Original Article

Probabilistic Statistics to Aid Novel Heavy Metal Analysis in Attention Deficit Hyperactivity Disorder (ADHD) – A Case-Control Study with Bayesian Statistics

Saurav Nayak[1], Suchanda Sahu[2], Joseph John[3]

[1]Senior Resident, [2]Additional Professor, Department of Biochemistry, [3]Professor, Department of Paediatrics, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India.

Abstract

Background and objectives: Probabilistic statistical methods with the help of Bayesian Statistics help to analyze biomedical data with a newer and wider perspective. Combined with the knowledge provided by previous data, the novel approach was undertaken to study the association of Attention Deficit Hyperactivity Disorder (ADHD) with heavy metals in children. ADHD is characterized by inattention, impulsivity, and hyperactivity. Being a widely prevalent neurodevelopmental disorder, it is attributed to both genetic and environmental etiology. Hair and urine samples were used instead of blood as two non-invasive sources for assessing heavy metals.

Materials and Methods: ADHD children diagnosed as per DSM-V criteria and age-matched healthy controls were recruited in this study. Hair and urine samples were analyzed for arsenic, cadmium, copper, lead, nickel, and zinc. The levels of heavy metals were measured using ICP-OES after acid digestion and extraction. Bayesian Statistics in JASP v0.15 was used for statistical analysis.

Results: The Bayes Factor, BF10 for the group with ADHD vs. healthy children, were significantly different for all heavy metals. In both hair and urine, lead, cadmium, nickel, and copper concentrations were higher in ADHD. However, zinc levels were lower in both samples for ADHD.

Conclusion: By the probabilistic method, heavy metals are higher in the urine and hair of children with ADHD.

Key Words: Attention Deficit Hyperactivity Disorder, Bayesian Statistics, ICP-OES, heavy metals

Article Summary: Submitted: 24-July-2023 **Revised:** 12-August-2023 **Accepted:** 02-September-2023 **Published:** 30-September-2023

Quick Response Code:

Web Site
http://ijmsnr.com/

DOI
10.55349/ijmsnr.202333512

Corresponding Author: Dr. Suchanda Sahu, Additional Professor, Department of Biochemistry, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India.
Email ID: biochem_suchanda@aiimsbhubaneswar.edu.in

Introduction

The current major public health challenges are neurodevelopmental diseases, according to the World Health Organization (WHO). They account for a significant part in affecting morbidity, mortality, disability, and quality of life. [1] Attention Deficit Hyperactivity Disorder (ADHD) is diagnosed as per the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V), into two major symptomatologic `purviews, that is, inattention and hyperactivity/impulsivity. The ADHD prevalence rate varies widely, with 9.5% global prevalence [2] and 1.5% to 7.2 % prevalence in certain populations in India. [3, 4] It is more common in boys than girls. [5] The brief attention span (Inattentiveness) is characterized by key indicators such as frequent distractions, inadvertent errors, frequent shifts in activities, and a general difficulty in comprehending and adhering to instructions. Hyperactivity and impulsiveness are prominently observed in children, manifested as an inability to maintain composure and stillness, accompanied by constant restlessness and excessive physical motion. These behaviors are often linked with challenges in academic performance, negative perceptions from peers regarding their learning pace, and instances of class expulsion or suspension, commonly associated with children diagnosed with ADHD. [6] Furthermore, individuals with ADHD also tend to exhibit reduced skill acquisition, leading to limited earning potential and employment prospects in adulthood. This results in poorer overall physical and mental well-being outcomes when compared to their non-ADHD counterparts. [1]

How to cite this article: Nayak S, Sahu S, John J. Probabilistic Statistics to Aid Novel Heavy Metal Analysis in Attention Deficit Hyperactivity Disorder (ADHD) – A Case-Control Study with Bayesian Statistics. Int J Med Sci and Nurs Res 2023;3(3):5-12.

While trace amounts of heavy metals are necessary for various physiological functions, excessive metal accumulation can have neurotoxic effects, detrimentally impacting cognitive function and disrupting neurodevelopmental processes. Lead poisoning poses a significant public health concern, particularly childhood poisoning, which is estimated to contribute to over half a million cases of intellectual impairment annually, thereby affecting neurodevelopmental physiological processes. [7]

Research indicates that children diagnosed with ADHD often exhibit diminished zinc intake and status, which could potentially contribute to the development of ADHD. This mechanism might involve the impairment of dopamine transport within the brain of affected children. [8] Several enzymes within the central nervous system, such as tyrosinase, peptidyl glycine-amidating monooxygenase, copper/zinc superoxide dismutase, ceruloplasmin, hephaestin, dopamine-hydroxylase, and cytochrome c oxidase, rely on adequate copper levels for proper functioning. Disruptions to this physiological equilibrium may lead to the onset of various neurological disorders. [9]

Currently, there is a lack of comprehensive data regarding the impact of cadmium and nickel on the prevalence of ADHD in children. As a result, this study aims to assess the potential effects of cadmium and nickel on ADHD status, contributing to a deeper understanding of their role in this context. The relationship between hair trace elements and their storage in the body is a significant factor to consider. Hair analysis has proven valuable in assessing exposure to various environmentally hazardous substances, capitalizing on this fundamental concept. It is important to acknowledge certain limitations while interpreting these findings. Notably, concentrations of specific trace elements like lead, cadmium, and zinc in the hair are believed to indicate recent exposure to these metals, underlining the potential insights gained through this method. [10]

Urine analysis offers a distinct, non-invasive approach for evaluating the presence of heavy metals in circulation, demonstrating a strong correlation with corresponding serum levels. It's crucial to bear in mind certain considerations while interpreting these results. Reflecting these points, concentrations of the studied trace elements in hair are indicative of chronic exposure, while urine concentrations offer insights into acute exposure, both with a significant degree of reliability.

The Bayesian paradigm of statistics provides a very nuanced approach to data analysis. [11] Specifically, Bayesian techniques enable researchers to incorporate background knowledge, called priors, into their analysis rather than repeatedly testing the null hypothesis and ignoring previous studies' insights. By contrast, frequentist (classical) statistical approaches frequently entail testing the null hypothesis. As a result, when comparing Bayesian and Frequentist approaches, the Bayesian approach is a robust tool for confirmatory strategy due to the plausibility of past research findings being reviewed in connection to new data. [12]

Materials & Methods

The present research was conducted at All India Institute of Medical Sciences in Bhubaneswar, Odisha, India, spanning from August 2019 to February 2021. The study strictly adhered to ethical standards, receiving proper clearance from the Institutional Ethical Committee vide Letter No. IEC/AIIMS BBSR/PG Thesis/2019-20/01. In accordance with the Indian Council of Medical Research's National Ethical Guidelines for Biomedical Research involving Children, informed consent and assent were diligently obtained from all participating subjects.

The study focused on children aged between 3 and 16 years diagnosed with ADHD by a medical psychiatrist utilizing the DSM-V Criteria. Controls were selected to match in terms of age and sex. Rigorous exclusion criteria were applied, disqualifying children with intellectual disabilities, any neurological or behavioral disorders, or those dealing with chronic illnesses.

The sample size was meticulously calculated to encompass 48 participants, with an allowable error of 5%, statistical power of 80%, and 95% confidence level.

Sample Collection and Processing: Hair samples were collected from the nape of the neck using a procedure adapted from the International Atomic Energy Agency's assessment of at-risk workers. Additionally, first-morning urine samples were obtained using a midstream clean-catch technique. [13] All collected samples were placed in containers that had been pre-treated with nitric acid and were subsequently stored at a temperature of -80°C for extraction purposes.

The extraction process for hair samples involved digesting them in a solution containing 6 mL of 65% nitric acid and 2 mL of 30% hydrogen peroxide. This mixture was heated to 180°C for a duration of 15 minutes, followed by a cooling period at room temperature for another 15 minutes. [14] In the case of urine samples, the extraction of heavy metals was carried out using a solution containing 0.1% (V/V) Triton-X-100 (Sigma, USA) and 1% ultrapure concentrated nitric acid. [15] The quantification of heavy metal levels was performed using a Perkin Elmer Avio™ 200 dual-view instrument equipped with Syngistix™ software, employing inductively coupled plasma optical emission spectrometry (ICP-OES). Both axial and radial views were utilized depending on the concentration of the specific heavy metal being analyzed. The analysis included the use of calibration standards and blanks for each heavy metal within the samples. To determine urine creatinine levels, Jaffe's Method was employed in a Beckman Coulter AU480 auto analyzer. Heavy metal concentrations in hair were expressed as micrograms per gram of hair (µg g-1 of hair), while heavy metal concentrations in urine were expressed as micrograms per gram of spot urine creatinine (µg g-1 of spot urine creatinine).

Alongside heavy metal analysis, basic demographic information was collected, including age, sex, weight, and height. The socioeconomic status of the participants' families was estimated using the Kuppuswamy Scale. All data were securely coded and stored in electronic format, ensuring complete anonymization and confidentiality. The data was exclusively accessed and utilized by the authors for the purpose of data analysis, without any personal identifiers.

Statistical Analysis: Data was subjected to the Shapiro-Wilk test and

and found to not follow a normal distribution. As a result, demographic data was presented using the Median (Interquartile Range) format, and counts were presented as percentages. To determine differences between groups, a Bayesian Independent Samples Mann Whitney U test was conducted. Given the absence of a proper prior distribution for all heavy metals in both hair and urine, a default width of 0.707 on Cauchy's Scale was used. This data interpretation was guided by Jeffery's Scheme of Bayes Factor interpretation` [16] with details provided in **Table-1**. Additionally, a conventional Mann Whitney U test was also performed and reported for comparison. All data analyses were carried out using JASP v0.15.

The Bayes Factor (BFij) values provide a scale of evidence for different hypotheses (Hi and Hj) based on their ln(BFij) values. Here's the interpretation of the Bayes Factor values and their corresponding ln(BFij) ranges:

This interpretation helps determine the strength of evidence in favour of one hypothesis over another based on the Bayes Factor values.

Table – 1 Distribution of Bayes Factor interpretation

BF_{ij}	$\ln(BF_{ij})$	Interpretation
>100	>4.61	Decisive evidence for H_i
30–100	3.4 to 4.61	Very strong evidence for H_i
10–30	2.30 to 3.40	Strong evidence for H_i
3–10	1.10 to 2.30	Substantial evidence for H_i
1–3	0 to 1.10	Not worth more than a bare mention
1/3–1	-1.1 to 0	Not worth more than a bare mention
1/10–1/3	-2.30 to -1.10	Substantial evidence for H_j
1/30–1/10	-3.40 to -2.30	Strong evidence for H_j
1/100–1/30	-4.61 to -3.40	Very strong evidence for H_j
<1/100	<-4.61	Decisive evidence for H_j

Results

The median age of the study participants was 8 years old. A majority of the subjects were male, accounting for 72.92% of the total. The participants had a median BMI (Body Mass Index) of 20.46. A significant proportion of the children came from families classified as Upper-Class (Kuppuswamy Scale), constituting 50% of the total participants in **Table-2**.

Table 2: Demographic details of the study population

Parameters		Median (IQR)
Sex	Male, n(%)	35 (72.92%)
	Female, n(%)	13 (27.08%)
Age (in years)		8 (6.5 - 10)
Height (in cm)		120 (110 - 135.5)
Weight (in kg)		32.5 (24.5 - 38)
BMI		20.46 (19.13 - 22.88)
SES	Upper Class, n(%)	24 (50.00%)
	Upper Middle Class, n(%)	12 (25.00%)
	Upper Lower Class, n(%)	6 (12.50%)
	Lower Middle Class, n(%)	6 (12.50%)

There was no statistically significant difference in age and anthropometric parameters of the cases and controls. In the controls, however, most subjects were from Upper Middle-Class families compared to the cases where most were of the Upper Class. The comparative data of both groups have been summarized in **Table-3**.

Table 3: Difference in demographic, anthropometric and socioeconomic factors of cases and controls

Parameters		Cases (n = 24)	Controls (n = 24)	p-Value
Sex	Male, n(%)	17 (70.83%)	17 (70.83%)	0.745 [a]
	Female, n(%)	7 (29.17%)	7 (29.17%)	
Age		8 (6.5 - 10)	8 (6.5 - 10)	0.739 [b]
SES	Upper Class, n(%)	18 (75.00%)	6 (25.00%)	-
	Upper Middle Class, n(%)	4 (16.66%)	8 (33.34%)	
	Upper Lower Class, n(%)	1 (4.17%)	5 (20.83%)	
	Lower Middle Class, n(%)	1 (4.17%)	5 (20.83%)	

a: Chi-Square Test
b: Mann Whitney U Test

Table – 4 Difference in heavy metals in hair and urine of cases and controls

Samples		Cases	Controls	p - Value [a]
Hair ($\mu g\ g^{-1}$ of hair)	Lead	3.12 (1.43 - 10.86)	1.13 (0.61 - 2.08)	**0.004**
	Cadmium	0.73 (0.35 - 1.08)	0.27 (0.16 - 0.6)	**0.020**
	Zinc	198.49 (124.95 - 248.12)	527.05 (404.19 - 1193.94)	**<0.001**
	Nickle	6.08 (3.74 - 12.26)	3.54 (1.87 - 6.02)	**0.016**
	Copper	14.01 (7.6 - 20.45)	7.43 (4.31 - 14.72)	**0.013**
Urine ($\mu g\ g^{-1}$ of spot urine creatinine)	Lead	5.49 (0.77 - 15.33)	1.03 (0.39 - 2.91)	**0.004**
	Cadmium	1.76 (0.57 - 2.11)	0.31 (0.13 - 0.61)	**0.001**
	Zinc	525.7 (199.52 - 962.2)	1374.09 (881.63 - 1808.84)	**<0.001**
	Nickle	3.86 (2.84 - 7.9)	1.67 (0.8 - 2.94)	**0.001**
	Copper	17.01 (8.53 - 40.79)	7.26 (4.27 - 12.56)	**0.007**

The median concentrations of lead, cadmium, nickel, and copper in hair were notably higher among the cases compared to the control group. Similarly, the median urinary levels of these metals were also found to be higher among the cases. Conversely, the zinc level was significantly lower in both hair and urine samples from the cases. Upon applying a conventional statistical analysis (Mann-Whitney U test), significant differences in the metal levels were observed between the two groups. A summary of the median levels along with the corresponding asymptotic significance values obtained from the test have been presented in **Table-4**.

The Bayesian Mann Whitney Test was run using a data augmentation algorithm in-built in JASP with 5 chains of 1000 iterations each. First, the two-tailed difference was checked, assuming the alternative hypothesis that the heavy metals' values in cases are not equal to controls. After that, to refine the results, a single-tailed difference was run as per the difference in the median values of heavy metals in groups. Thus, for lead, cadmium, nickel and copper, the alternative hypothesis stated that the median values of cases were greater than controls; however, it was the opposite for zinc. In both the scenarios, the null hypothesis was assumed to be cases and controls having no difference in their heavy metal levels in hair and urine.

The BF_{10} and log (BF_{10}) for all the heavy metals in both hair and urine suggested a significant difference between these two groups. However, it could be well quantified using the BF_{10} that how much did these values differ on a probabilistic scale. The error percentage on Bayes Factor for all the parameters was less than 10%. The Bayesian analysis has been summarized in **Tables-5 and 6**.

Discussion

Our study aimed to find the difference between heavy metal levels in ADHD cases and healthy controls. As evident from the results, there exists a significant difference in those levels. The Bayes Factor equipped with Jeffrey's interpretation scheme provides new insight into how the data might be interpreted. The p-value driven data can qualitatively state whether the alternate hypothesis proposed can or cannot be accepted. However, Bayesian statistics gives us more profound evidence as to how much these data differ. In this study, it is seen that the difference ranges from moderate to decisive for all the heavy metals in the hair and urine. It was also seen that when the assumption was changed from generalized to more specific difference, the level of evidence in most cases improved.

A similar study was conducted by Li et al. in Guangzhou province of China amongst schoolchildren where urinary lead and cadmium levels were seen in ADHD children. According to that study, there exists an increased urinary level of these metals in the ADHD population. [17] A higher level of hair lead in children was reported in a study of school children from Massachusetts, in which it was also discussed that hair lead levels strike a more potent warning sign than tooth-enamel or serum lead levels. [18] Similar to these findings, our study also demonstrated an evident relation between increased hair and urine lead and cadmium levels in ADHD children. For the one-tailed hypothesis, the lead levels were 13.5 and 24.9 times more likely to be higher in hair and urine samples, respectively, of children with ADHD. The same was 2.3 and 40.5 times for cadmium levels in the hair and urine of children.

Table 5: Bayes Factor calculation for heavy metals in hair and urine assuming alternate hypothesis, Cases ≠ Controls

Sample	Heavy Metals	BF10	log BF10	Median	95% CI	Evidence
Hair	Lead	7.519	2.017	0.705	0.157, 1.288	Moderate
	Cadmium	1.185	0.17	0.45	-0.097, 1.025	Moderate
	Zinc	749.391	6.619	-1.262	-1.907, -0.631	Decisive
	Nickel	3.042	1.113	0.603	0.044, 1.161	Moderate
	Copper	3.524	1.26	0.615	0.075, 1.205	Moderate
Urine	Lead	10.056	2.308	0.767	0.194, 1.355	Strong
	Cadmium	29.351	3.379	0.85	0.297, 1.473	Very Strong
	Zinc	30.433	3.416	-0.907	-1.521, -0.327	Very Strong
	Nickel	21.493	3.068	0.862	0.278, 1.472	Strong
	Copper	4.364	1.473	0.668	0.095, 1.271	Moderate

Table 6: Bayes Factor calculation for heavy metals in hair and urine assuming the one-tailed alternate hypothesis

Sample	Heavy Metals	BF10	log BF10	Median Posterior Distribution	95% CI for Posterior Distribution	Evidence
Hair	Lead [a]	13.529	2.605	0.719	0.180, 1.327	Strong
	Cadmium [a]	2.372	0.864	0.485	0.055, 1.045	Anecdotal
	Zinc [b]	486	6.187	-1.248	-1.881, -0.626	Decisive
	Nickel [a]	6.294	1.84	0.606	0.112, 1.185	Moderate
	Copper [a]	6.968	1.941	0.617	0.122, 1.207	Moderate
Urine	Lead [a]	24.875	3.214	0.763	0.210, 1.362	Strong
	Cadmium [a]	40.518	3.702	0.866	0.285, 1.485	Very Strong
	Zinc [b]	49.534	3.903	-0.877	-1.486, -0.300	Very Strong
	Nickel [a]	44.538	3.796	0.856	0.277, 1.447	Very Strong
	Copper [a]	10.818	2.381	0.666	0.150, 1.285	Strong

a: Cases > Controls
b: Cases < Controls

Copper accumulation has been linked to various neurological issues, including localized brain swelling, neurodegeneration, demyelination of nerve fibers, and increased proliferation of glial cells. These processes can contribute to the development of disorders characterized by necrosis, cystic changes, interstitial focus, and cavernous vacuolation. Such conditions are believed to play a role in the onset of ADHD among children. [17]

Several studies focused on children have indicated elevated serum copper levels, as evident from a high copper-to-zinc ratio and increased hair copper concentrations. [19, 20] In alignment with these findings, our study also identified substantially higher copper levels in both hair and urine samples of ADHD-afflicted children. Specifically, copper levels were 6.9 times higher in the hair of ADHD children and 10.8 times greater in their urine samples. These observations align with previous research conducted by Goodlad et al. and Huang et al., both of which reported similar elevated copper levels within the ADHD population. [21, 22]

There is a limited body of prior research addressing the relationship between nickel and ADHD. Notably, a study conducted by Li et al. involving school children found a significantly higher nickel level among children with ADHD. [17] The brain is widely recognized as a primary target for nickel-induced neurotoxicity. Among the various potential mechanisms underlying this neurotoxicity, oxidative stress is a pivotal factor. [23] Our study contributes to the understanding that ADHD might indeed play a role in the aetiology of ADHD itself. Specifically, our findings suggest that nickel levels could be 6.3 times. higher in the hair of children with ADHD and a substantial 44.5 times higher in their urine samples.

Zinc holds vital importance for brain and neural tissue development and growth, as it facilitates the conversion of dietary pyridoxine to its active form, pyridoxal phosphate. This activation process is crucial for converting tryptophan into 5-hydroxytryptamine, a neurotransmitter closely linked to ADHD. However, the existing literature on the relationship between zinc and ADHD is somewhat conflicting. Skalny et al. and Tippairote et al. investigated hair zinc levels and found elevated levels in the ADHD group, though the differences were not statistically significant. [20 – 24] In contrast, our study yielded decisive evidence of lower hair zinc levels and very strong evidence of reduced urinary zinc in the ADHD group. Our findings align with the work of Bilici et al. and Quist and Kennedy, both of whom reported significantly lower levels of hair and urine zinc in the ADHD population. [25, 26]

Hence, across the spectrum of heavy metals analyzed, there is a significant difference in their levels in ADHD children compared to the healthy controls. ADHD is a multifactorial disease with its aetiology not being clear. It has ample scope for heavy metal profiling playing an important role in screening, diagnosis, and subsequent management and monitoring of ADHD children. In this regard, Bayesian Statistics provides a novel methodology to look at the sourced data, away from the yes or no approach of the frequentist statistics towards a more probabilistic scenario. This will further help in proper quantification of the cause, thus providing a deeper and better understanding of the role of heavy metal in ADHD scenarios and improving the scope for more integrative management of ADHD and such other diseases in children. A better understanding of the disease causation and process, and timely and apt treatment and behavioural therapy will provide an improved scope of learning and skill development in these children, thus allowing for better academic, vocation and income opportunities and reducing the rate of other associated ill effects, both academic and social. These children may have a chance, highly likely probabilistically, to lead a normal life into adulthood.

Conclusion

ADHD is a complex disorder with a multifactorial nature and an unclear underlying cause. In this context, heavy metals have been proposed as potential contributors to its development. Our study aimed to explore this association, and our findings revealed elevated levels of lead, cadmium, nickel, and copper in both hair and urine samples from children with ADHD when compared to a carefully selected group of healthy controls, matched for age and sex. Notably, the ADHD group exhibited significantly lower levels of zinc in both hair and urine samples.

These results underscore the potential involvement of heavy metals in the intricate web of factors contributing to ADHD. However, it's important to note that ADHD is a complex condition influenced by a range of genetic, environmental, and physiological factors. Our study adds valuable insight into this intricate relationship and emphasizes the relevance of considering heavy metal exposure in understanding ADHD's underlying mechanisms.

Thus, it can be suggested that the determination of heavy metals in a non-invasive way by the hair and urine analysis will provide for a useful screening, diagnostic and monitoring tool for ADHD. The use in other associated syndromes like Autism Spectrum Diseases might also be studied. The use of the Bayes Factors provided a deeper insight into the level of evidence as to how much more likely it is to detect particular heavy metals in ADHD children's hair and urine, which is not possible by usual methods of p-value used in frequentist analysis. Thus, these probabilistic methods can be used in other analysis formats to understand subsequent studies better.

Limitations of the study: The want to conduct a larger study will be there to accept the probabilistic approach of our study.

Source of funding: None

Conflict of Interest: The authors declare that they have no conflict of interest in the research conducted.

Acknowledgment: We are grateful to Dr Suravi Patra, Additional Professor, Department of Psychiatry, AIIMS Bhubaneswar for all her help.

Authors' Contributions: All the authors contributed to the conceptualization, conduct of the study and in preparation of the manuscript.

References

1. Gourie-Devi M, Gururaj G, Satishchandra P, Subbakrishna DK. Prevalence of Neurological Disorders in Bangalore, India: A Community-Based Study with a Comparison between Urban and Rural Areas. Neuroepidemiology 2004;23(6):261–268. **DOI:** https://doi.org/10.1159/000080090
2. Polanczyk GV, Willcutt EG, Salum GA, Kieling C, Rohde LA. ADHD prevalence estimates across three decades: an updated systematic review and meta-regression analysis. Int J Epidemiol 2014;43(2):434–442. **Available from:** https://academic.oup.com/ije/article-lookup/doi/10.1093/ije/dyt261
3. Jaisoorya TS, Beena KV, Beena M, Ellangovan K, George S, Thennarasu K, et al. Prevalence and Correlates of Self-Reported ADHD Symptoms in Children Attending School in India. J Atten Disord 2020;24(12):1711–1715. **Available from:** http://doi.org/10.1177/1087054716666951
4. Juneja M, Sairam S, Jain R. Attention Deficit Hyperactivity Disorder in Adolescent School Children. Indian Pediatr 2014;51(2):151-152. **PMID:** 24632700
5. Rucklidge JJ. Gender Differences in Attention-Deficit/Hyperactivity Disorder. Psychiatr Clin North Am 2010;33(2):357–373. **Available from:** https://linkinghub.elsevier.com/retrieve/pii/S0193953X10000213
6. Attention deficit hyperactivity disorder (ADHD) – Symptoms. nhs.uk. 2021. **Available from:** https://www.nhs.uk/conditions/attention-deficit-hyperactivity-disorder-adhd/symptoms/
7. Sharma P, Chambial S, Shukla KK. Lead and Neurotoxicity. Indian J Clin Biochem. 2015;30(1):1–2. **DOI:** http://doi.org/10.1007/s12291-015-0480-6
8. Lepping P, Huber M. Role of Zinc in the Pathogenesis of Attention-Deficit Hyperactivity Disorder: Implications for Research and Treatment. CNS Drugs. 2010;1. **DOI:** http://doi.org/10.2165/11537610-000000000-00000
9. Zucconi GG, Cipriani S, Scattoni R, Balgkouranidou I, Hawkins DP, Ragnarsdottir KV. Copper deficiency elicits glial and neuronal response typical of neurodegenerative disorders. Neuropathol Appl Neurobiol 2007;33(2):212–225. **DOI:** http://doi.org/10.1111/j.1365-2990.2006.00793.x
10. Chłopicka J, Zachwieja Z, Zagrodzki P, Frydrych J, Słota P, Krośniak M. Lead and cadmium in the hair and blood of children from a highly industrial area in Poland. Biol Trace Elem Res 1998;62(3):229–234. **Available from:** http://doi.org/10.1007/BF02783973
11. Van de Schoot R, Kaplan D, Denissen J, Asendorpf JB, Neyer FJ, van Aken MAG. A Gentle Introduction to Bayesian Analysis: Applications to Developmental Research. Child Dev 2014;85(3):842–860. **DOI:** https://doi.org/10.1111/cdev.12169
12. Kaplan D, Depaoli S. Bayesian Statistical Methods. Oxford University Press; 2013. **Available from:** http://oxfordhandbooks.com/view/10.1093/oxfordhb/9780199934874.001.0001/oxfordhb-9780199934874-e-020
13. Van de Schoot R, Kaplan D, Denissen J, Asendorpf JB, Neyer FJ, van Aken MAG. A Gentle Introduction to Bayesian Analysis: Applications to Developmental Research. Child Dev 2014;85(3):842–860. **DOI:** https://doi.org/10.1111/cdev.12169
14. Kaplan D, Depaoli S. Bayesian Statistical Methods. Oxford University Press; 2013. **Available from:** http://oxfordhandbooks.com/view/10.1093/oxfordhb/9780199934874.001.0001/oxfordhb-9780199934874-e-020
15. Smodiš B, Davidsson L, International Atomic Energy Agency. Assessment of levels and 'health-effects' of airborne particulate matter in mining, metal refining and metal working industries using nuclear and related analytical techniques. Vienna: International Atomic Energy Agency; 2008.
16. Fiore M, Barone R, Copat C, Grasso A, Cristaldi A, Rizzo R, et al. Metal and essential element levels in hair and association with autism severity. J Trace Elem Med Biol 2020;57:126409. **Available from:** https://linkinghub.elsevier.com/retrieve/pii/S0946672X1930358X
17. Liu N, Guan Y, Li B, Yao S. Biomonitorization of concentrations of 28 elements in serum and urine among workers exposed to indium compounds. Hu Y, editor. PLOS ONE 2021;16(2):e0246943. **Available from:** https://dx.plos.org/10.1371/journal.pone.0246943
18. Robert CP, Chopin N, Rousseau J. Harold Jeffreys's Theory of Probability Revisited. Stat Sci 2009;24(2):141-172. **Available from:** http://arxiv.org/abs/0804.3173
19. Li Y, Cha C, Lv X, Liu J, He J, Pang Q, et al. Association between 10 urinary heavy metal exposure and attention deficit hyperactivity disorder for children. Environ Sci Pollut Res 2020;27(25):31233–31242. **Available from:** http://link.springer.com/10.1007/s11356-020-09421-9
20. Tuthill RW. Hair Lead Levels Related to Children's Classroom Attention-Deficit Behavior. Arch Environ Health Int J 1996;51(3):214–220. **Available from:** http://www.tandfonline.com/doi/abs/10.1080/00039896.1996.9936018
21. Robberecht H, Verlaet AAJ, Breynaert A, De Bruyne T, Hermans N. Magnesium, Iron, Zinc, Copper and Selenium Status in Attention-Deficit/Hyperactivity Disorder (ADHD). Molecules 2020;25(19):4440. **Available from:** https://www.mdpi.com/1420-3049/25/19/4440
22. Skalny AV, Mazaletskaya AL, Ajsuvakova OP, Bjørklund G, Skalnaya MG, Notova SV, et al. Hair trace element concentrations in autism spectrum disorder (ASD) and attention deficit/hyperactivity disorder (ADHD). J Trace Elem Med Biol 2020;61:126539. **Available from:** https://linkinghub.elsevier.com/retrieve/pii/S0946672X20301048
23. Goodlad JK, Marcus DK, Fulton JJ. Lead and Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms: A meta-analysis. Clin Psychol Rev 2013;33(3):417–425. **Available from:** https://linkinghub.elsevier.com/retrieve/pii/S0272735813000123
24. Huang S, Hu H, Sánchez BN, Peterson KE, Ettinger AS, Lamadrid-Figueroa H, et al. Childhood Blood Lead Levels and Symptoms of Attention Deficit Hyperactivity Disorder (ADHD): A Cross-Sectional Study of Mexican Children. Environ Health Perspect 2016;124(6):868–874. **Available from:** https://ehp.niehs.nih.gov/doi/10.1289/ehp.1510067

25. Das KK, Das SN, Dhundasi SA. Nickel, its adverse health effects & oxidative stress. Indian J Med Re 2008;128(4):412-425. **PMID:** 19106437
26. Tippairote T, Temviriyanukul P, Benjapong W, Trachootham D. Hair Zinc and Severity of Symptoms Are Increased in Children with Attention Deficit and Hyperactivity Disorder: a Hair Multi-Element Profile Study. Biol Trace Elem Res 2017;179(2):185–194. **Available from:** http://link.springer.com/10.1007/s12011-017-0978-2
27. Bilici M, Yıldırım F, Kandil S, Bekaroğlu M, Yıldırmış S, Değer O, et al. Double-blind, placebo-controlled study of zinc sulfate in the treatment of attention deficit hyperactivity disorder. Prog Neuropsychopharmacol Biol Psychiatry 2004;28(1):181–190. **Available from:** https://linkinghub.elsevier.com/retrieve/pii/S0278584603002471
28. Quist JF, Kennedy JL. Genetics of Childhood Disorders: XXIII. ADHD, Part 7: The Serotonin System. J Am Acad Child Adolesc Psychiatry 2001;40(2):253–256. **Available from:** https://linkinghub.elsevier.com/retrieve/pii/S0890856709603774