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Environmental Impact of the Heavy Metal Intoxication on Metabolic, Physiological and Nutritional Profiles in Children with Autism Spectrum Disorder in Morocco

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ABSTRACT

Autism spectrum disorders (ASD) arise from a complex interplay between genetic predisposition and various environmental factors, leading to diverse and intricate conditions. Over recent decades, there has been a noticeable increase in autism prevalence. Thus, the main objective of this exploratory investigation is to evaluate the effects of heavy metal poisoning on the metabolic and nutritional profiles of 20 children diagnosed with autism spectrum disorder (ASD) in Morocco. Based on the descriptive statistical methods and the chi-square (χ^2) test, we analyzed the results of the study, which involved around 100 biological parameters conducted in the United States (Mosaic Diagnostics) .The results revealed that variations in neurotransmitter production, dysbiosis, yeast overgrowth, elevated oxidative stress, mitochondrial dysfunction, increased oxalate levels, and deficiencies in vitamins and minerals among the children were connected to heavy metal poisoning and intoxication. These findings underscored the link between metabolic profiles and heavy metal poisoning, highlighting the need for individualized and multidisciplinary care approaches for children with ASD. Further research and controlled clinical trials are necessary to validate these interventions comprehensively.

Keywords: autism spectrum disorders, nutritional and metabolic profile, heavy metals, neurotransmitters, intestinal dysbiosis, mitochondrial activity.

INTRODUCTION

Autism spectrum disorders (ASD) are characterized by difficulties in social communication, restricted and focused interests, speech deficits, and language delays (Vogindroukas et al., 2022). The DSM-uses these first two characteristics to diagnose ASDs. People with ASDs may also present with other signs such as intellectual disability, anxiety, depression, attention deficit disorder, hyperactivity, impulsivity, seizures, gastrointestinal problems, sensory disorders, aggression, metabolic problems, sleep disorders, motor difficulties, and altered immunity, resulting in a significant diversity in the clinical characteristics of people with ASDs (Wiggins et al., 2019). The WHO describes it as a complex condition affecting various aspects of life, such as social interaction, communication, learning, and behavior (Bamicha and Drigas, 2022). Autism spectrum disorder (ASD) is recognized in approximately 1 in 54 children in the United States, according to the Centers for Disease Control and Prevention. Prevalence rates vary in Europe, from 1 in 210 to 1 in 32, and in Asia, from 1 in 322 to 1 in 20 (Xu et al., 2019).

The history and explanation of ASD are linked to the development of the understanding of metals as a risk factor (Hertz-Picciotto et al., 2018). Since the work of Leo Kanner and Hans Asperger , the debate has revolved around the contribution of genetic and environmental factors to ASD (Jackman and Zwaigenbaum, 2023). Recent advances in genetics, particularly in DNA sequencing, highlight the importance of genetic aspects (Goldfeder et al., 2016). The Simons

Foundation Autism Research Initiative database lists 1,095 genes linked to ASD, underscoring the strength of the genetic evidence up to October 2022 (Caporale et al., 2022). In contrast, few environmental factors linked to ASD have been identified over the decades, which may be the result of a lack of research tools and techniques. This creates controversy about the respective roles of hereditary and environmental factors in ASD. Despite this, people with ASD generally present the same main symptoms, suggesting a complex combination of hereditary and environmental factors affecting a potentially common brain mechanism (Tordjman et al., 2014). This pathological mechanism is linked to abnormal synaptic processes, according to current data. Over the last decade, there has been an uptick in ASD diagnoses, possibly due to increased exposure to pollution and metals (Bjørklund et al., 2018). The 'poor excretory theory' posits that autistic children might have difficulty expelling metals from their bodies. Discussions on heavy metal pollution and ASD have been ongoing since the 1970s, primarily because of the higher lead levels in autistic children (Gorini et al., 2014). Although genetic and environmental factors (Fig. 1) are involved, the exact causes of ASD remain partially understood because of the timing of exposure and limited biological sampling (Hertz-Picciotto et al., 2018).

Recent studies on ASD and metals underscore the significance of integrating exposure data with lifestyle and social factors. They utilize diverse biological samples like hair, nails, and teeth to track metal exposure history, with blood and urine serving for short-term exposure assessment (Yu et al., 2024). Initial epidemiological findings indicating the potential involvement of toxic heavy metals in ASD have validated the concept of 'heavy metal pollution' as a contributing factor (Ding et al., 2023). While the exact definition of a 'heavy metal' may vary concerning ASD and human health, it typically denotes elevated levels of harmful metals such as lead (Pb), nickel (Ni), cadmium (Cd), and mercury (Hg). Current evidence indicates a notable connection between disruptions in the intestinal microbiota and lead exposure in ASD development (Tizabi et al., 2023a). Lead, a hazardous heavy metal, correlates with various health issues, including anemia, encephalopathy, and gastrointestinal disorders, notably linked to ASD's cognitive and behavioral traits (Saghazadeh and Rezaei, 2017). Exposure to lead and mercury can disrupt the intestinal flora, which is critical for overall well-being. The intestinal microbiota crucially impacts multiple physiological and psychological functions (Tizabi et al., 2023b), while heavy

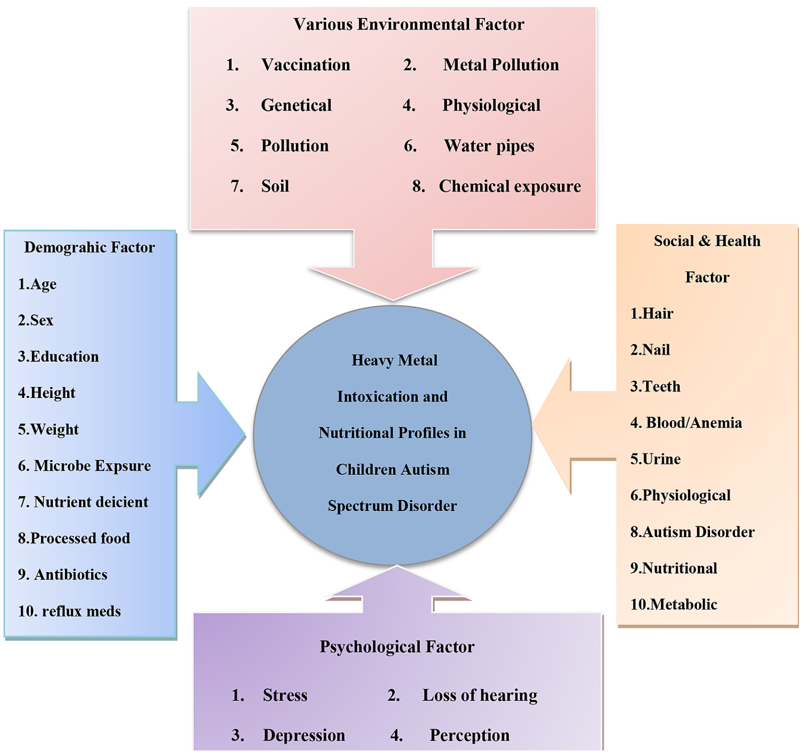

Figure 1. The heavy metal intoxication and nutritional profiles in children with autism spectrum disorder were influenced by various factors

metal exposure can induce toxic effects, even at minimal levels, affecting various biological systems, especially neurobiological and enzymatic ones. This leads to prolonged cellular oxidative stress, potentially impairing cell communication and leading to apoptosis. Conversely, trace elements are beneficial, mitigating oxidative stress and participating in enzymatic and hormonal reactions (Wróblewski et al., 2023).

Morocco, along with several other countries like the USA, various African nations, and India, contends with metal pollution resulting from its prevalent semi-arid climate in many regions, compounded by industrial contamination (Azdem et al., 2024; Azhari et al., 2022) Consequently, there's a noticeable risk to children, considering the observed rise in ASD cases in recent years (Neggers, 2014). Unraveling the mechanisms behind this problem presents a considerable challenge to researchers due to the intricacies involved in conducting a comprehensive analysis within a single study. The primary goal of this exploratory study is to investigate possible connections between heavy metal exposure in children diagnosed with ASD and their nutritional and metabolic profiles. This investigation involves conducting stool microbiology tests to evaluate microbiota composition, analyzing urine for organic acid levels, and measuring heavy metal concentrations in hair samples.

The specific aims of this exploratory investigation are to evaluate the effects of heavy metal poisoning on the metabolic and nutritional profiles of 20 Moroccan children diagnosed with autism spectrum disorder (ASD) by:

1. Analyzing the concentrations of both toxic and essential metals in the hair samples of children with autism.
2. Assessing the influence of heavy metal exposure on factors such as intestinal dysbiosis, neurotransmitter production, disruptions in mitochondrial activity, and elevated levels of oxalates.

MATERIAL AND METHOD

Data collection

This exploratory study involved children diagnosed with autism spectrum disorders from diverse urban regions in Morocco, including Tangiers, Oujda, Rabat, Salé, Casablanca, El Jadida, Guelmim, Agadir, and Marrakech. The study was conducted from September 2019 to July 2023. We used a random sampling method to collect 20 (the limited number of samples was a result of insufficient parental consent) samples from these urban areas based on parental consent, comprising 80% boys and 20% girls aged between 2 and 9 years old. Our investigation focused on evaluating the metabolic and nutritional profiles of these children, analyzing nearly 100 biological parameters

The primary objective of this exploratory investigation was to assess the effects of heavy metal poisoning on the metabolic and nutritional profiles of 20 children diagnosed with autism spectrum disorder (ASD) in Morocco. This aimed to enhance our understanding of the physiological, metabolic, and nutritional dimensions of autism. Additionally, we conducted hair tests to evaluate heavy metal intoxication and explored its impact on the nutritional and metabolic profiles. Data collection extended for nearly 4 years, starting in September 2019 and concluding in July 2023:

Upon recruitment, each participant underwent an initial consultation, which included several steps:

- Obtaining informed consent from parents, documented in both French and Arabic
- Measuring weight and height using professional tools
- Referring children to a doctor for the prescription of microbiological tests for feces, hair, and organic acids

After receiving medical prescriptions, participants were directed to a laboratory responsible for sending samples of stool, hair, blood, and urine to the United States for analysis. Upon receipt of the results via email, a comprehensive description and analysis of all parameters were conducted.

Statistical analysis

Descriptive statistics analysis is indeed a valuable tool in scientific research for several reasons. Firstly, it efficiently gathers and organizes large amounts of data, making it manageable and structured. This organized approach leads to easily understandable data outputs, such as frequency distributions, percentages, and averages (Cooksey, 2020). Additionally, descriptive statistics help in determining the standard deviation, which gives insights into the variability of the data. One of the significant advantages of descriptive statistics is its ability to provide numerical summaries that capture occurrences within sample data. This summarization process condenses extensive data into a concise format, making it easier to interpret and draw conclusions from. Descriptive statistics are particularly useful when complex research models are not required, as they focus on immediate data and single factors, allowing researchers to derive useful insights efficiently (Alabi and Bukola, 2023). Moreover, descriptive statistics can also spark innovative research ideas by identifying patterns and trends within the data. By laying out the basic numerical summaries, they serve as a foundation for further research, encouraging exploration of statistical processes and expanding knowledge across various fields. In the context of this study, descriptive statistical analyses were conducted using IBM SPSS Statistics 26 on a dataset containing from the results of the analysis of 100 parameters from 9 regions of Morocco, which was utilized for the chi-squared test. The chi-squared test is a statistical method used to determine the presence of a significant association between categorical variables (Hazra and Gogtay, 2016). It is commonly applied to analyze data presented in contingency tables, showing the frequency distributions of multiple categorical variables. Significance in the chi-squared test is attributed when the p-value is below 0.05, based on two hypotheses: H0 (the null hypothesis) and H1 (the alternative hypothesis). The null hypothesis (H0) assumes no association between the studied variables, indicating their independence, while the alternative hypothesis (H1) suggests a significant association, implying their interdependence (Stunt et al., 2021).

RESULTS

This section explains a comprehensive exploration of potential links between heavy metal exposure in children diagnosed with ASD and their nutritional and metabolic profiles. It begins by elucidating the characteristics of the sample population, incorporating the frequencies of the studied variables through descriptive analysis. Following this, the section delves into examining the impacts of heavy metal exposure on critical factors like intestinal dysbiosis, neurotransmitter production, disruptions in mitochondrial activity, and the presence of excess oxalates, utilizing the chi-squared test.

Descriptive statistics

The study examined 20 pediatric cases diagnosed with autism spectrum disorder (ASD) in various Moroccan cities. Notably, there was a significant male predominance, comprising 85% of the cases, consistent with global trends showing a higher ASD prevalence among males. Age-wise, the cases ranged from 3 to 9 years, with a concentration between 4 and 7 years, typical for ASD diagnoses. Geographically, the cases were spread across diverse cities like Agadir, Rabat, Tanger, Casablanca, Oujda, Mohamedia, Nador, Marrakech, and El Jadida, indicating ASD's prevalence throughout urban Morocco.

Regarding severity, the cases varied, with 45% severe, 35% moderate, and the rest mild. This spectrum underscores ASD's heterogeneous nature and the diverse challenges individuals face. As for associated factors: Intestinal dysbiosis (Fig. 2a) showed signs in all cases (100%), suggesting gut microbiota dysregulation's potential role in ASD pathogenesis. While, heavy metal intoxication presented a significant proportion (95%) of exposure to heavy metals like lead, mercury, and arsenic, known environmental ASD contributors (Fig. 2b). In addition, elevated oxalate levels were observed in 80% of cases (Fig. 2c), linking dietary or endogenous oxalates to oxidative stress and inflammation. 100% of cases exhibited mitochondrial activity disruptions (Fig. 2d), implying mitochondrial dysfunction's involvement in ASD and its impact on energy production and cellular processes 65% (Fig. 2e).

These findings highlight the multifaceted nature of ASD, including gender disparities, age-related patterns, geographic spread, severity variability, and significant associations with intestinal dysbiosis, heavy metal intoxication, oxalate excess, and mitochondrial dysfunction. Addressing these factors is crucial for comprehensive ASD management and tailored interventions for improved outcomes in affected individuals.

The association between the categorical variables

In our study, the chi-squared (χ^2) test was employed due to the categorical nature (yes/no) of the variables. This test aimed to investigate the presence of independence or significant association between two categorical variables, specifically heavy metal toxicity and other variables, within the sample cases.

According to the results in Table 1 (Fig. 3a), the test yielded a value of 25.098 with 9 degrees of freedom, indicating a significant difference

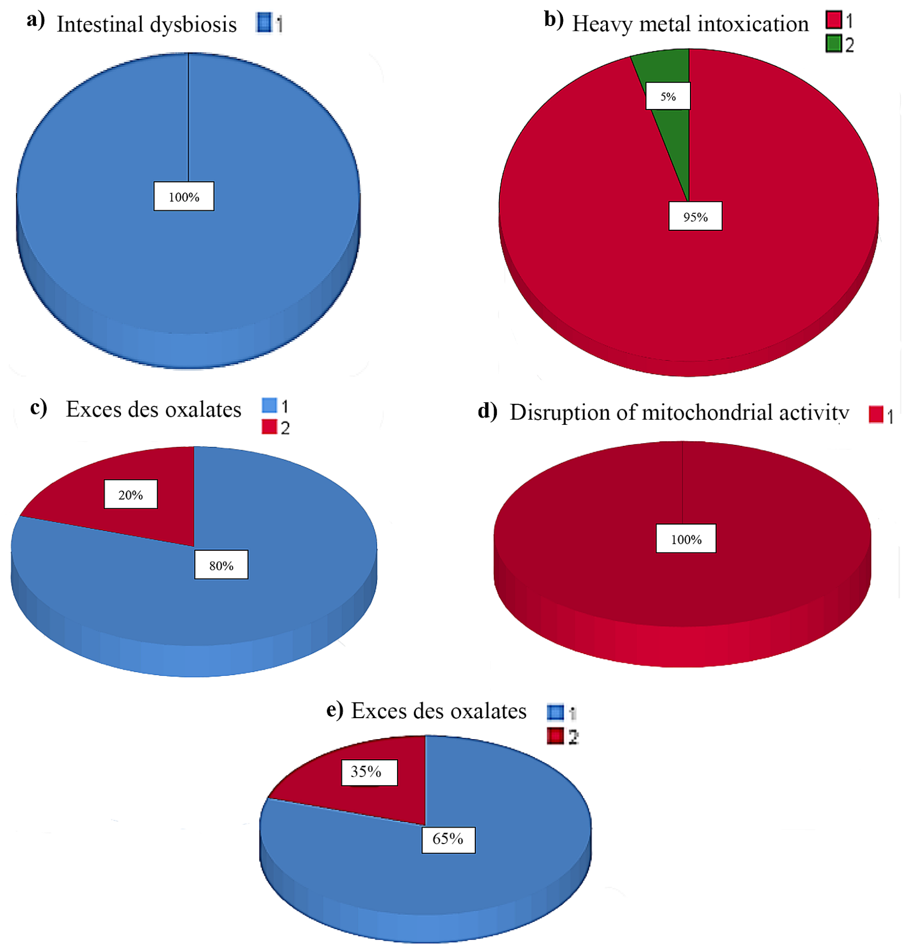

Figure 2. Descriptive statistics: frequency pie chart: a) intestinal dysbiosis, b) heavy metal poisoning, c) oxalate excess, d) disruption of mitochondrial activity, e) production

Table 1. Khi-2 test: assessment of heavy metal intoxication and intestinal dysbiosis effects on stool microbiota.

Specification	Value	ddl	Asymptotic significance (two-sided)
Pearson chi-square	25,398a	9	***p = 0,003
Likelihood ratio	12,635	9	0,180
Linear by linear association	13,688	1	0,000
N of valid observations	20		

Note: *** Significant association between two categorical variables is less than at 1%: a) 15 cells (93.8%) have a theoretical number less than 5. The minimum theoretical number is 0.05.

between the observed frequencies and those expected under the null hypothesis of independence between the variables. The very low p-value of 0.003 indicates that this difference is extremely unlikely to occur by mere chance, leading to the rejection of the null hypothesis and the conclusion that there is a significant association between intestinal dysbiosis and heavy metal intoxication. Additionally, we used the likelihood ratio to assess whether the adjusted model is significantly better than the null model. The obtained likelihood ratio was 12.635 with 9 degrees of freedom. A significant value below the threshold of 0.05, such as the p-value of 0.180, indicates that the adjusted model is significantly better than the null model, thus confirming the significant association between intestinal dysbiosis and heavy metal intoxication. The Cramer's V measure, presented in Table 3, is a normalized version of the Phi measure. A score of 1.000 indicates a perfect correlation between the variables, meaning they are closely associated. The very low p-value of 0.000 confirms that this correlation is extremely unlikely to occur by mere chance. This measure is also used to assess the correlation between nominal variables. A Cramer's V score of 1.000 indicates a strong correlation between the variables, comparable to the Phi measure. The very low p-value of 0.000 underscores the high statistical significance of this correlation (Table 1).

The cross-tabulation (Table 2) reveals a statistically significant correlation between heavy metal intoxication and neurotransmitter production (Fig. 3b), as indicated by the notable outcomes derived from Pearson's chi-square test (8.235, p = 0.041) and the likelihood ratio (7.911, p = 0.048). Cramer's V measure further reinforces this observation, demonstrating a robust correlation with a score of 0.717 and a p-value of 0.000. These statistical analyses collectively underscore a substantial interdependence between heavy metal intoxication and production. In conclusion, our comprehensive statistical analysis reveals a robust and statistically significant relationship between heavy metal intoxication and production. These findings contribute valuable insights into the intricate interplay between environmental exposures and biological processes, highlighting potential avenues for further research and intervention in addressing the impact of heavy metal toxicity on production-related outcomes. While the contingency (Table 3) demonstrates a significant association between heavy metal intoxication and disruption of mitochondrial activity (Fig. 3c). Both Pearson's chi-square test (8.235, p = 0.041) and the likelihood ratio (7.911, p = 0.048) have p-values below the threshold of 0.05, indicating a significant association between heavy metal intoxication and disruption of mitochondrial activity. These results suggest that the probability of obtaining these results by pure chance is very low. Additionally, Cramer's V measure confirms a high correlation between these variables

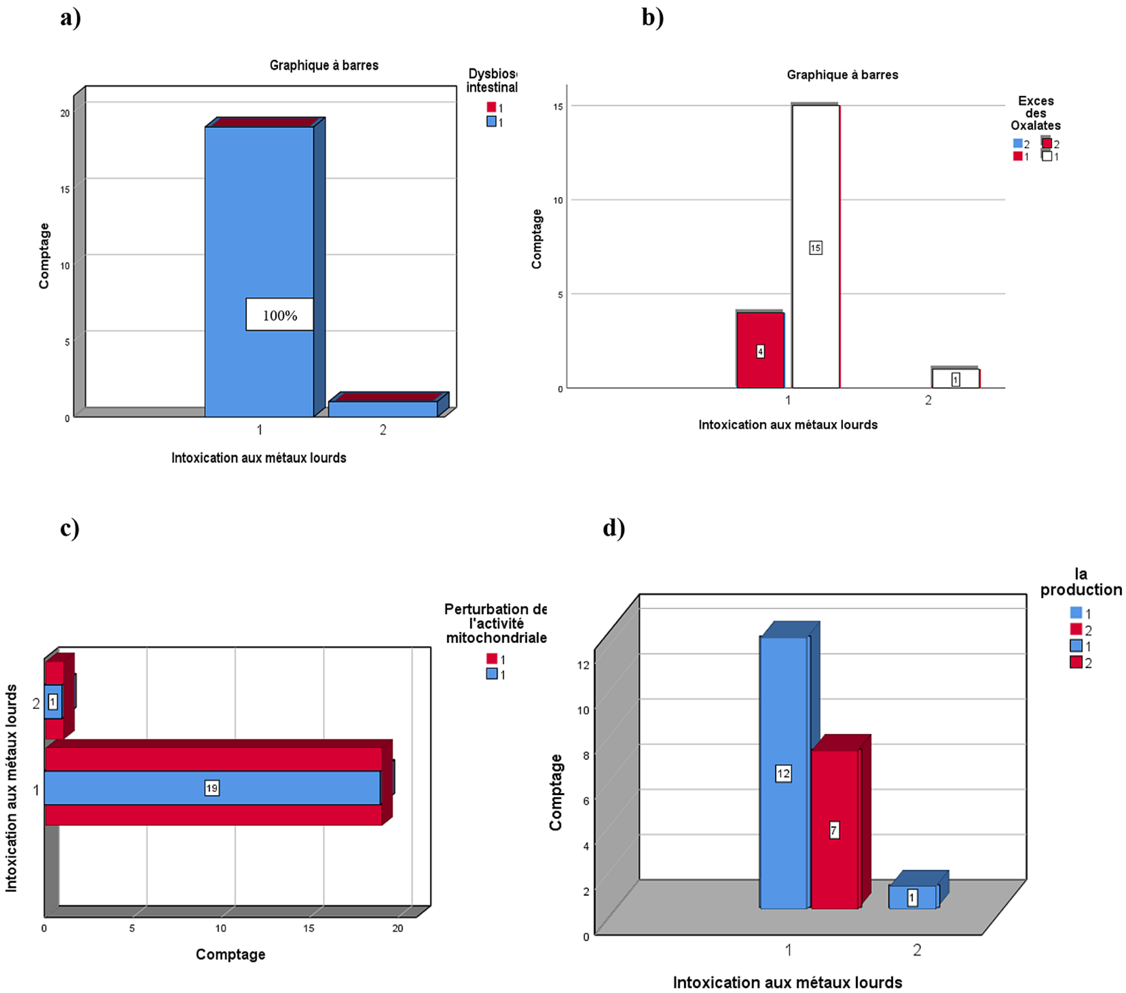

Figure 3. Relationship between variables in the following cases: a) heavy metal intoxication and intestinal dysbiosis on stool microbiological, b) heavy metal intoxication and neurotransmitter production, c) heavy metal intoxication and mitochondrial activity, d) heavy metal intoxication and excess oxalates.

Table 2. Khi-2 test: assessment of heavy metal intoxication and neurotransmitter production.

Specification	Value	ddl	Asymptotic significance (two-sided)
Pearson chi-square	8,235a	3	***p = 0,041
Likelihood ratio	7,911	3	0,048
Linear by linear association	4,592	1	0,032
N of valid observations	20		

Note: *** Significant association between two categorical variables is less than at 1%: a) 7 cells (87.5%) have a theoretical number less than 5. The minimum theoretical number is 15.

Table 3. Khi-2 test: assessment of heavy metal intoxication and mitochondrial activity

Specification	Value	ddl	Asymptotic significance (two-sided)
Pearson chi-square	5,690a	1	***p = 0,017
Continuity correctionb	3,712	1	0,054
Likelihood ratio	6,221	1	0,013
Fisher's exact test			
Linear by linear association	5,406	1	0,020
N of valid observations	20		

Note: *** Significant association between two categorical variables is less than at 1%: a) 2 cells (50.0%) have a theoretical number less than 5. The minimum theoretical number is 3.60. b) calculated only for a 2×2 table.

with a score of 0.717 and a p-value of 0.000. A value of 1 for Cramer's V would indicate a perfect correlation, so a score of 0.717 indicates a strong correlation. In summary, these statistical results reveal a notable dependence between heavy metal intoxication and disruption of mitochondrial activity. The high correlation and significant p-values indicate that this association is likely not due to chance and may have significant implications for understanding the effects of heavy metals on mitochondrial activity. The significant results (Table 4) obtained from the association of Pearson's chi-square test (34.132, p = 0.000) and the likelihood ratio (8.50, p = 0.000) robustly and statistically significantly demonstrate a strong association between heavy metal intoxication and oxalate excess (Fig. 3d). This implies that variations in intestinal heavy metal intoxication have a significant impact on oxalate excess levels, and vice versa. Furthermore, Cramer's V measure revealed a high score of 1.000 with a p-value of 0.000, thus confirming a very close correlation between intestinal heavy metal intoxication and oxalate excess. This high correlation indicates that these variables are closely linked, and there is a notable dependence between them.

DISCUSSION

Our investigation centered on a cohort of 20 children diagnosed with autism spectrum disorder (ASD). Among these participants, 80% were male and 20% were female, aged between 2 and a half to 9 years. This timeframe aligns with the onset phase of ASD symptoms and subsequent diagnosis, typically occurring between ages 2 and 4. The male predominance among recruited children affirms ASD's higher incidence in males than females (Napolitano et al., 2022). We performed an exhaustive analysis of the metabolic and nutritional profiles of these children, examining nearly 100 biological parameters encompassing various categories such as urinary organic acids (76 parameters), dysbiosis (19 parameters), oxalates (3 parameters), mitochondria (11 parameters), neurotransmitter production (8 parameters), oxidative stress markers (7 parameters), heavy metals and essential elements (39 parameters), and stool microbiology tests (7 parameters). This comprehensive approach aimed to enhance our comprehension of autism from physiological, metabolic, and nutritional standpoints.

Several studies employing various sample types have indicated that children diagnosed with ASD possess elevated concentrations of toxic

Table 4. Khi-2 test: assessment of heavy metal intoxication and excess oxalates

Specification	Value	ddl	Asymptotic significance (two-sided)
Pearson chi-square	34,132a	12	***p = 0,001
Likelihood ratio	22,099	12	0,036
Linear by linear association	14,984	1	0,000

Note: *** Significant association between two categorical variables is less than at 1%: a) 15 cells (93.8%) have a theoretical number less than 5. The minimum theoretical number is 0.05.

metals in their bodies compared to neurotypical counterparts (Fiłon et al., 2020; Kim et al., 2016; Saghazadeh and Rezaei, 2017; Sulaiman et al., 2020). Furthermore, certain investigations have reported a correlation between the severity of ASD symptoms and the levels of heavy metals accumulated.

The results of the hair analysis revealed heavy metal intoxication in 95% of the sample. Toxic metals and metalloids such as arsenic (As), lead (Pb), chromium (Cr), and cadmium (Cd), generally considered as potential environmental risk factors in ASD, were quantified in the hair of children with ASD. This approach uses hair as a biological matrix representative of the body's metal impregnation, offering the advantage of being non-invasive, painless, and particularly practical for a non-cooperative population such as children with ASD (Chojnacka and Mikulewicz, 2023)

Previous studies examining the link between toxic metals found in hair and children with ASD yielded similar outcomes. For instance, Elsheshtawy et al. (2011) discovered substantial differences in the levels of these substances in the hair of children with autism compared to controls. They also noted a positive correlation between CARS score (childhood autism rating scale) and both mercury and copper, while intelligence quotient showed a significant negative correlation with lead levels in the hair. Zinc levels did not correlate with either CARS score or intelligence quotient among 32 children with autism and 32 controls (aged 4.1 ± 0.8 years). Conversely, Geier et al. (2012) found that higher concentrations of hair mercury were significantly associated with increased ASD severity, although no significant correlations were observed for other hair toxic metals in 18 cases. This is consistent with the findings of (Fido and Al-Saad, 2005), who studied 40 boys with ASD and 40 controls (aged 4.2 ± 2.2 years) and discovered significantly higher levels of lead, mercury, and uranium in the hair of children with autism compared to controls. However, no notable differences were found between the two groups concerning the other five toxic elements, and the ratio between nutritional elements and toxic metals among children with autism fell within the normal range. Moreover, Austin et al. (2022) conducted a prospective study in Japan using a predictive algorithm that detected ASD risk as early as 1 month with high sensitivity (96.4%), specificity (75.4%), and accuracy (81.4%) in a cohort of 486 individuals, including 175 cases. These findings underscore the systemic dysregulation of elemental metabolism in autism and highlight the potential for utilizing hair samples to predict the onset of ASD as early as 1 month of age. while, according to a metallomics investigation by (Yasuda et al., 2013), 8.5% of ASD children aged 0 to 15 exhibited elevated cadmium loads in hair samples.

The findings from our descriptive cross-sectional study reveal notable disruptions in the metabolic and nutritional profiles of the 20 children included in our sample. These disruptions encompassed heavy metal intoxication, observed in 19 of the children, generalized intestinal dysbiosis, micronutrient deficiency present in all children, elevated oxidative stress in 60% of the sample, impaired fat energy production and assimilation, as well as disturbed mitochondrial activity observed across the entire sample. Additionally, 80% of the children exhibited a significant excess of oxalates, and 70% showed reduced production of neurotransmitters like dopamine and noradrenaline due to nutritional deficiencies of phenylalanine and tyrosine.

Numerous studies confirm the striking similarities between the cellular pathologies associated with ASD and those induced by excessive exposure to mercury, lead, and cadmium. These similarities include the competition with essential metals, particularly zinc, oxidative stress, lipid peroxidation, mitochondrial dysfunction, neuroinflammation, gliosis, and axonal demyelination typically observed following exposure to high levels of toxic metals. These affected processes are highly active during brain development, and toxic metals may impede normal brain development through various mechanisms like DNA methylation, histone modifications, microRNA expression, alterations in protein properties, or disruption of gut-brain signaling by affecting microbiota composition (Błażewicz and Grabrucker, 2022). The effects related to ASD may manifest primarily during the prenatal period. Moreover, mercury, lead, and various POPs (Persistent organic pollutants) could lead to an imbalance between free radicals and antioxidants, resulting in neurodevelopmental aberrations. Oxidative stress is also suggested as a mechanism for mitochondrial dysfunction, with certain chemicals potentially causing mitochondrial toxicity and subsequently disrupting ATP levels in neural cells. This connection has been supported by (Ijomone et al., 2020).

The findings also indicate disruptions in the metabolic pathway of tryptophan, the precursor to serotonin, a hormone vital for well-being and psychological health, impacting 65% of the studied children. Several human studies corroborate the notion that disturbances in serotonin (5-hydroxytryptamine or 5-HT) production play a role in the onset of autism in certain individuals. These findings underscore the intricate nature of heavy metal intoxication's effects on diverse metabolic and nutritional aspects, emphasizing the necessity for a multidisciplinary approach to tackle these challenges in children with ASD.

Similarly, Abdulamir et al. (2018) conducted a study involving 60 male autistic children categorized into mild, moderate, and severe groups (20 in each) to assess serotonin levels and SERT (serotonin transporters). These levels were compared with those of 26 healthy control children. The results demonstrated a significant increase in serotonin and SERT levels among autistic children compared to gender- and age-matched controls. Specifically, serotonin levels were 80.63 ± 21.83 ng/ml in mild cases, 100.39 ± 23.07 ng/ml in moderate cases, and 188.7 ± 31.72 ng/ml in severe cases of autism. Serotonin transporter levels were 10.13 ± 4.51 ng/ml in mild cases, 13.15 ± 4.71 ng/ml in moderate cases, and 16.32 ± 6.7 ng/ml in severe cases of autism. This elevation in both serotonin and SERT levels correlated with the severity of autism.

Additionally, Essa et al. (2013) reported significant metabolic and nutritional disruptions in 20 children, including heavy metal intoxication, intestinal dysbiosis, micronutrient deficiencies, and oxidative stress, often associated with mitochondrial disorders. Mutations in mitochondrial DNA (mtDNA) have been linked to various clinical features, including those observed in autism spectrum disorder. Studies suggest that disrupted bioenergetics metabolism is a factor in autism, as evidenced by elevated lactate levels and brain metabolism abnormalities in some patients. Mitochondrial respiratory chain disorders have also been associated with autism, possibly due to oxidative changes affecting mitochondrial function. These disruptions in energy metabolism may contribute to brain dysfunction in autism, given the significance of energy production for cellular processes like neurotransmitter synthesis and signaling. Mitochondrial dysfunction and impaired energy metabolism are implicated in various neuropsychiatric disorders, underscoring their role in brain function and neurodevelopment. Adiponectin and leptin levels, which regulate energy metabolism, have also been linked to the pathophysiology of autism, suggesting a complex interplay between metabolism, neurodevelopment, and ASD.

CONCLUSIONS

Autism spectrum disorders are characterized by two key features observed in individuals with ASD: social deficits and repetitive, stereotyped behaviors. Despite the involvement of numerous candidate genes and various non-genetic factors in ASD, the precise understanding of its origins remains complex. As neurological processes underlie these behaviors, it is plausible that all potential causes disrupt these underlying processes, showing convergence not only at the behavioral level but also at the molecular level. This raises the fundamental question of the neuronal mechanism behind ASD symptoms and how such a wide range of hereditary and non-genetic factors could contribute to a shared process.

Therefore, the primary aim of this exploratory study is to employ descriptive and cross-sectional methodologies to investigate the nutritional and metabolic profiles of 20 Moroccan children diagnosed with autism spectrum disorder. Utilizing descriptive statistical methods and the chi-square (χ^2) test, we analyzed the results of the study, which encompassed approximately 100 biological parameters conducted in the United States.

The results indicated that the variations observed in neurotransmitter production, dysbiosis, yeast overgrowth, heightened oxidative stress, mitochondrial dysfunction, elevated oxalate levels, and deficiencies in vitamins and minerals among the children were associated with heavy metal poisoning and intoxication. These findings can aid decision-makers in monitoring and managing the various factors contributing to ASD. The insights from this study may hold relevance for regions with similar characteristics, both within Morocco and globally. In perspective, it's crucial to note that ongoing research on metals should prioritize investigating speciation forms, metal concentration, exposure pathways, and durations. This approach will lead to a more nuanced comprehension of the diverse effects of metals on different molecular targets.

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REFERENCES

1. Abdulamir, H.A., Abdul-Rasheed, O.F., Abdulghani, E.A. 2018. Serotonin and serotonin transporter levels in autistic children. Saudi Medical Journal, 39(5), 487–494. https://doi.org/10.15537/smj.2018.5.21751
2. Alabi, O., Bukola, T. 2023. Introduction to descriptive statistics. In recent advances in biostatistics [Working Title]. IntechOpen. https://doi.org/10.5772/intechopen.1002475
3. Austin, C., Curtin, P., Arora, M., Reichenberg, A., Curtin, A., Iwai-Shimada M., Wright, R., Wright, R., Remnelius, K.L., Isaksson, J., Bolte, S., Nakayama, S. 2022. Elemental dynamics in hair accurately predict future autism spectrum disorder diagnosis: An international multi-center study. https://doi.org/10.21203/rs.3.rs-1307805/v1
4. Azdem, D., Mabrouki, J., Moufti, A., El Hajjaji, S., Fatni, A. 2024. Assessment of heavy metal contamination in seawater in Agadir coastline, Morocco. Desalination and Water Treatment, 317, 100129. https://doi.org/10.1016/j.dwt.2024.100129
5. Azhari, H.E., Cherif, E.K., Sarti, O., Azzirgue, E.M., Dakak, H., Yachou, H., Esteves Da Silva, J.C.G., Salmoun, F. 2022. Assessment of surface water quality using the water quality index (IWQ), multivariate statistical analysis (MSA) and Geographic Information System (GIS) in Oued Laou Mediterranean Watershed, Morocco. Water, 15(1), 130. https://doi.org/10.3390/w15010130
6. Bamicha, V., Drigas, A. 2022. ToM & ASD: The interconnection of theory of mind with the social-emotional, cognitive development of children with autism spectrum disorder. The use of ICTs as an alternative form of intervention in ASD. Technium Social Sciences Journal, 33, 42–72. https://doi.org/10.47577/tssj.v33i1.6845
7. Bjørklund, G., Skalny, A.V., Rahman, Md. M., Dadar, M., Yassa, H.A., Aaseth, J., Chirumbolo, S., Skalnaya, M.G., Tinkov, A.A. 2018. Toxic metal(loid)-based pollutants and their possible role in autism spectrum disorder. Environmental Research, 166, 234–250. https://doi.org/10.1016/j.envres.2018.05.020
8. Błażewicz, A., Grabrucker, A.M. 2022. Metal profiles in autism spectrum disorders: A crosstalk between toxic and essential metals. International Journal of Molecular Sciences, 24(1), 308. https://doi.org/10.3390/ijms24010308
9. Caporale, N., Leemans, M., Birgersson, L., Germain, P.L., Cheroni, C., Borbély, G., Engdahl, E., Lindh, C., Bressan, R.B., Cavallo, F., Chorev, N.E., D'Agostino, G.A., Pollard, S.M., Rigoli, M.T., Tenderini, E., Tobon, A.L., Trattaro, S., Troglio, F., Zanella, M., Testa, G. 2022. From cohorts to molecules: Adverse impacts of endocrine disrupting mixtures. Science, 375(6582), eabe8244. https://doi.org/10.1126/science.abe8244
10. Chojnacka, K., Mikulewicz, M. 2023. Chemical elements in hair and their association with autism spectrum disorder: A comprehensive systematic review. Pollutants, 3(4), 587–602. https://doi.org/10.3390/pollutants3040038
11. Cooksey, R.W. 2020. Descriptive statistics for summarising data. Illustrating statistical procedures: Finding meaning in quantitative Data (p. 61–139). Springer Singapore. https://doi.org/10.1007/978-981-15-2537-7_5
12. Ding, M., Shi, S., Qie, S., Li, J., Xi, X. 2023. Association between heavy metals exposure (cadmium, lead, arsenic, mercury) and child autistic disorder: A systematic review and meta-analysis. Frontiers in Pediatrics, 11, 1169733. https://doi.org/10.3389/fped.2023.1169733
13. Elsheshtawy, E., Tobar, S., Sherra, K., Atallah, S., Elkasaby, R. 2011. Study of some biomarkers in hair of children with autism. Middle East Current Psychiatry, 18(1), 6–10. https://doi.org/10.1097/01.XME.0000392842.64112.64

14. Essa, M.M., Subash, S., Braidy, N., Al-Adawi, S., Lim, C. K., Manivasagam, T., Guillemin, G. J. 2013. Role of NAD +, oxidative stress, and tryptophan metabolism in autism spectrum disorders. International Journal of Tryptophan Research, 6s1, IJTR. S11355. https://doi.org/10.4137/IJTR.S11355
15. Fido, A., Al-Saad, S. 2005. Toxic trace elements in the hair of children with autism. Autism, 9(3), 290–298. https://doi.org/10.1177/1362361305053255
16. Fiłon, J., Ustymowicz-Farbiszewska, J., Krajewska-Kułak, E. 2020. Analysis of lead, arsenic and calcium content in the hair of children with autism spectrum disorder. BMC Public Health, 20(1), 383. https://doi.org/10.1186/s12889-020-08496-w
17. Geier, D., Kern, J., King, P., Sykes, L., Geier, M. 2012. Hair toxic metal concentrations and autism spectrum disorder severity in young children. International Journal of Environmental Research and Public Health, 9(12), 4486–4497. https://doi.org/10.3390/ijerph9124486
18. Goldfeder, R.L., Priest, J.R., Zook, J.M., Grove, M.E., Waggott, D., Wheeler, M. T., Salit, M., Ashley, E.A. 2016. Medical implications of technical accuracy in genome sequencing. Genome Medicine, 8(1), 24. https://doi.org/10.1186/s13073-016-0269-0
19. Gorini, F., Muratori, F., Morales, M.A. 2014. The role of heavy metal pollution in neurobehavioral disorders: A focus on autism. Review Journal of Autism and Developmental Disorders, 1(4), 354–372. https://doi.org/10.1007/s40489-014-0028-3
20. Hazra, A., Gogtay, N. 2016. Biostatistics series module 4: Comparing groups - categorical variables. Indian Journal of Dermatology, 61(4), 385. https://doi.org/10.4103/0019-5154.185700
21. Hertz-Picciotto, I., Schmidt, R.J., Krakowiak, P. 2018. Understanding environmental contributions to autism: Causal concepts and the state of science. Autism Research, 11(4), 554–586. https://doi.org/10.1002/aur.1938
22. Ijomone, O.M., Olung, N.F., Akingbade, G.T., Okoh, C.O.A., Aschner, M. 2020. Environmental influence on neurodevelopmental disorders: Potential association of heavy metal exposure and autism. Journal of Trace Elements in Medicine and Biology, 62, 126638. https://doi.org/10.1016/j.jtemb.2020.126638
23. Jackman, A., Zwaigenbaum, L. 2023. The history of autism spectrum disorder. In D.D. Eisenstat, D. Goldowitz, T.F. Oberlander, J.Y. Yager (Éds.), Neurodevelopmental Pediatrics (p. 215–226). Springer International Publishing. https://doi.org/10.1007/978-3-031-20792-1_12
24. Kim, K.-N., Kwon, H.-J., Hong, Y.-C. 2016. Low-level lead exposure and autistic behaviors in school-age children. NeuroToxicology, 53, 193–200. https://doi.org/10.1016/j.neuro.2016.02.004
25. Napolitano, A., Schiavi, S., La Rosa, P., Rossi-Espagnet, M.C., Petrillo, S., Bottino, F., Tagliente, E., Longo, D., Lupi, E., Casula, L., Valeri, G., Piemonte, F., Trezza, V., Vicari, S. 2022. Sex differences in autism spectrum disorder: diagnostic, neurobiological, and behavioral features. Frontiers in Psychiatry, 13, 889636. https://doi.org/10.3389/fpsyt.2022.889636
26. Neggers, Y.H. 2014. Increasing prevalence, changes in diagnostic criteria, and nutritional risk factors for autism spectrum disorders. ISRN Nutrition, 1–14. https://doi.org/10.1155/2014/514026
27. Saghazadeh, A., Rezaei, N. 2017. Systematic review and meta-analysis links autism and toxic metals and highlights the impact of country development status : Higher blood and erythrocyte levels for mercury and lead, and higher hair antimony, cadmium, lead, and mercury. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 79, 340–368. https://doi.org/10.1016/j.pnpbp.2017.07.011
28. Stunt, J., Van Grootel, L., Bouter, L., Trafimow, D., Hoekstra, T., De Boer, M. 2021. Why we habitually engage in null-hypothesis significance testing: A qualitative study. PLOS ONE, 16(10), e0258330. https://doi.org/10.1371/journal.pone.0258330
29. Sulaiman, R., Wang, M., Ren, X. 2020. Exposure to aluminum, cadmium, and mercury and autism spectrum disorder in children: A systematic review and meta-analysis. Chemical Research in Toxicology, 33(11), 2699-2718. https://doi.org/10.1021/acs.chemrestox.0c00167
30. Tizabi, Y., Bennani, S., El Kouhen, N., Getachew, B., Aschner, M. 2023a. Interaction of heavy metal lead with gut microbiota: implications for autism spectrum disorder. Biomolecules, 13(10), 1549. https://doi.org/10.3390/biom13101549
31. Tizabi, Y., Bennani, S., El Kouhen, N., Getachew, B., Aschner, M. 2023b. Interaction of heavy metal lead with gut microbiota: implications for autism spectrum disorder. Biomolecules, 13(10), 1549. https://doi.org/10.3390/biom13101549
32. Tordjman, S., Somogyi, E., Coulon, N., Kermarrec, S., Cohen, D., Bronsard, G., Bonnot, O., Weismann-Arcache, C., Botbol, M., Lauth, B., Ginchat, V., Roubertoux, P., Barburoth, M., Kovess, V., Geoffray, M.-M., Xavier, J. 2014. Gene x environment interactions in autism spectrum disorders: role of epigenetic mechanisms. Frontiers in Psychiatry, 5. https://doi.org/10.3389/fpsyt.2014.00053
33. Vogindroukas, I., Stankova, M., Chelas, E.-N., Proedrou, A. 2022. Language and speech characteristics in autism. Neuropsychiatric Disease and Treatment, Volume 18, 2367–2377. https://doi.org/10.2147/NDT.S331987

34. Wiggins, L.D., Rice, C.E., Barger, B., Soke, G.N., Lee, L.-C., Moody, E., Edmondson-Pretzel, R., Levy, S.E. 2019. DSM-5 criteria for autism spectrum disorder maximizes diagnostic sensitivity and specificity in preschool children. Social Psychiatry and Psychiatric Epidemiology, 54(6), 693–701. https://doi.org/10.1007/s00127-019-01674-1
35. Wróblewski, M., Wróblewska, J., Nuszkiewicz, J., Pawłowska, M., Wesołowski, R., Woźniak, A. 2023. The role of selected trace elements in oxidoreductive homeostasis in patients with thyroid diseases. International Journal of Molecular Sciences, 24(5), 4840. https://doi.org/10.3390/ijms24054840
36. Xu, G., Strathearn, L., Liu, B., O'Brien, M., Kopelman, T.G., Zhu, J., Snetselaar, L.G., Bao, W. 2019. Prevalence and treatment patterns of autism spectrum disorder in the United States, 2016. JAMA Pediatrics, 173(2), 153. https://doi.org/10.1001/jamapediatrics.2018.4208
37. Yasuda, H., Yasuda, Y., Tsutsui, T. 2013. Estimation of autistic children by metallomics analysis. Scientific Reports, 3, 1199. https://doi.org/10.1038/srep01199
38. Yu, E.X., Dou, J.F., Volk, H.E., Bakulski, K.M., Benke, K., Hertz-Picciotto, I., Schmidt, R.J., Newschaffer, C.J., Feinberg, J.I., Daniels, J., Fallin, M.D., Ladd-Acosta, C., Hamra, G.B. 2024. Prenatal metal exposures and child social responsiveness scale scores in 2 prospective studies. Environmental Health Insights, 18, 11786302231225313. https://doi.org/10.1177/11786302231225313.