Research Article

Correlation Between Environmental Metal Toxicity And Lysosome Malfunction, Neuroinflammation, And Neurotransmitter Imbalance In Autism Spectrum Disorder

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Abstract

Background: The research problem can be seen in the absence of an all-inclusive approach towards investigating the link between environmental toxicity (Pb, Hg) and the dysfunctions within the body systems. The current diagnosis does not take into account lysosomal dysfunction (LAMP1, alpha-synuclein), inflammation within the nervous system (IL-17), and neurotransmitters/metabolic disorders (GABA, OPLAH, Ca). To elucidate interconnected pathophysiological processes in autism spectrum disorder (ASD), the development of multidisciplinary biomarker techniques is being looked at.
Methods: Using an event-manipulated design, serum levels of environmental and metabolic neuroinflammatory, lysosomal, and neurotransmitter markers were measured in adolescents with ASD, their neurotypical siblings, and unrelated healthy controls.
Results: The results indicate that prolonged environmental exposure to lead (Pb) acts as a major systemic stressor (P=0.002), significantly disrupting vital calcium (Ca^{2+}) homeostasis (P=0.002). This toxic steel load is coupled with a profound depletion of the metabolic enzyme OPLAH (P<0.001), signaling a reduction within the γ-glutamyl cycle and cellular glutathione (GSH) synthesis. This metabolic instability is due to autophagic-lysosomal dysfunction and structural stress. (P <0.001) and an inverse decrease in soluble α-synuclein precipitation (P < 0.001). In the long term, this clearance disorder acts as an endogenous trigger that activates microglia and astrocytes, driven by the marked elevation of the inflammatory cytokine IL-17 (P<0.001). Finally, it suppresses hyper-inflammatory detection and leads to inhibition of toxicity, GABA (P <0.001).
Discussion & Conclusion: The spiky function reduces excitatory/inhibitory (E/I) imbalance and, importantly, demonstrates the amazing diagnostic accuracy of this panel through receiver operating characteristic (ROC) evaluation, with OPLAH serving as the most sensitive discriminator (AUC = 0.932). Furthermore, the diagnostic neurotypical skill for system expertise is especially maintained with metabolic neurotransmitters. Compared with single markers. This multi-biomarker panel provides a fantastic, clinically viable tool for direction-precise medical interventions, environmental fitness monitoring, and early preventive screening.

Key words: IL-17, OPLAH, LAMP1, α synuclin and GABA.

Introduction

Autism spectrum disorder (ASD) is a complicated neurodevelopmental condition characterized by determined impairments in social communication and interaction, along with stereotyped behaviors and limited interests. Gradually, the frequency of ASD diagnosis increases in its prevalence worldwide, with current estimates of one child in every hundred being diagnosed [1]. The causes of autism spectrum disorder remain unclear despite significant progress, reflecting its complex origins, which stem from both genetic and non-genetic factors, including environmental and immunological ones [2].
LAMP1 Lysosomes are small organelles that function as the cell's cleaning mechanism, dissolving and degrading waste and damaged materials. The importance of lysosomes in maintaining cellular homeostasis, and the dysfunction of lysosomes can lead to synaptic and neuronal dysfunction, which may affect behavior and brain function [3].
Proteolytic enzymes, such as lysosomal proteins and cathepsins, play a critical role in maintaining neuronal integrity. Lysosomal impairment may contribute to the neurodegenerative dysfunctions and neurodevelopmental disorders associated with autism spectrum disorders [4]. Additionally, there is a strong association between autism spectrum disorder and neuroinflammation, as the latter contributes to the development of autism spectrum disorder. with the latter contributing to the development of ASD. This occurs through the stimulation of both microglia and astrocytes, as well as elevated levels of cytokines, which lead to increased inflammation and affect the functions of the central

nervous system [5]. Chronic neuroinflammation can negatively affect neuronal maturation, nerve transmission, and the formation of neural circuits. Elevated levels of inflammatory markers have been observed in patients with ASD, indicating an immune dysfunction that may contribute to both the onset and severity of the disorder. Moreover, damage to nerve cells may increase because of the dysfunction of lysosomes and neuroinflammation [6].

Alpha Synuclein stands out as an essential protein that connects proteotoxicity, synaptotoxicity, and inflammation in ASD. Although alpha-syn has been extensively explored in relation to neurodegenerative disorders, its relevance in the context of neurodevelopmental disorders has become increasingly prominent [7].

OPLAH is the enzyme that transforms 5-oxoproline into L-glutamate through an ATP-dependent process. Many patients with ASD have disturbances in the glutathione pathway. The lack of OPLAH causes an increase in 5-oxoproline in cells, indicating a problem in the cell's "recycling" system that prevents proper levels of glutathione from protecting against oxidative stress. Since OPLAH helps in making L-glutamate from 5-oxoproline, the functioning of this enzyme also affects the Glutamate-GABA ratio. Abnormal functioning of the enzyme OPLAH may lead to an abnormal flux of glutamate, which is a key factor in the cause of excitation-inhibition imbalance associated with sensory processing disorder and seizures seen in individuals with ASD. The reason why the activity of this enzyme needs ATP makes it secondary evidence of mitochondrial stress within the neuron. Levels of OPLAH are responsive to the inflammatory status of the body. During inflammation, there is an increased demand for glutathione. Low level of activity of the OPLAH enzyme, along with high cytokine concentrations [8].

Neurotransmitters are critical for proper brain function; they impact neuronal communication, behavior, cognition, and emotional responses. Alterations in neurotransmitter systems, particularly those involving serotonin, dopamine, gamma-aminobutyric acid (GABA), and glutamate, are regularly determined in individuals with ASD [9]. These imbalances may also contribute to the moderate behavioral and cognitive markers seen in individuals with ASD. Furthermore, disruption of neurotransmitter signaling may be associated with oxidative stress, mitochondrial dysfunction, and neuroinflammatory strategies, reflecting the complex biological mechanisms involved in ASD pathophysiology [10]. In addition to inner organic factors, environmental effects, especially the contribution of heavy metals, have attracted great interest. Environmental metals such as lead, mercury, cadmium, and aluminum have been identified as neurotoxic pollutants that can cross the blood-brain barrier and affect neuronal properties. These metals can result in oxidative stress, altered mitochondrial properties, disrupt neurotransmitter signaling, and cause neuroinflammation. In addition, heavy metals can overtake lysosomes, contributing to lysosomal dysfunction and toxicity in the cells. A developing body of evidence indicates that children with ASD may also have particular levels of certain environmental metals, which aid their ability to be involved in the pathophysiology of their issues [11]. Lysosomal dysfunction, neuroinflammation, neurotransmitter imbalance, and exposure to environmental metals may respond synergistically, promoting neuronal injury and developmental conditions [12]. Concurrently, interactions between neuroinflammatory strategies and neurotransmitter regulation are emerging as a key field of study. Neuronal conversation and synaptic efficiency can be affected through the effects of inflammatory mediators on neurotransmitter synthesis, release, and reuptake [13]. Persistent inflammatory pain can disrupt the stability between excitatory and inhibitory signals, which is critical for proper brain function. These disruptions are considered a key mechanism behind some of the behavioral cognitive development associated with autism spectrum disorders, and furthermore, inflammatory signaling may have an impact on neuroplasticity and neural connectivity, thus contributing to long-term plastic change [14]. These environmental factors can exacerbate natural imbalances. Exposure to neurotoxic metals, even at low concentrations, can affect biological pathways critical to the development of the mind. These metals can result in the generation of reactive oxygen species, impaired antioxidant defense mechanisms, and disrupted intracellular signaling pathways [15].

Subjects and Methods

A case-control study consisted of 60 children with ASD and 120 age- and sex-matched children without ASD. The 120 control subjects were separated into two groups: 60 siblings of children with ASD (Sibling group) and 60 unrelated children (Unrelated control group). All children with ASD finished every biochemical analysis test. They were between the ages of three and twelve. These children with ASD were enrolled in specialist autism treatment facilities in Iraq, namely in the province of Najaf. After obtaining written informed consent from the participants' parents or guardians, blood samples were taken from them prior to any study procedures. In compliance with the Declaration of Helsinki's ethical guidelines, the study procedure was closely followed. The University of Kufa IRB/Ethics Committee granted

ethical approval for the study (Approval No. Ref. H K/167; Date: December 15, 2025). All participating children's parents/guardians received a thorough explanation of the study's objectives and methodology before their participation. Before any study procedures were carried out, written informed consent was obtained from each guardian.[16]

Exclusion Criteria: Children with ASD who appeared to have a mental disorder were not included in this study. The study did not include children who took any form of supplement, particularly those that prevent oxidative stress.

Blood Samples: Each child in the ASD and control groups had five milliliters of venous blood extracted using plastic syringes and a disposable needle. After clotting the blood for ten minutes in a serum tube and centrifuging it for ten minutes at 3000 × g, the serum was extracted, transferred into fresh disposable tubes, and stored at -70°C until it was tested.

Methods:

ELISA Technique. Quantitative assignment of serum biomarkers, including interleukin-17 (IL-17), 5-oxoprolinase (OPLAH), lysosome-associated membrane protein 1 (LAMP1),α-synuclein, and Gamma-Aminobutyric acid (GABA), as performed using a high-sensitivity, solid-phase sandwich and invasive enzyme immunoassay kit purchased from Elabscience Biotechnology Co., Ltd. For sandwich-based whole assays (IL-17, OPLAH, LAMP1, and α-synuclin), serum samples, and specific requirements were pipetted into wells of microtiter plates precoated with biotinylated antibodies specifically for each target biomarker. After initial incubation, avidin-horseradish peroxidase (HRP) conjugate was added to bind the immobilized complex. For invasive assays (GABA), a biotinylated target antigen competed with endogenous GABA in the sample for binding sites on the precoated antibody plate. In both assays, the catalytic activity was detected by adding TMB substrate solution, whose color changed in relation to (or inverse relation to, in case of GABA) the amount of the biomarker. The reaction was stopped by Acid Stop Solution, resulting in a change of color to yellow. Optical density (OD) readings of all wells were taken spectrophotometrically at 450 nm by an automated ELISA microplate reader. Sample concentrations were determined by mathematical interpolation of OD readings against a rigorously plotted 4-parameter logistic (4-PL) standard curve for each test run.
Flame Atomic Absorption Spectroscopy (FAAS) Technique. Quantification of trace elements and heavy metals, namely Lead (Lead, µg/dL), Mercury (Mercury, µg/L), and Calcium (Calcium, mg/dL), was performed using Flame Atomic Absorption Spectroscopy (FAAS). FAAS provides an accurate and specific measurement of optical radiation absorption by atoms in the gaseous phase. For spectroscopic analysis, blood samples were subjected to a rigorous wet acid digestion with ultra-pure nitric acid (HNO_3, 65% v/v) in a closed-system vessel for interference removal and liberation of protein-bound metals from the organic matrix. The obtained solutions were diluted with deionized water up to a constant volume. The solutions were then sprayed into the hot air-acetylene flame in FAAS equipment. The element-specific hollow cathode lamps (HCLs) served as the source of optical radiation emitting a specific wavelength for each target analyte: 283.3 nm for Lead, 253.7 nm for Mercury, and 422.7 nm for Calcium. As the gaseous phase with dissolved atoms passed through the optical path of the spectrometer, the absorption of light occurred on the resonance lines. The light attenuation depended on the analyte concentration according to the Beer-Lambert law and was measured by a photomultiplier detector. Calibration curves were confirmed on a daily basis using certified reference materials (CRMs).

Analysis of Statistics

Statistical distributions classify variables into two groups: non-parametric variables and those having a normal distribution. For variables with an irregular distribution by Kolmogorov-Smirnov, the data were displayed as median ± S. Error. The control groups, patients, and subgroups were compared using ANOVA. To assess the correlation between the parameters, Spearman's correlation coefficients were calculated. P-values were considered statistically significant if they were less than 0.05. The statistical analyses were conducted using IBM USA and SPSS Statistics V. 26, and the figures were created using Microsoft Office 2016's Excel.[17]

Results.

Autism spectrum disorder (ASD) is a diverse collection of neurodevelopmental diseases characterized by repetitive behaviors since infancy and difficulty with social communication. Since ASD is produced by a variety of circumstances, neurobiology in the modern world tries to utilize a multimodal biomarker profiling approach to comprehend the complex interactions between environmental factors and genetic predisposition. Finding diagnostic and prognostic indicators for autism requires investigating many pathophysiological pathways, such as disruptions in the lysosomal route, neuroinflammation, or neurotransmitter dysfunction, in conjunction with exposure to environmental metals.

Demographic

Table 1: The demographic characteristics of autism, Siblings, and the unrelated control group

Group / Parameter	Autism	Siblings	Control	Chi^2 / P Value
Sex (M/F)	56/4	42/18	46/14	0.004
Location (Urban/Rural)	59/1	60/1	60/0	0.366
Age (Mean ± SD)	6.48±2.50	7.25±2.54	6.78±2.46	0.243

The demographics of the research subjects, including children with ASD, their healthy siblings, and the controls, were identified and meticulously matched to obtain higher internal validity and remove any confounders. Table 1 shows that the three groups were matched in terms of age (mean age: ASD 6.48±2.50, siblings 7.25±2.54, and controls 6.78±2.46; P=0.243 and geography P=0.366, with more participants from metropolitan regions. Since both neurodevelopment and metal exposure rely on age and geography, respectively, matching these two factors is crucial. More significantly, it has been statistically demonstrated that there are gender disparities among the participants under study (P=0.004), with a preponderance of males among those with ASD (56 males and 4 females). Established epidemiological studies on the frequency of autism spectrum disorder in many regions of the world are consistent with this notable gender difference (14:1 male to female). In terms of profiling, understanding the impact of gender-based biological differences on autistic symptoms will be aided by the study of biochemical components.

Elevated Lead, Neuroinflammation, and Lysosomal Dysfunction Parallel to Neurotransmitter and Calcium Depletion in Autism

Table 2: Comparison of Heavy Metals, Electrolytes, and Neuroimmune Biomarkers in Children with Autism, Their Siblings, and Healthy Controls

	Autism		**Siblings**		**Control**		**P Value**
	Median	**(25-75)%**	**Median**	**(25-75)%**	**Median**	**(25-75)%**	
Lead µg/dL	5.17	4.84-5.62	4.93	4.75-5.78	4.77	4.57-5.18	0.002
Mercury µg/L	3.50	2.87-3.76	3.29	3.04-3.46	3.32	3.05-3.51	0.12
Calcium mg/dL	6.80	5.33-8.60	6.80	5.60-9.60	8.05	6.83-9.40	0.002
IL-17(pmol/L)	17.58	15.81-19.19	12.57	11.47-13.54	14.21	12.85-15.33	0.0001
OPLAH µmol/L	1.63	1.24-2.36	5.08	3.53-6.43	4.53	3.58-6.38	0.0001
LAMP1 ng/ml	41.33	40.30-42.37	30.10	29.52-30.57	29.80	29.45-30.46	0.0001
α synuclin ng/ml	25.42	22.67-28.37	28.72	26.37-34.32	30.25	27.96-32.37	0.0001

GABA pg/mL	245.73	199.38-319.66	315.39	279.46-405.53	352.09	240.61-439.07	0.0001

Comparison of Minerals and Heavy Metals. The three groups' blood Pb levels differed significantly (p=0.002), according to a statistical analysis of heavy metals. Post hoc analysis showed that children with autism had higher median lead concentrations (5.17 µg/dL; IQR: 4.84-5.62) than their siblings without autism (4.93 µg/dL), whereas control individuals had the lowest amounts (4.77 µg/d). The groups' blood mercury (Hg) concentrations, however, did not differ significantly (p=0.120), with medians ranging from 3.29 µg/L to 3.50 µg/L. Regarding important elements, the concentration of calcium (Ca) in the serum varied significantly (p=0.002). It's interesting to note that both the autism and sibling groups had lower median calcium values (6.80 mg/dL) than the healthy controls (8.05 mg/dL, IQR = 6.83–9.40). Regarding IL-17, this inflammatory cytokine showed significant variations across all cohorts (p<0.00). The median IL-17 levels in the autism group were substantially higher (17.58 pmol/L; IQR = 15.81-19.19) than in the control group (14.21 pmol/L) and the sibling group (12.57 pmol/L). However, compared to the sibling group, the autism group's 5-oxoprolinase (OPLAH) level was considerably lower (1.63µmol/L; IQR=1.24-2.36). The lysosomal activity surrogate biomarker, Lysosome-associated membrane protein 1 (LAMP1), demonstrated a significantly greater expression level in autism participants alone (41.33 ng/mL; IQR: 40.30–42.37), consistent with the dysmetabolism seen, whereas both siblings (30.1). Gamma-aminobutyric acid (GABA) and α-synuclein levels varied significantly between the cohorts, according to the neurobiological assessment (p<0.001). The autistic group's median α-synuclein concentrations (25.42 ng/mL; IQR: 22.67–28.37) were considerably lower than those of the sibling (28.72 ng/mL) and control cohorts (30.25 ng/mL). The GABA concentrations of the autistic subjects were significantly lower (245.73 pg/mL; IQR: 199.38–319.66) than those of their unaffected siblings (315.39 pg/mL) and the healthy controls (352.09 pg/mL; IQR: 240.61–439.07), indicating a severe deficit in inhibitory neurotransmission.

Correlation

Table 3: Correlation matrix of multidimensional biochemical and environmental biomarkers within the ASD cohort.

Variables	Spearman's rho	Lead µg/dL	Mercury µg/L	Calcium mg/dL	IL-17 (pmol/L)	OPLAH µmol/L	LAMP1 ng/ml	α synuclin ng/ml	GABA pg/mL
Lead µg/dL	**r**	**1**	**0.147**	**-.521****	**.365****	**-.327***	**.477****	**-.496****	**-.422****
	P Value	**.**	**0.262**	**0**	**0.004**	**0.011**	**0**	**0**	**0.001**
Mercury µg/L	**r**	**0.147**	**1**	**0.012**	**0.175**	**0.108**	**-0.048**	**.349****	**-0.165**
	P Value	**0.262**	**.**	**0.925**	**0.182**	**0.413**	**0.717**	**0.006**	**0.206**
Calcium mg/dL	**r**	**-.521****	**0.012**	**1**	**-.363****	**.575****	**-.363****	**.370****	**.655****
	P Value	**0**	**0.925**	**.**	**0.004**	**0**	**0.004**	**0.004**	**0**
IL-17 (pmol/L)	**r**	**.365****	**0.175**	**-.363****	**1**	**-0.227**	**0.112**	**-0.161**	**-.459****
	P Value	**0.004**	**0.182**	**0.004**	**.**	**0.08**	**0.393**	**0.219**	**0**
OPLAH µmol/L	**r**	**-.327***	**0.108**	**.575****	**-0.227**	**1**	**-.410****	**.558****	**.514****
	P Value	**0.011**	**0.413**	**0**	**0.08**	**.**	**0.001**	**0**	**0**
LAMP1 ng/ml	**r**	**.477****	**-0.048**	**-.363****	**0.112**	**-.410****	**1**	**-.568****	**-.327***
	P Value	**0**	**0.717**	**0.004**	**0.393**	**0.001**	**.**	**0**	**0.011**

α synuclin ng/ml	**r**	**-.496****	**.349****	**.370****	**-0.161**	**.558****	**-.568****	**1**	**0.19**
	P Value	**0**	**0.006**	**0.004**	**0.219**	**0**	**0**	**.**	**0.146**
GABA pg/mL	**r**	**-.422****	**-0.165**	**.655****	**-.459****	**.514****	**-.327***	**0.19**	**1**
	P Value	**0.001**	**0.206**	**0**	**0**	**0**	**0.011**	**0.146**	**.**

Spearman's rank correlation between environmental metals, inflammatory factors, neurotransmitters, and lysosomal enzymes was performed in order to comprehend the interconnected pathways involved in ASD etiology (Table 3). Numerous highly significant and consistent biological clusters were discovered. Lead (Pb) was a key disruptor with extremely high systemic correlations. Lead and calcium concentrations were shown to have a highly significant negative association (r=-0.521; p<0.001), suggesting a potential mechanism whereby high amounts of one can interfere with another. However, there was no discernible relationship between the content of Mercury (Hg) and either Lead (r=0.147; p=0.262) or Calcium (r=0.012; p=0.925). The accumulation of lead (r = 0.365, P = 0.004) and the concentration of calcium (r = -0.363, P = 0.004) were significantly positively correlated with the neuroinflammatory biomarker IL-17. More notably, there was a strong negative association (r = -0.459, P < 0.001) between the concentration of GABA and increased neuroinflammation caused by IL-17 and decreased inhibitory neurotransmission. Furthermore, the proper functioning of calcium homeostasis was significantly positively correlated with the amount of GABA (r = 0.655, P < 0.001). and was negatively affected by exposure to lead (r = -0.422, P = 0.001). The behavior of lysosome activity in relation to LAMP1 was very abnormal. LAMP1 had a negative correlation with calcium (r=−0.363, P=0.004) and a strong positive correlation with lead levels (r=0.477, P<0.001). Notably, there was a strong negative correlation between LAMP1 and α-synuclein levels (r=−0.568, P<0.001). This suggests that variations in protein clearance pathways are closely linked to lysosome failure in terms of altered LAMP1 dynamics. Notably, α-synuclein had a strong negative correlation with lead (r=−0.496, P<0.001) but a positive correlation with mercury levels (r=0.349, P=0.006). OPLAH, an important biomarker in the γ-glutamyl cycle and glutathione metabolism, showed a great degree of integration in the physiological homeostasis. OPLAH was observed to positively correlate with calcium (r=0.575, P<0.001), α-synuclein (r=0.558, P<0.001), and GABA (r=0.514, P<0.001). On the other hand, elevated lysosomal stress marker LAMP1 (r=−0.410, P=0.001) and high lead levels (r=−0.327, P=0.011) were observed to considerably lower OPLAH.

ROC curve analysis

An ROC curve analysis was used to assess the efficacy and discriminatory power of the quantified neuro-inflammatory, lysosomal, metabolic, and neurotransmitter indicators between children with ASD and control subjects.

Table 4: ROC curve parameters evaluating the diagnostic efficacy and discriminative power of neuroinflammatory, lysosomal, metabolic, and synaptic biomarkers in children with ASD versus controls

Parameters	Cut off	Sensitivity %	Specificity%	AUC	95% CI of AUC	P Value
IL-17 pmol/L	15.236	81.7	78.3	0.867	0.811- 0.924	0.0001
LAMP1 ng/ml	30.918	88.3	88.3	0.884	0.813- 0.955	0.0001
OPLAH µmol/L	3.267	88.3	88.3	0.932	0.896- 0.968	0.0001
α synuclin ng/ml	27.632	71.7	71.7	0.737	0.660- 0.813	0.0001
GABA pg/mL	301.81	66.7	65.8	0.722	0.643-0.801	0.0001

As shown in Table 4, the ROC curves include a number of metrics, including the AUC, optimal threshold value (determined using Youden's index), sensitivity, specificity, and 95% CI. The diagnostic capability of all the evaluated markers was very high (P<0.001).

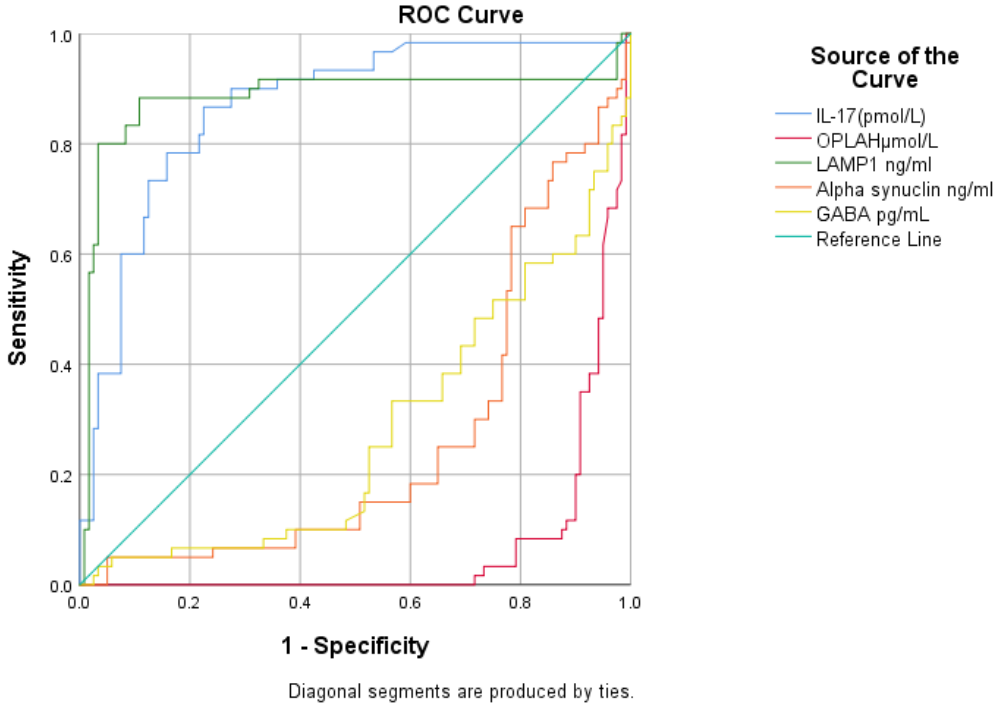

Figure 1: ROC profiles of neuroinflammatory, metabolic, lysosomal, and neurotransmitter pathways as diagnostic indicators for ASD.

The most potent single biomarker among those evaluated was OPLAH, a significant enzyme involved in glutathione and one-carbon metabolism. With an AUC of 0.932 (95% CI: 0.896–0.968, P<0.001), it demonstrated outstanding diagnostic ability. OPLAH demonstrated a good balance between sensitivity and specificity, with 88.3% when the ideal cutoff value was set at 3.267 mol/L, suggesting significant metabolic abnormalities underlying ASD pathophysiology. The inflammatory cytokine IL-17 and the lysosomal protein LAMP1 both showed extremely encouraging diagnostic outcomes, confirming the link between neuroinflammation and deficiencies in lysosome clearance in the development of autism.

With an AUC of 0.884 (95% CI: 0.813–0.955), the LAMP1 protein demonstrated excellent diagnostic effectiveness. It demonstrated an impressive balance between high sensitivity and high specificity of 88.3% when the cut-off point was set at 30.918 ng/ml.

IL-17's AUC was 0.867 (95% CI: 0.811–0.924). IL-17 effectively distinguished ASD cases with a sensitivity of 81.7% and a specificity of 78.3% by using the cut-off point of 15.23 pmol/L.

GABA, an inhibitory neurotransmitter, and alpha-synuclein, a marker of protein aggregation, produced acceptable to high diagnostic yield results; as a result, they can be regarded as complimentary indicators rather than single-screening agents. The α-synuclein AUC was determined to be 0.737 (95% CI: 0.660–0.813). At a cut-off concentration of 27.632 ng/mL, it demonstrated 71.7% sensitivity and specificity. GABA's AUC was determined to be 0.722 (95% CI: 0.643–0643–0.801). It provided 66.7% sensitivity and 65.8% specificity at a cut-off concentration of 301.81 pg/mL.

Discussion

In order to reduce confounding effects in multidimensional biomarker profiling, a highly controlled baseline is established by the demographic characterization of our experimental subjects (Table 1). The absence of a statistically significant variation in geographic regions and age (p=0.243), ASD, Siblings, and the unrelated control group were all exactly matched (p=0.366). This homogeneity guarantees that later changes in neuroinflammatory, lysosomal, and metabolic biomarkers ultimately represent the pathophysiology of autism rather than artifacts of heterogeneous environmental bio exposure or neurodevelopmental maturation. Both urbanization and age heavily modulate biochemical and trace metal dynamics[18]. Conversely, there was still a significant and statistically significant mating divergence (P = 0.004), which was marked by a strong male preponderance in the ASD system (56 males vs. 4 females; ratio ≈14:1) [19]. There is a lot of mountain climbing in accurate scientific model sizes, but this proposed gender bias is quite comparable with existing international epidemiological data, which consistently show a higher incidence of ASD in males, around a 4:1 ratio worldwide. Mechanistically, the cohort's pronounced gender imbalance provides key context for evaluating a multi-biomarker matrix. The "female shield effect" to ASD may possibly involve sex-specific differential regulation of neuroinflammatory pathways, microglial activation, and certain synaptic neurotransmitters, according to mounting evidence from the vast body of neurobiological literature. Indicate neurotransmitter toxicity and oxidative stress in addition to other evidence by upregulating glutathione synthesis, a route directly connected to the OPLAH metabolic biomarker assessed in our study and, thus, the lysosomal malfunction identified in our findings. The male-to-female ratio is significantly skewed in favor of the interrelated cascade of toxic metal buildup (such as lead exposure) and neuroinflammation [20]. To construct a comprehensive version of ASD pathogenesis, comparative analyses of biological environmental parameters examine the known robust, statistically sound alterations across groups (Table 2). All examined biomarkers, except for mercury (P = 0.12), displayed well-shaped variation (P < 0.01) that mapped the interrelated cascade of environmental toxicity, neuroinflammation, neurotransmitter imbalance, and lysosomal clearance failure. The results indicate that children with ASD (median: 5.17 µg/dL) and their siblings (median: 4.93 µg/dL) have significantly higher blood lead phase (Pb) levels than unrelated controls (median: 4.77 µg/dL; P=0.002). When compared to controls (median: 8.05 mg/dL; P = 0.002), this buildup is linked to a concurrent and substantial depression in systemic calcium levels in ASD and siblings (median: 6.80 mg/dl), compared to controls (median: 8.05 mg/dL; P = 0.002). Mechanistically, lead is a well-known neurotoxin that immediately interrupts calcium (Ca2+) for domestic binding to important proteins, along with calmodulin and in the form of voltage-gated calcium channels. Increased lead loading in neurodevelopmental settings, especially among siblings. This frequency of normal-environmental exposure to Pb calcium precursors is extremely significant because it directly explains the breakdown of calcium-established homeostatic mechanisms that specifically affect synaptic signaling and enzymatic integrity in the autistic brain [21].

The pro-inflammatory cytokine IL-17 was significantly elevated in the ASD group (Median: 17.58 pmol/L) compared to both siblings (Median: 12.57 pmol/L) and controls (Median: 14.21 pmol/L; P < 0.001), confirming a severe neuroinflammatory condition. Remarkably, OPLAH levels are severely depleted in ASD children (Median: 1.63 µmol/L), which is significantly lower than the healthy baselines observed in siblings (Median: 5.08 µmol/L) and controls (Median: 4.53 µmol/L; P < 0.001). OPLAH contains a vital enzyme. γ-glutamyl cycle, which produces glutamate from 5-oxoproline, a direct precursor to the synthesis of glutathione (GSH) and one-carbon metabolism. A catastrophic breakdown in the cellular antioxidant defense system is indicated by the acute recurrence of OPLAH in the ASD population. Excessive oxidative stress brought on by this metabolic barrier serves as the main trigger for microglial activation and the ensuing overproduction of IL-17. It's interesting to note that the sibling association maintained a high OPLAH average, suggesting that ASD patients may have compensatory metabolic inflexibility. those for the entire neuroinflammation cascade[22,23]. Significant changes were observed in lysosomal integrity as measured by LAMP1 (Lysosome-associated membrane protein 1). In contrast to stable baselines in siblings and controls (≈30 ng/ml), LAMP1 levels only increased in the ASD group (Median: 41.33 ng/ml; P< 0.001. At the same time, α -synuclein levels were significantly lower in the ASD group (Median: 25.42 ng/ml) than in the controls (Median: 30.25 ng/ml; P<0.001) and siblings (Median: 28.72 ng/ml). An effective biomarker of lysosomal stress or compensatory biogenesis, which is frequently brought on by the buildup of undegraded cellular waste, is LAMP1 overexpression. Reduced soluble α and accelerated LAMP1 have an inverse connection. Synuclein protects against sudden interference with protein clearance processes like chaperone-mediated autophagy (CMA). Lysosomal acidification and enzymatic activity can be interfered with by heavy metals like Pb and oxidative stress caused by OPLAH depletion. This changes the mobile processing or clearance of synaptic proteins, for example, and causes an

accumulation of faulty lysosomes (high LAMP1). α There is no question that synuclein has a role in the structural neurodevelopmental deficits associated with ASD [24.25]. The principal inhibitory neurotransmitter GABA was considerably lower in the ASD group (Median: 245.73 pg/mL) than in siblings (Median: 315.39 pg/mL) and controls (Median: 352.09 pg/mL; P<0.001), reflecting the clinical phenotype of brain hyper-excitability in autism. A bridge between environmental, metabolic, and neuroinflammatory stimuli is provided by this decrease of GABAergic tone. First, Pb induction significantly impairs intercellular calcium signaling, which is crucial for GABA production and vesicle release. Second, it has been demonstrated that the primary neuroinflammatory cytokine IL-17 rapidly modifies synaptic transmission by impairing the characteristics of GABAergic interneurons through the downregulation of GABA receptor expression. Thus, the neuroinflammatory milieu (high IL-17) that results from metabolic weariness (low OPLAH) and environmental stressors (high Pb) inhibits GABA, causing the typical excitatory/inhibitory imbalance observed in ASD [26,27]. The multi-biomarker Spearman correlation matrix serves as the foundation for our investigation into the systemic pathophysiology of ASD (Table 3). This matrix shows a dense community where metabolic exhaustion, lysosomal collapse, neuroinflammation, and clinical synaptic dysfunction are all immediately bridged by the burden of environmental chemicals. Blood lead (Pb) and calcium levels have a wide space within the correlation matrix ($r = -0.521$, $p < 0.001$). Molecular mimicry of noxious heavy metals was demonstrated by this compelling organic preparation. Lead competitively occupies the Ca^{2+} binding domains of intracellular proteins and essential channels [28]. This disruption of calcium homeostasis is accompanied by a weak cascade. On top of this, imaginative and predictable systemic downstream deficiencies within the calcium-stable cell mechanism can occasionally be explained by means of depletion of calcium, an essential second messenger that is pushed without delay by Pb formation. An important basis for understanding the metabolic structural changes within the autism population is provided by this molecular displacement [29]. OPLAH, an important metabolic enzyme for the γ-glutamyl cycle and glutathione (GSH) biosynthesis, showed a strong specific dependence on precise calcium phases (r=0.575, P<0.001) and was greatly attenuated through Pb exposure. This indicates that. Pb-induced calcium displacement disrupts the enzymatic machinery required for the maintenance of mobile antioxidant defense [30].

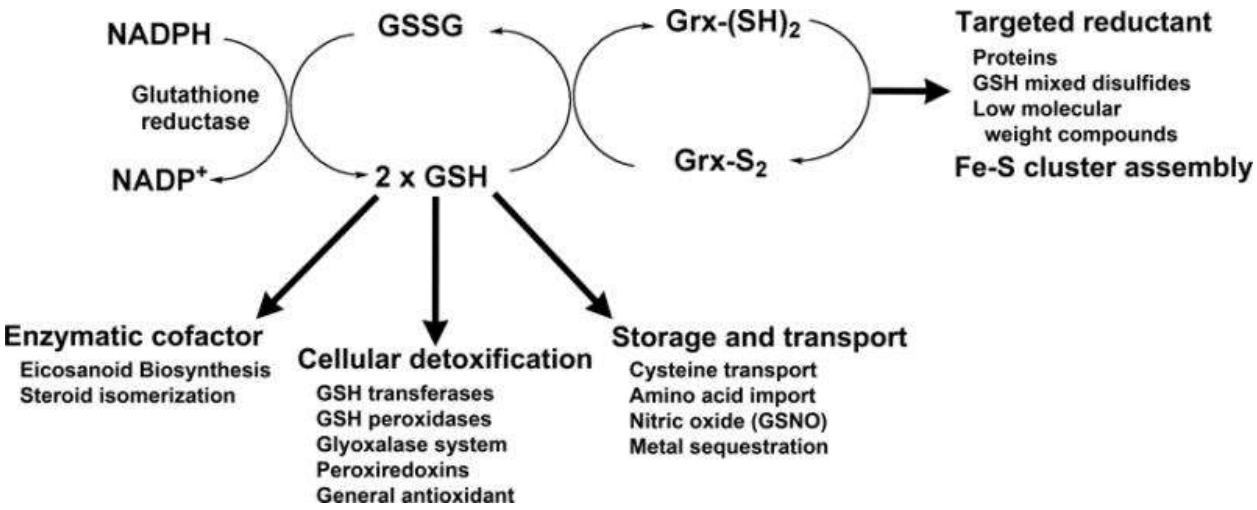

Figure 2. Roles of glutathione and enzymes that preserve redox homeostasis [30]

As demonstrated by the high positive association between Pb phases and LAMP1 ($r = 0.477$, $P < 0.001$) and the reverse situation between OPLAH and LAMP1 ($r = -0.410$, $P = 0.001$), the continuous stress was transferred to the lysosomal compartment. According to this biological hypothesis, compensatory lysosomal biogenesis or crucial autophagy-lysosome pathway (ALP) mobilization takes place as oxidative stress increases as a result of OPLAH depletion (indicated by folded LAMP1). The cell's internal milieu changes from one of active metabolic production to one of persistent clearance stress [31]. The substantial negative connection between LAMP1 and α-synuclein ($r=-0.568$, $P<0.001$) further demonstrates this lysosomal constriction. Soluble monomeric α-synuclein is broken down under normal physiological conditions by macro-autophagy and chaperone-mediated autophagy (CMA).

However, typical clearance pathways are inhibited when lysosomal acidification and hydrolase leaks are disrupted by heavy metal toxicity (Pb) and downstream oxidative stress [32]. High levels of LAMP1 are associated with a significant depletion of soluble α-synuclein, which is probably due to intracellular aggregation or systemic clearance failure, a well-established feature of neurodegenerative processes and new neurodevelopmental models of autism. It's interesting to note that Mercury (Hg) levels showed a selective positive correlation only with α-synuclein ($r=0.349$, $P=0.006$). This suggests that whereas Pb targets lysosomal structural stress, Hg may directly interact with the biochemical conformation of particular synaptic proteins, highlighting the need for a multidimensional analytical approach [33]. The severe disruption of neurotransmission is the last phenotypic result of this metabolic and lysosomal breakdown. The pro-inflammatory cytokine IL-17 had a negative association with calcium ($r=-0.363$, $P=0.004$) and a direct positive correlation with Pb exposure ($r=0.365$, $P=0.004$), indicating that neuroinflammation is fueled by the environmental-biochemical axis [34]. Importantly, a significant negative connection with GABA levels ($r=-0.459$, $P<0.001$) indicated that this elevated neuroinflammatory state via IL-17 was firmly linked to a severe reduction in inhibitory neurotransmission. GABA was inhibited by Pb buildup ($r=-0.422$, $P=0.001$) and showed a substantial dependence on calcium homeostasis ($r=0.655$, $P<0.001$) [35]. IL-17 signaling cascades directly interfere with GABA transport, downregulate GABA levels, and decrease microglial and astroglial activity. When Pb displacement causes a decrease in calcium-dependent vesicular GABA release through mechanistic inhibition of hibiscus, a receptor expression occurs in the postsynaptic membrane. biological basis for the excitatory/inhibitory imbalance that characterizes ASD's clinical manifestation [36]. Beyond a statistical validation, the diagnostic efficiency determined by receiver operating characteristics (ROC) analysis (Table 4, Fig. 1) offers profound insight into the hierarchical predominance of particular metabolic and cellular pathways within the clinical manifestation of ASD. The map of the objective and temporal load of environmental stress, metabolic weariness, and neurosynaptic failure by comparing the geometric behavior of various curve paths with their corresponding areas under the curve (AUC). The mild metabolism of ASD is characterized by persistent disruption of the γ-glutamyl cycle, with a bias for secondary result, according to the mathematical dominance of OPLAH as the top diagnostic discriminant (AUC=0.932). In the lower suitable quadrant of Figure 1, the OPLAH trajectory forms an inverted curve. This spatial orientation in adolescents with ASD indicates excessive, systemic downregulation of the enzyme [37]. This apparent threshold reduction is factored into the land with physiologically mobile antioxidant decline. OPLAH is responsible for scavenging 5-oxoproline and providing glutamate for the subsequent synthesis of glutathione (GSH). The cell loses its primary defense against oxidative stress when the OPLAH phase falls below the therapeutic limit. This metabolic instability makes neurodevelopmental pathways unusually sensitive to environmental pollutants, which may also help explain why even low exposure to heavy metals like lead can have unusually highly detrimental consequences for those children[38].The conventional upper left curves in Figure 1 demonstrate the incredible diagnostic power of LAMP1 (AUC=0.884) and IL-17 (AUC=0.867), which are well known from the way cells react to this metabolic disturbance. Importantly, LAMP1 responds to lysosomal stress caused by the abrupt upward pressure exceeding the physiological threshold. Cellular compensatory responses conceived under the LAMP1 subtype of waste enhancement failure occur when heavy metal accumulation and oxidative stress deplete the GSH pathway, impair lysosomal acidification, and result in a backup in chaperone-mediated autophagy (CMA) by driving lysosomal biogenesis. This contractile autophagy state acts as an endogenous catalyst for an extended period of time. It turns on astrocytes and microglia, accounting for the significant upward stimulus in IL-17 production seen in ASD [39]. This natural reporting concludes with the help of the diagnostic profiles of GABA (AUC=0.722) and α-synuclein (AUC=0.737), which combine cellular stress with synaptic specificity. In Figure 1, both markers result in inverted curves below the reference line, indicating large molecular enrichment within the synaptic ASD environment. The coordinated below shows how upstream inflammatory metabolic stresses interfere with neurotransmission: Protein accumulation: Because soluble α-synuclin is retained in insoluble intracellular aggregates and disrupts regular synaptic vesicle recycling, the loss of lysosomal clearance efficiency (excessive LAMP1) falls below the soluble α-syncline threshold Excitatory/inhibitory imbalance: This structural disruption disrupts GABAergic interneurons and inhibits GABA production when combined with lead-induced calcium displacement and IL-17-mediated transmission[38]. Lastly, the multi-curve ROC architecture demonstrates that a single distant biomarker cannot adequately describe ASD. Rather, the profile follows a pure pathological cascade: lysosomal stress (high LAMP1) and chronic neuroinflammation (excessive IL-17) are caused by an early decline in metabolic defense (low OPLAH) and

an increase in heavy metal load. These factors ultimately lead to critical dysregulation of neuronal synapses and synaptic α drive decomposition [40].

Conclusion

By establishing an in-depth multi-biomarker profile for ASD, our images change the diagnostic model from a distant signal to a synchronized pathogenic cascade. The number one systemic stressor that interferes with Ca^{2+} homeostasis (OPL) is widely acknowledged (Pb) exposure. Riding upstream degradation is a symptom of driver degradation. This metabolic instability leads to autophagic lysosomal shrinkage (decreased soluble α-synuclein, increased LAMP1), which allows astrocytes and microglia to pursue inflammatory follicular cytokines as endogenous catalysts to induce toxicity, GABAE/suppressive function, or significant inhibitory tone. Importantly, the ROC magnitude confirms first-class diagnostic accuracy in this panel, with OPLAH increasing as a significant discriminator (AUC=0.932). Furthermore, the unique preservation of metabolic and neurotransmitter flexibility in neurotypical siblings provides a unique basis for records that entitle the phenotypic to access. Lastly, the use of this multi-biomarker panel instead of a remote marker for environmental health monitoring and early detection of ASD. provides a powerful, clinically safe tool for clinical intervention and pathway precision.

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Authors Contribution

Each author made an equal contribution to this work. The final version has been read and approved by all of them.

Ethical Statement

The study procedure was strictly adhered to in compliance with the ethical principles of the Declaration of Helsinki. The study gained ethical approval from the University of Kufa IRB/Ethics Committee (Approval No. Ref. H K/167; Date: December 15, 2025). Before participation, the study's aims and methodology were explicitly described to the parents/guardians of all participating children. All guardians provided written informed consent before the conduct of any study

Conflict of Interest Statement

The authors declare no conflicts of interest.

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