a substrate and cofactor (6R). -L-erythro-5,6,7,8-tetrahydrobiopterin (BH4) [14].

Heavy metal exposure has been linked to deleterious neurodevelopmental consequences in children, namely an increased risk of attention-deficit hyperactivity disorder (ADHD) [15]. Excess metals impede neurodevelopmental processes and have neurotoxic consequences, reducing cognitive function. Heavy metals may thus have a role in ADHD susceptibility. Given the rising frequency of ADHD, it is critical to investigate the environmental elements that may influence its development. Among these considerations, certain metals with proven neurotoxic effects have gained attention [16]. Cadmium is a neurotoxin, and the exact mechanism of cognitive impairment is uncertain. It has been theorized to impair synaptic transmission, bind to calmodulin, or induce an imbalance in oxidative stress [17].

The aim of this study is to examine the relationship between cadmium (Cd) level and tyrosine hydroxylase in children and adults with ADHD and compare them with normal controls.

Materials and Methods

Study design and patients

This study was designed to be a case-control study. A total of 90 subjects were recruited for this case-control study and divided into two groups: Group 1 included 60 patients with inattention, hyperactivity, impulsivity, and ADHD, with an age range of 4-18 years, and Group 2 represented the control group, which comprised 30 volunteers without any ADHD and matched the age to the patient groups, were nonsmoking, and were nonalcoholic. The diagnosis of ADHD was made by psychiatrists in the hospitals based on standardized clinical criteria defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM) [6]. The diagnostic information was documented in the patients' medical records and used for recruitment purposes. The patients were collected from the Children's Teaching Hospital in Dhi Qar Health Directorate in Dhi Qar Governorate and Al-Hassan Al-Mujtaba Hospital in Karbala Health Directorate in Karbala Governorate, Iraq. A special questionnaire form containing descriptive information was completed for each patient.

Inclusion criteria:

Males and girls (ages 4-18) were included. Subjects must have a diagnosis of ADHD, which is determined by clinical evaluation standards.

Exclusion criteria:

Included patients were those with a history of head trauma with loss of consciousness, organic brain diseases, seizures, or neurosurgical intervention. Others were those with any major medical ailment identified by the researcher, mental retardation, pregnancy, and nursing.

Subjects having a recent history (within the last six months) of illicit substance or alcohol dependence and sensory issues, including deafness or blindness.

Blood sample collection and storage

The study involved obtaining 5 mL of whole blood from each subject, which was divided into two parts. The first part was used for venipuncture to obtain three-ml blood samples, which were then placed in gel tubes at room temperature for ten minutes. A 3000 x 2000 RPM centrifuge was used to separate the serum for ten minutes. The second part involved dividing the serum samples into three parts in the Eppendorf laboratory and storing them at -20°C for later use for further investigation.

Determination of cadmium in serum

Cadmium (Cd) determination in serum was conducted using Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS), which allows for highly sensitive detection of trace metals in biological fluids. The element cadmium was obtained in standard solution form (1000 ppm stock solution) (Merck KGaA, Darmstadt, Germany) and further diluted with deionized water and nitric acid (0.2%) to prepare four standard working solutions of 2.5, 5, 10, and 20 parts per billion (ppb). These standards were used to construct a calibration curve, which served as the reference for quantifying cadmium levels in serum samples.

Sample and procedure

Serum was obtained from venous blood collected in trace metal-free Vacutainer tubes and centrifuged at 3000 rpm for 10 minutes. Approximately 20 µL of serum was injected into a pyrolytically coated graphite tube, the atomizer unit of the GFAAS instrument (Shimadzu AA-6300, Japan). The graphite tube was subjected to a programmed temperature cycle for drying, ashing, and atomization, which vaporizes cadmium from the matrix and enables detection via atomic absorption at a 228.8 nm wavelength.

Ethical approval

The study protocols were approved by the Ethical Committee of the College of Medicine, University of Karbala, on October 7, 2024, under approval number 24-60. Written informed consent was obtained from all participants or their legal guardians before enrollment and blood sample collection.

Statistical analysis

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) version 28.0 (IBM Corp., Armonk, NY, USA). Data with a normal distribution were reported as mean and standard deviation (mean ± SD). Two groups were compared using independent samples t-tests. The Pearson correlation coefficient (r) was employed to determine the strength and direction of linear correlations among continuous variables. To explore predictors of tyrosine hydroxylase (TH) levels, multiple linear regression analysis was applied, incorporating relevant biochemical and demographic variables. A p-value < 0.05 was considered statistically significant for all analyses.

Results

Demographic data characteristic

The demographic characteristics of patients and healthy control groups are summarized in Table 1. The age range of participants was between 4 and 18 years old, with the gender distribution among ADHD patients. The sample included 63.33% males and 36.67% females, reflecting the higher prevalence of ADHD typically reported in males in epidemiological studies. In terms of weight, the average body weight of participants was recorded and compared between groups to account for potential physiological differences.

Comparison of biochemical markers between ADHD patients and control group

The findings of the independent t-test revealed no statistically significant differences in some biomarkers between the ADHD and control groups. The ADHD and control groups had identical mean TH index values ($p < 0.05$). In contrast, there were statistically significant variations in cadmium between the ADHD and control groups ($p = 0.0001$), showing that the values were comparable between the two groups.

Spearman’s correlation analysis was performed to assess the relationship between tyrosine hydroxylase (TH) activity and the concentrations of cadmium (Cd) among the study participants ($n = 90$). A statistically significant correlation ($r = -0.230$, $p = 0.029$), indicating that higher Cd concentrations may be associated with reduced TH activity (Table 2). Among the included variables in the multiple linear regression model, Cd and treatment type were found to be statistically significant predictors of tyrosine hydroxylase (TH) activity. Cadmium was negatively associated with TH ($B = –0.054$, $p = 0.016$) (Table 3).

Discussion

Cadmium is a heavy metal present in the earth's crust that is distributed in the environment both naturally and by human activities such as fossil fuel combustion, waste incineration, smelting methods, and the use of phosphate fertilizers. Uptake of ambient cadmium in plants and animals’ results in human exposure through food or tobacco smoke, and occupational exposures can also occur [18]. Cd is known to cause nephrotoxicity, and emerging data suggest that exposure to Cd may have negative neurodevelopmental effects.

Table 1: Descriptive statistics of age, weight, and biochemical markers among ADHD patients and control group

Type	Character	Minimum	Maximum	Mean	Stander Deviation
Patient (n=60)	Age (4-18 years)	4	18	7.20	2.791
	Weight	11	47	18.57	7.422
	TH (ng/mL)	0.13	19.14	11.9482	3.72815
	Cadmium (µg/dL)	0.00	48.22	8.8325	13.88380
Control (n=30)	Age (4-18 years)	4	15	7.50	2.764
	Weight	18	47	28.08	7.951
	TH (ng/mL)	5.88	19.76	12.6789	2.92483
	Cadmium (µg/dL)	0.00	.00	.0000	.00000

Table 2: Correlation between tyrosine hydroxylase (TH) and heavy metals (Pb, Al, and Cd) among study participants (n = 90).

Character	Correlations		
		Tyrosine hydroxylase	Cadmium
Tyrosine hydroxylase	Correlation Coefficient	1.000	-0.230*
	Significant (2-tailed)	-	0.029
	Total No.	90	90
Cadmium	Correlation Coefficient	-0.230*	1.000
	Significant (2-tailed)	0.029	-
	Total No.	90	90

*Correlation is significant at the 0.05 level (2-tailed).

Table 3: Multiple linear regression coefficients for predicting tyrosine hydroxylase (TH) activity

Coefficients					
Model	Unstandardized Coefficients		Standardized Coefficients	t-test	p- value
	B	Stander Error	Beta		
Constant	20.118	1.894		10.619	.000
Weight	0.079	0.043	0.157	1.837	0.072
Cadmium	-0.054	0.022	-0.201	-2.485	0.016
Treatment	-7.332	0.760	-0.767	-9.653	0.000

Animal studies have reported the impact of Cd on the electrophysiological characteristics of the brain, alterations in neurotransmitter function, and negative neurobehavioral results [19].

A recent analysis of urine Cd and ADHD in 1994-2004 revealed a possible non-significant decrease in ADHD risk in children who had urinary Cd levels above the first quartile, with OR of 0.50, 0.52, and 0.67 for the second, third, and fourth quartiles, respectively. He discovered a nearly zero connection between a medical diagnosis of ADHD and continuous or categorical blood cadmium levels [20].

The results of this study showed statistically significant differences in the demographic prevalence. In males, it was found that comments agree with ADHD, which is less commonly diagnosed in girls than in males, particularly in youth. Even when girls are diagnosed with ADHD, the diagnosis is frequently delayed compared to males, and females are less likely to be provided medication [21]. There could be a gender bias in the diagnosis and treatment of ADHD. This study examined gender differences in the strength and presentation of ADHD symptoms, behavioral difficulties, and learning deficits in males and females with and without clinically diagnosed ADHD [22]. Girls with ADHD had fewer hyperactive and impulsive symptoms and more inattentive symptoms than boys with the illness [23].

Cadmium (Cd) is generally known as an environmental neurotoxin. Exposure to Cd has been linked to motor impairments, a reduction in dopaminergic neurons, and neuropathological alterations in the midbrain [24]. Elevated blood cadmium levels have been linked to impaired cognitive function and motor abilities. Combined exposure to metals in drinking water changes the dopamine system, changing the activity of tyrosine hydroxylase (TH). Toxicology is often determined by exposure to metal combinations [25]. Several animal studies have looked at how cadmium affects electrophysiological parameters, neurotransmitter function indices, and neurobehavioral outcomes. Studies on children have also revealed a link between elevated cadmium levels and mental impairment [26].

Conclusions

ADHD is a prevalent neurodevelopmental disorder that can persist into adolescence and adulthood, with inattention and impulsivity often remaining even when hyperactivity decreases. The study found significantly higher levels of heavy metals (Pb, Al, and Cd) in ADHD patients compared to healthy controls. Cadmium showed a significant negative correlation with tyrosine hydroxylase activity, suggesting that cadmium exposure may impair dopamine synthesis. Cadmium levels and treatment type were identified as significant predictors of TH activity, while Pb, Al, and vitamin D showed no significant predictive effect. These findings highlight the possible role of environmental factors (heavy metals) and pharmacological treatment in the dopaminergic dysfunction observed in ADHD. Screening for heavy metal exposure and ensuring balanced nutritional factors, such as vitamin D may support better ADHD management.

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References

1. Salari N, Ghasemi H, Abdoli N, Rahmani A, Shiri M H, Hashemian A H, et al. The global prevalence of ADHD in children and adolescents: a systematic review and meta-analysis. Ital J Pediatr. 2023;49:48.
2. Coghill D, Santosh P, Huss M, Zuddas A, Millar T, Robertson B, et al. The management of ADHD in children and adolescents: bringing evidence to the clinic: perspective from the European ADHD Guidelines Group (EAGG). Eur Child Adolesc Psychiatry. 2021;30:1–25.
3. Mechker K, Banaschewski T, Hohmann S, Häge A. Evidence-based pharmacological treatment options for ADHD in children and adolescents. Pharmacol Ther. 2022;230:107940.
4. Holton KF, Nigg JT. The association of lifestyle factors and ADHD in children. J Atten Disord. 2020;24(11):1511–1520.
5. **Storebø OJ, Ramstad E, Krogh HB, Nilausen TD, Skoog M, Holmskov M, et al.** Social skills training for attention deficit hyperactivity disorder (ADHD) in children aged 5 to 18 years. *Cochrane Database Syst Rev.* 2019;6:CD008223.
6. Spender C, Marton I, Wiener J, Rogers M, Moore C, Spender C., Rokeach A, et al. Friendship characteristics of children with ADHD. J Atten Disord. 2015;19(10):872–881.
7. Ilipilă L, Arnold L E. The role of adrenergic neurotransmitter reuptake inhibitors in the ADHD armamentarium. Expert Opin Pharmacother. 2024;25(8):945–956.
8. Chen H, Li J, Huang Z, Fan X, Wang X, Chen X, et al. Dopaminergic system and neurons: role in multiple neurological diseases. Neuropharmacology. 2024;260:110133.doi:10.1016/j.neurpharm.2024.110133.
9. Xing B, Li Y-C, Gao W-J. Norepinephrine versus dopamine and their interaction in modulating synaptic function in the prefrontal cortex. Brain Res. 2016;1641:217–233.
10. Dunkley P R, Dickson P W. Tyrosine hydroxylase phosphorylation in *vivo*. J Neurochem. 2019;149(6):706–728.
11. Vecchio LM, Sullivan P, Dunn AR, Bermejo MK, Fu R, Masoud ST, et al. Enhanced tyrosine hydroxylase activity induces oxidative stress, causes accumulation of autotoxic catecholamine metabolites, and augments amphetamine effects *in vivo*. J Neurochem. 2021;158(4):960–979. doi:10.1111/jnc.15432.
12. Korner G, Noain D, Ying M, Hole M, Flydal M I, Bindoff L A, et al. Brain catecholamine depletion and motor impairment in a Th knock-in mouse with type B tyrosine hydroxylase deficiency. Brain. 2015;138(10):2948–2963.
13. Alam G, Richardson JR. Regulation of tyrosine hydroxylase: relevance to Parkinson's disease. In: genetics, neurology, behavior, and diet in Parkinson's disease. Academic Press; 2020: 51–66.
14. Mowlem FD, Rosenqvist MA, Martin J, Lichtenstein P, Asherson PJE, Larsson H. Sex differences in predicting ADHD clinical diagnosis and pharmacological treatment. Eur Child Adolesc Psychiatry. 2019;28(4):481–489. doi:10.1007/s00787-018-1211-3.
15. Slobodin O, Davidovitch M. Gender differences in objective and subjective measures of ADHD among clinic-referred children. Front Hum Neurosci. 2019;13:441.
16. Peasgood T, Bhardwaj A, Biggs K, Brazier JE, Coghill D, Cooper CL, et al. The impact of ADHD on the health and well-being of ADHD children and their siblings. Eur Child Adolesc Psychiatry. 2016;25(11):1217–1231. doi:10.1007/s00787-016-0841-6.
17. Pyatha S, Kim H, Lee D, Kim K. Co-exposure to lead, mercury, and cadmium induces neurobehavioral impairments in mice by interfering with dopaminergic and serotonergic neurotransmission in the striatum. Front Public Health. 2023;11:1265864. doi:10.3389/fpubh.2023.1265864.
18. Ciesielski T, Weuve J, Bellinger DC, Schwartz J, Lanphear B, Wright RO. Cadmium exposure and neurodevelopmental outcomes in U.S. children. Environ Health Perspect. 2012;120(5):758–763.
19. Kim W, Jang Y, Lim YH, Kim BN, Shin CH, Lee YA, et al. The effect of prenatal cadmium exposure on attention-deficit/hyperactivity disorder in 6-year-old children in Korea. J Prev Med Public Health. 2020;53(1):29–36. doi:10.3961/jpmph.19.175.
20. Kim S, Arora M, Fernandez C, Landero J, Caruso J, Chen A. Lead, mercury, and cadmium exposure and attention deficit hyperactivity disorder in children. Environ Res. 2013;126:105–110. doi:10.1016/j.envres.2013.08.008.
21. Farmani R, Mehrpour O, Kooshki A, Nakhaee S, Braun JM, Froehlich T, et al. Exploring the link between toxic metal exposure and ADHD: a systematic review of Pb and Hg. J Neurodev Disord. 2024;16(1):44. doi:10.1186/s11689-024-09555-8.

22. Tamegart L, Abbaoui A, Makbal R, Zroudi M, Bouizgarne B, Bouyatas M M, et al. *Crocus sativus* restores dopaminergic and noradrenergic damages induced by lead in Meriones shawi: a possible link with Parkinson's disease. Acta Histochem. 2019;121(2):171–181. doi:10.1016/j.acthis.2018.12.003.
23. Aydin DB, Nasıroğlu S, Aydın E, Elmas B, Özdemir Ö, Erel Ö, et al. Heavy metal and thiol/disulfide in children with attention-deficit and hyperactivity disorder. Psychiatry Clin Psychopharmacol. 2024;34(1):50–57. doi:10.9758/cpn.2024.34.1.50.
24. Xu Y, Hong H, Lin X, Tong T, Zhang J, He H, et al. Chronic cadmium exposure induces Parkinson-like syndrome by eliciting sphingolipid disturbance and neuroinflammation in the midbrain of C57BL/6J mice. Environ Pollut. 2023;337:122606. doi:10.1016/j.envpol.2023.122606.
25. Kaur S, Canals-Sans J, Morales-Hidalgo P, Alda J A, Arija V, et al. Investigating the association between heavy metals and attention deficit hyperactivity disorder in children: an exploratory study. J Atten Disord. 2025;29(6):423–436. doi:10.1177/10870547251315276.