Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6, 3808-3815
2024
Publisher: Learning Gate
DOI: 10.55214/25768484.v8i6.2824

Evaluation of neurotransmitters (Gamma-aminobutyric acid and serotonin) and apoptosis factor (Caspase-7) as biomarkers for the diagnosis of autism spectrum disorder

Noor Ahmed Hameed[1], Shrouk Abd Alrazak Hassan[2], Hamid Jaddoa Abbas[3*]
[1,2]Medical Laboratory Technology Deptartment, College of Health & Medical, Technology, Southern Technical University, Basrah, Iraq; nourhan4788@gmail.com (N.A.H.) shrouk.albraheem@stu.edu.iq. (S.A.A.H.)
[3]Al-Faiha'a Teaching Hospital, Al- Zehra'a Medical College, University of Basrah, Basrah, Iraq; hamedjadoa@yahoo.com (H.J.A.)

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disability characterized by impaired communication and social skills along with restricted interests and repetitive behaviors. Biomarkers such as γ-aminobutyric acid (GABA), serotonin (5-Hydroxytryptamine, 5-HT) and caspase-7, showing potential for early diagnosis and management of ASD. To evaluate the effectiveness of GABA, serotonin and caspase-7 as novel biomarkers to predict autism at an early stage. A case- control study which started from November 2023 to June 2024, that involved 77 children's patients, newly diagnosed with autism. The control group was comprised of 61 age- and sex-matched apparently healthy children, they had no history of autism and any other chronic diseases. Serum samples were analyzed for GABA, serotonin and caspase-7 by using enzyme linked immune-sorbent assay (ELISA) technique. The statistical package for social sciences (SPSS- 26) was used for data analyses. The study revealed significantly elevated GAMA and caspase-7 levels; while, no significant differences of serotonin level between the autistic children's patients and the control group. This study provides a groundbreaking revelation of the biochemical markers (GAMA and caspase-7) and autism spectrum disorder, the myriad metabolic and neurochemical dysfunctions of which form a complex tapestry of ASD.

Keywords: *5-Hydroxytryptamine, Autism spectrum disorder, Caspase7, γ-aminobutyric acid.*

1. Introduction

Autism is a neurodevelopmental disease marked by deficits in communication and social abilities, as well as restricted interests and repetitive activities [1]. Clinical symptoms often emerge by the age of three; however, impairments in speech, social responsiveness, and play may be evident as early as 6 to 12 months of age [2]. The genesis of ASD likely involves environmental variables that induce physiological anomalies in genetically predisposed people. Notwithstanding this fact, the precise etiology remains unclear; ASD is a complex condition [3]. Neurotransmitters play a fundamental role in the causes of autism and the variation of its symptoms and are considered important markers in diagnosing this disorder [4]. Neurotransmitters are pivotal in the etiology of autism and the variability of its symptoms, serving as significant indicators in the diagnosis of this illness [5]. In immature brains, GABA receptors differ from those in adult brains. GABA serves as the principal excitatory neurotransmitter during cerebral development, affecting proliferation, migration, synaptic maturation, differentiation, and apoptosis [6]. Changes in the gabaminergic and glutaminergic systems alter the excitatory/inhibitory balance and may contribute to autistic behaviors and numerous neurodevelopmental problems [7]. Evidence increasingly suggests that the neurophysiological processes underlying neuropsychiatric diseases, including schizophrenia, depression, and anxiety, include disruption of inhibitory neurotransmission [8]. Extensive research has shown that the

* Correspondence: hamedjadoa@yahoo.com

neurophysiological basis of epilepsy arises from aberrant excitatory neuronal firing in certain brain areas due to a lack of GABAergic inhibition [9]. GABAergic dysfunction may offer a plausible explanation for the high comorbidity with epilepsy or the heightened vulnerability to epilepsy seen in individuals with autism [10].

Serotonin (5-HT), a neurotransmitter belonging to the monoamine family, regulates neuronal proliferation, migration, and differentiation. It influences cortical plasticity in adults and significantly contributes to early cortical development as a result [11]. Disruptions, even temporary aberrations, of the 5-HT pathway during development can result in enduring changes in brain function and behavior. Autism spectrum disorder is a neurodevelopmental syndrome with increasing evidence of serotonin (5-HT) involvement [12]. Numerous investigations have shown that the serotonergic system encompasses the neurotransmitters most frequently linked to the pathophysiology of autism. Seven families of serotonin receptors (5-HT1–5-HT7) are involved in mediating the effects of this neurotransmitter, which predominantly influences mood, sleep, memory, learning capacity, muscular contraction balance, and endocrine activities [13]. The expression levels and activity of the tissue serotonin transporter (SERT) govern serotonin levels, both intracellularly and extracellularly [14]. The serotonin transporter significantly influences the control of neuronal serotonin levels. Numerous internal and environmental factors can substantially impact the functionality and expression levels of SERT from early embryonic development through adolescence. Reduced serotonin levels may correlate with several characteristics in autistic children, including sleep difficulties and cognitive and social deficits. Other investigations have shown increased levels of SERT and/or serotonin in autistic children relative to controls [11].

On the other hand, research indicated that caspase 7 play a role in neuroinflammation, a key factor in ASD pathology [15]. Caspase 7, an effector caspase (with caspase 3 and 6), participates in apoptotic and inflammation. Caspase 7 is considered to be functionally redundant with caspase 3, which may be directly triggered by caspase 9 to break cellular substrates during apoptosis [16]. In the context of ASD, caspase-7 might be relevant for several reasons [17], although direct studies linking caspase-7 specifically to autism are limited, but there are some potential connections:

1. Apoptosis dysregulation: There is growing evidence that dysregulation of apoptosis may be implicated with neurodevelopmental disorders, including ASD. Abnormal cell death processes during brain development could affect neuronal connectivity, which is a hallmark of ASD [18].

2. Neuroinflammation: Since caspases can influence inflammation pathways, an imbalance in caspase-7 activity could be linked to the inflammatory processes that may affect brain development and function in ASD [19].

3. Synaptic development and Plasticity: Emerging research suggests that caspases might be involved in synaptic pruning (the process of eliminating excess neurons and synapses during development) [20]. Disruptions in synaptic pruning have been implicated in ASD, and caspase-7 could potentially play a role in these processes through its action in apoptosis [21].

2. Material and Methods

A case- control study was carried in specialized autism centers; Usraty Center, Tooba Center and Al-Faihaa Teaching Hospital Center, Basra province, Iraq; from November 2023 to June 2024. The study involved newly diagnosed with autism seventy- seven patients, their aged were between 5 and 14 years; who diagnosed by a specialist psychiatrist. Patients who suffer from mental illnesses or other chronic diseases and patients who are below or above 5- 14 years were excluded. Whole blood was collected for determinations of parameters levels by standard methods. IBM SPSS Statistics version 26.0 was used for data analyses, p values greater than 0.05 which the set of statistical significance.

3. Results

Table 1, was shown that children with ASD had significant (P value < 0.05) high levels of gamma-aminobutyric acid and caspase 7; and non-significant (P value > 0.05) of serotonin compared with the control.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6: 3808-3815, 2024
DOI: 10.55214/25768484.v8i6.2824

Table 1.
Anthropometric and biochemical characteristics of study participants.

Variables		ASD (n=77) Mean ± SD	Control (n=61) Mean± SD	P. Value
Ages (Years)		7.468± 2.25	7.661± 2.517	0.643*
Sex (n%)	Males	61 (97.2%)	36 (59.01%)	0.086**
	Females	16 (20.8%)	25 (%)	
	Total	77 (100%)	61 (100%)	
HB (gm/dL)		11.006± 1.064	11.752± 1.199	0.0001*
FBS (mg/dL)		81.469± 10.886	94.325±15.630	0.0001*
B. Urea (mg/dL)		26.678± 6.481	25.030± 5.806	0.133*
CPK (IU/L)		94.18± 53.36	88.143± 25.402	0.434*
LDH (IU/L)		375.208± 212.407	268.696± 271.401	0.012**
GABA (pg/ml)		130.545± 106.879	20.149± 34.044	0.0001**
CASP7 (ng/ml)		10.126± 8.126	0.497± 0.608	0.0001**
Serotonin (ng/ml)		3.825± 24.553	0.320± 0.482	0.228*

Table 2 was revealed no significant statistical differences between males' group and female group for all of the parameters (P values > 0.05).

Table 2.
Comparison of biochemical characteristics in patients according to sex.

Variables	Males (n=61)-ASD Mean ± SD	ASD - Females (n=16) Mean ± SD	P. Value
Age (Years)	7.672±2.315	6.688±1.852	0.120*
HB (gm/dL)	10.946±1.121	11.238±0.799	0.333*
FBS (mg/dL)	80.852±9.783	83.819±14.496	0.325*
B. Urea (mg/dL)	26.774±6.763	26.313±5.453	0.802*
CPK (IU/L)	92.934±50.540	98.938±64.647	0.692**
LDH (IU/L)	377.197±223.117	367.625±171.433	0.874**
GABA (pg/ml)	127.051±95.507	143.86±145.436	0.579**
CASP7(ng/ml)	10.414±7.807	9.027±9.442	0.574**
Serotonin(ng/ml)	4.682±27.567	0.557±0.505	0.553**

Table 3 showed ROC curve plotting and corresponding AUC calculations of accuracy parameters of each of the variables revealed that GABA, CASP7and Serotonin were significant identifies of ASD.

Table 3.
The ROC curve and AUC analyses for the values of serum biomarkers for the diagnosis of ASD.

Variables	Area under the ROC curve (AUC)	P - value (AUC=0.5)	Best cut-off criterion	Sensitivity (%)	Specificity (%)	Efficiency
GABA (pg/ml)	0.978	0.0001	30.550	98.1	90.2	94.1
CASP7 (ng/ml)	0.971	0.0001	0.9400	94.8	78.8	86.8
Serotonin (ng/ml)	0.798	0.0001	0.203	81.8	61.7	71.7

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6: 3808-3815, 2024
DOI: 10.55214/25768484.v8i6.2824

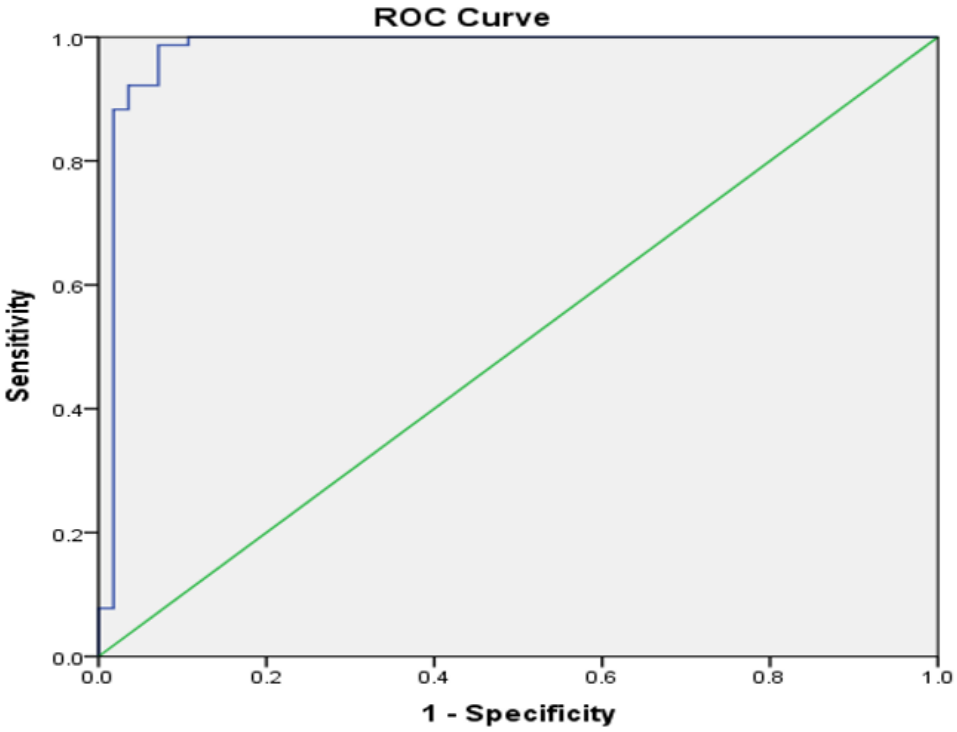

Figure 1.
Receiver operational curves for GABA.

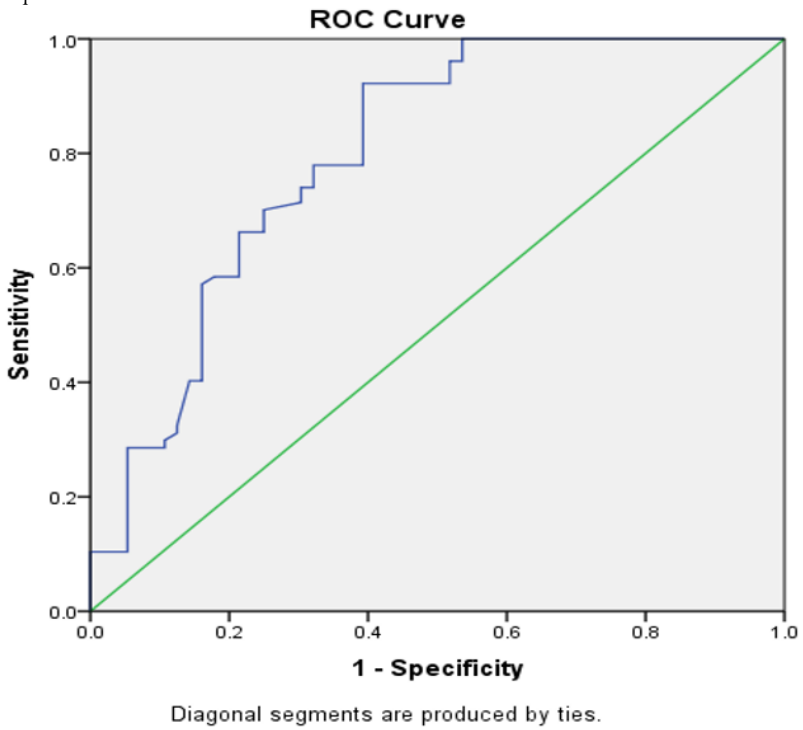

Figure 2.
Receiver operational curves for serotonin.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6: 3808-3815, 2024
DOI: 10.55214/25768484.v8i6.2824

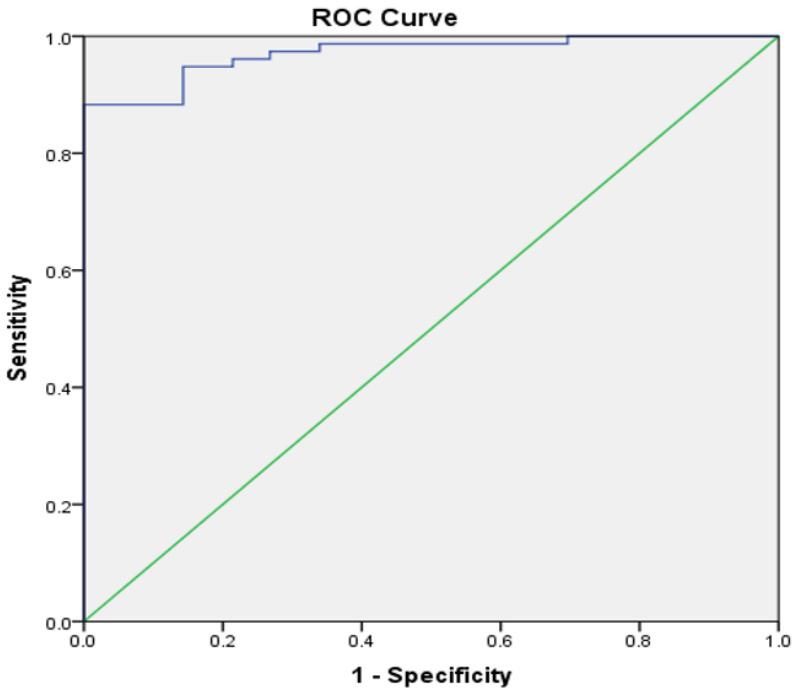

Figure 3.
Receiver operational curves for CASP 7.

Table 4.
Correlations among the variables for patients.

Variables		RBS	Urea	CK	LDH	GABA	CASP7	Serotonin
Hb	R*	0.042	0.035	0.414	0.059	-0.054	-0.146	0.074
	P value	0.715	0.765	0.001	0.612	0.642	0.206	0.521
RBS	R		0.217	-0.029	0.050	-0.099	0.079	0.083
	P value		0.058	0.799	0.667	0.390	0.493	0.472
Urea	R			-0.100	-0.186	-0.030	-0.097	0.185
	P value			0.388	0.106	0.797	0.399	0.108
CK	R				0.300	0.014	0.142	0.001
	P value				0.008	0.901	0.217	0.992
LDH	R					-0.082	0.004	0.070
	P value					0.477	0.975	0.548
GABA	R						0.176	-0.245
	P value						0.127	0.032
CASP7	R							0.198
	P value							0.084

Note: *Spearman's correlation coefficient.

4. Discussion

Autism is a complex developmental disability. Hence much attention has been given to it due to confusion created by faint and vague sources. Studies being conducted today are directing more and more towards a big tent which includes genetic, environmental, and neurological factors that underlie or contribute to the development of autism [22], [23]. However, it has not been ascertained why autism results to its development in the first place. ASD is determined by the symptomatology that can be mild or severe and which involves speech, social and behavioral profiles [24] . Geschwind (2021) notes that this condition is highly heterogeneous in that the presentation of phenotypes is diverse and therefore

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6: 3808-3815, 2024
DOI: 10.55214/25768484.v8i6.2824

makes it very difficult to come up with a definite cause, or molecular pathway, associated with the illness. Science is on the move to provide a distinction between the chemical, physical, and hereditary processes that define ASD [25], [26]. Thus, autism spectrum disorder is still a significant priority for scientific research. The move of appreciating the fact that there cannot be a single theory out to explain all the observed cases is the fundamental concept in this case [27]. Gamma-aminobutyric acid is the principal inhibitory neurotransmitter, although excitatory neurotransmitters are also crucial in the human brain; their balanced interplay is essential for neuronal function. Prior studies indicate that GABA levels may be modified in individuals with autism [28]. Approximately 80 percent of neurons in the cerebral cortex, which envelops the brain's surface, convey excitatory impulses predominantly through the release of the neurotransmitter glutamate. The remaining 20 percent, referred to as interneurons, are inhibitory [29] . They function through gamma-aminobutyric acid. The appropriate equilibrium of these two signal types allows neuronal activity in certain situations while suppressing it in others [30]. In the context of ASD, for example, there is a neurological dysfunction of the excitatory/inhibitory balance. One of the reasons for many of the neurological signs observed in the course of the disease is excessively high levels of excitatory signaling [31]. As for GABA, the authors explain that the levels of GABA, which are alleged to be increased in specific autistic children, might indicate the brain's attempt to restore balance by increasing inhibitory transmission in response to a vast amount of excitatory input [32]. The literature on neurotransmitters shows that the two major neurotransmitter systems affected in the autistic brain are the glutamatergic and GABAergic systems. The explanation given is that the GABAergic neurotransmitters overcompensate for the dysfunction of glutamatergic neurotransmitters [33]. Apoptosis is a crucial process in tissue homeostasis which is otherwise popularly referred to as programmed cell death [34]. There was a high caspase-7 level in the children with autism than in normal children, this could probably mean that the turnover or apoptosis is happening faster in autistic children. This may indies to inflammation, immunization, or neurological stress which are parameters associated with ASD. It has been proved that caspase-7 is involved in immune mechanisms that might be dysregulated in children with autism [35]. This is the case given the fact that there is a dearth of recent papers that focus the role of caspase-7 in autistic states. According to the findings of the present study, the results revealed that GABA concentration were higher in both boys and girls with ASD than in children without this disorder in the control group, this agreed with [36]. Batie *et al.*, rightly mentioned, that the biochemical pathways appear to be imbalanced in both boys and girls diagnosed with ASD. This is so yet the behavioral and cognitive symptoms of ASD present in different ways in males' children and in girls. This conclusion is supported by other related research that found out that metabolic or neurochemical differences that exist between the sexes do not result from autism. In other words, it demonstrated that the fundamental biochemical properties of autism are forcing dynamics that are beyond sex-specific biological differences. Previous studies indicate that serotonin is regarded as the most significant neurotransmitter. Identified in the initial trimester of human central nervous system development, any disruption, including temporary deviations, of the 5-HT system can result in lasting changes in brain function and behavior [37]. In the present study, serotonin levels showed no significant differences between the groups. The serotonin system's abnormalities may be connected to difficulties in emotional regulation and social interaction, but it does not exhibit the same level of dysregulation as GABA in the context of autism. This difference may be due to genetic factors, prenatal influences, or neuroinflammatory processes that affect GABA more prominently in autistic brains. GABA had significant negative correlations with serotonin. GABA and serotonin often balance each other in terms of neural excitation and inhibition [38]. High GABA levels might correlate with lower serotonin activity in certain regions of the brain. Area under the curve from the ROC curve provided a numerical value that summarizes the overall diagnostic ability of the test or biomarker. A high AUC would suggest that the diagnostic test or biomarker used in the study is effective and could be applied clinically or in further research [39]. The present study was identified the efficiencies of GABA (89.7), serotonin (71.7), caspase-7 (94.1). Al hence, these biomarkers protentional help in diagnosis of ASD and identify kids with the condition at the earliest stage of their complaint [40].

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6: 3808-3815, 2024
DOI: 10.55214/25768484.v8i6.2824

5. Conclusions

There is a significant increase in the vital biomarker; GABA and decrease caspase 7; while, no significant statistical difference in serotonin level. The study successfully identified essential biochemical markers (GABA and caspase 7), that could be used for diagnosing and early detection of autism spectrum disorders.

Ethical approval: The current research was approved by the ethical consideration committee of the Training and Human Development Unit, Basrah Health Department, Ministry of Health/ Environment, Iraq.
Authors contribution: Samah Fadhil Hadi contributed to data collection, writing, and analysis. Shrouk A. Hassan Al. Ibraheem and Hamid Jaddoa Abbas contributed to the manuscript concept, results, analysis, manuscript submission, revision, and galley proof.
Acknowledgment: The authors would like to thank all the specialized autism centers; Usraty Center, Tooba Center and Al-Faihaa Teaching Hospital Center, Basra province, Ira.

References

[1] T. Hirota and B. H. King, "Autism spectrum disorder: a review," *Jama*, vol. 329, no. 2, pp. 157–168, 2023.
[2] A. Genovese and M. G. Butler, "Clinical assessment, genetics, and treatment approaches in autism spectrum disorder (ASD)," *Int. J. Mol. Sci.*, vol. 21, no. 13, p. 4726, 2020.
[3] M. Thorsen, "Oxidative stress, metabolic and mitochondrial abnormalities associated with autism spectrum disorder," *Prog. Mol. Biol. Transl. Sci.*, vol. 173, pp. 331–354, 2020.
[4] A. Kardani, A. Soltani, R. D. E. Sewell, M. Shahrani, and M. Rafieian-Kopaei, "Neurotransmitter, antioxidant and anti-neuroinflammatory mechanistic potentials of herbal medicines in ameliorating autism spectrum disorder," *Curr. Pharm. Des.*, vol. 25, no. 41, pp. 4421–4429, 2019.
[5] D. Rashmi, R. Zanan, S. John, K. Khandagale, and A. Nadaf, "γ-aminobutyric acid (GABA): Biosynthesis, role, commercial production, and applications," *Stud. Nat. Prod. Chem.*, vol. 57, pp. 413–452, 2018.
[6] S. Behuet, J. N. Cremer, M. Cremer, N. Palomero-Gallagher, K. Zilles, and K. Amunts, "Developmental changes of glutamate and GABA receptor densities in Wistar rats," *Front. Neuroanat.*, vol. 13, p. 100, 2019.
[7] R. Marotta *et al.*, "The neurochemistry of autism," *Brain Sci.*, vol. 10, no. 3, p. 163, 2020.
[8] T. Prévot and E. Sibille, "Altered GABA-mediated information processing and cognitive dysfunctions in depression and other brain disorders," *Mol. Psychiatry*, vol. 26, no. 1, pp. 151–167, 2021.
[9] G. L. Sarlo and K. F. Holton, "Brain concentrations of glutamate and GABA in human epilepsy: A review," *Seizure*, vol. 91, pp. 213–227, 2021.
[10] H. Zhao *et al.*, "GABAergic system dysfunction in autism spectrum disorders," *Front. cell Dev. Biol.*, vol. 9, p. 781327, 2022.
[11] H. A. Abdulamir, O. F. Abdul-Rasheed, and E. A. Abdulghani, "Serotonin and serotonin transporter levels in autistic children," *Saudi Med. J.*, vol. 39, no. 5, p. 487, 2018.
[12] E. Daly, M. D. Tricklebank, and R. Wichers, "Neurodevelopmental roles and the serotonin hypothesis of autism spectrum disorder," in *The Serotonin System*, Elsevier, 2019, pp. 23–44.
[13] N. K. Popova, A. S. Tsybko, and V. S. Naumenko, "The implication of 5-HT receptor family members in aggression, depression and suicide: similarity and difference," *Int. J. Mol. Sci.*, vol. 23, no. 15, p. 8814, 2022.
[14] L. Maroteaux and F. Kilic, "Frontiers of serotonin beyond the brain," *Pharmacol. Res.*, vol. 140, pp. 1–6, 2018.
[15] P. Wójcik, M. K. Jastrzębski, A. Zięba, D. Matosiuk, and A. A. Kaczor, "Caspases in Alzheimer's disease: mechanism of activation, role, and potential treatment," *Mol. Neurobiol.*, vol. 61, no. 7, pp. 4834–4853, 2024.
[16] X. Li *et al.*, "Apoptotic caspase-7 activation inhibits non-canonical pyroptosis by GSDMB cleavage," *Cell Death Differ.*, vol. 30, no. 9, pp. 2120–2134, 2023.
[17] D. Dong, H. R. Zielke, D. Yeh, and P. Yang, "Cellular stress and apoptosis contribute to the pathogenesis of autism spectrum disorder," *Autism Res.*, vol. 11, no. 7, pp. 1076–1090, 2018.
[18] J. Upadhyay, J. Patra, N. Tiwari, N. Salankar, M. N. Ansari, and W. Ahmad, "Dysregulation of multiple signaling neurodevelopmental pathways during embryogenesis: a possible cause of autism spectrum disorder," *Cells*, vol. 10, no. 4, p. 958, 2021.
[19] D. Siniscalco, S. Schultz, A. L. Brigida, and N. Antonucci, "Inflammation and neuro-immune dysregulations in autism spectrum disorders," *Pharmaceuticals*, vol. 11, no. 2, p. 56, 2018.
[20] A. Mukherjee and D. W. Williams, "More alive than dead: non-apoptotic roles for caspases in neuronal development,

plasticity and disease," *Cell Death Differ.*, vol. 24, no. 8, pp. 1411–1421, 2017.
[21] F. J. Castora, "Mitochondrial function and abnormalities implicated in the pathogenesis of ASD," *Prog. Neuro-Psychopharmacology Biol. Psychiatry*, vol. 92, pp. 83–108, 2019.
[22] I. Hertz-Picciotto, R. J. Schmidt, and P. Krakowiak, "Understanding environmental contributions to autism: Causal concepts and the state of science," *Autism Res.*, vol. 11, no. 4, pp. 554–586, 2018.
[23] M. Lai *et al.*, "A machine learning approach for retinal images analysis as an objective screening method for children with autism spectrum disorder," *EClinicalMedicine*, vol. 28, 2020.
[24] E. B. Clarke, J. B. McCauley, A. Lutz, M. Gotelli, S. J. Sheinkopf, and C. Lord, "Understanding profound autism: Implications for stigma and supports," *Front. Psychiatry*, vol. 15, p. 1287096, 2024.
[25] T. Barlattani *et al.*, "Autism spectrum disorders and psychiatric comorbidities: a narrative review," *J. Psychopathol.*, 2023.
[26] C. D. de la Bâtie *et al.*, "Autism spectrum disorders in propionic acidemia patients," *J. Inherit. Metab. Dis.*, vol. 41, pp. 623–629, 2018.
[27] R. Chapman, "The reality of autism: On the metaphysics of disorder and diversity," *Philos. Psychol.*, vol. 33, no. 6, pp. 799–819, 2020.
[28] T. Kolodny, M. Schallmo, J. Gerdts, R. A. E. Edden, R. A. Bernier, and S. O. Murray, "Concentrations of cortical GABA and glutamate in young adults with autism spectrum disorder," *Autism Res.*, vol. 13, no. 7, pp. 1111–1129, 2020.
[29] M. P. Mattson, *Sculptor and Destroyer: Tales of Glutamatethe Brain's Most Important Neurotransmitter*. MIT Press, 2023.
[30] L. Trobiani *et al.*, "The neuroligins and the synaptic pathway in Autism Spectrum Disorder," *Neurosci. Biobehav. Rev.*, vol. 119, pp. 37–51, 2020.
[31] J. Olloquequi *et al.*, "Excitotoxicity in the pathogenesis of neurological and psychiatric disorders: Therapeutic implications," *J. Psychopharmacol.*, vol. 32, no. 3, pp. 265–275, 2018.
[32] S. Maier *et al.*, "Increased prefrontal GABA concentrations in adults with autism spectrum disorders," *Autism Res.*, vol. 15, no. 7, pp. 1222–1236, 2022.
[33] L. Rylaarsdam and A. Guemez-Gamboa, "Genetic causes and modifiers of autism spectrum disorder," *Front. Cell. Neurosci.*, vol. 13, p. 385, 2019.
[34] S. Kari, K. Subramanian, I. A. Altomonte, A. Murugesan, O. Yli-Harja, and M. Kandhavelu, "Programmed cell death detection methods: a systematic review and a categorical comparison," *Apoptosis*, vol. 27, no. 7, pp. 482–508, 2022.
[35] A. M. Khemakhem, R. E. Frye, A. El-Ansary, L. Al-Ayadhi, and A. Ben Bacha, "Novel biomarkers of metabolic dysfunction is autism spectrum disorder: potential for biological diagnostic markers," *Metab. Brain Dis.*, vol. 32, pp. 1983–1997, 2017.
[36] H. Al-Otaish, L. Al-Ayadhi, G. Bjørklund, S. Chirumbolo, M. A. Urbina, and A. El-Ansary, "Relationship between absolute and relative ratios of glutamate, glutamine and GABA and severity of autism spectrum disorder," *Metab. Brain Dis.*, vol. 33, pp. 843–854, 2018.
[37] P. Lin *et al.*, "A comparison between children and adolescents with autism spectrum disorders and healthy controls in biomedical factors, trace elements, and microbiota biomarkers: a meta-analysis," *Front. Psychiatry*, vol. 14, p. 1318637, 2024.
[38] Y.-Y. Yin *et al.*, "The role of the excitation: inhibition functional balance in the mPFC in the onset of antidepressants," *Neuropharmacology*, vol. 191, p. 108573, 2021.
[39] I. Unal, "Defining an optimal cut-point value in ROC analysis: an alternative approach," *Comput. Math. Methods Med.*, vol. 2017, no. 1, p. 3762651, 2017.
[40] A. El-Ansary, L. Al-Ayadhi, M. Al-Hakbany, V. Polyakova, and S. Suchkov, "The relative usefulness of the identification and analysis of biomarkers for the diagnosis of autism spectrum disorders in early childhood and the implementation of personalized precision medicine," *Int. J. Autism Challenges Solut.*, vol. 1, no. 1, pp. 51–71, 2024.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 6: 3808-3815, 2024
DOI: 10.55214/25768484.v8i6.2824