Medical Science Journal for Advance Research

Vol. 7, No. 2, June 2026
e-ISSN: 2774-4892

Evaluation of Mitochondrial Dysfunction, Oxidative Stress and Electron Transporters as Biomarkers for The Diagnosis of Autism Spectrum Disorder

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ABSTRACT

Background: *Autism spectrum disorder (ASD) is a neuro- developmental disorder commonly seen in children. It is characterized by age inappropriate, impaired social communication and the presence of stereotypic behavior. This disorder is hypothesized to result from cerebral dysfunction arising from a complex interaction between genetic, epigenetic and environmental factors. Biomarkers such as glutathione (GSH), glutathione S transferase (GST), GST: GSH ratio and Co Q10, showing potential for early diagnosis and management of ASD.* ***Objective:*** *to evaluate the effectiveness of GSH, GST, GST:GSH ratio and Co Q10 as novel biomarkers to predict autism at an early stage.* ***Methods:*** *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 GSH, GST, GST:GSH ratio and Co Q10 by using enzyme linked immune sorbent assay (ELISA) technique. The statistical package for social sciences (SPSS- 26) was used for data analyses.* ***Results:*** *The study revealed significantly elevated GSH, GST, GST:GSH ratio and Co Q10 levels between the autistic children's patients and the control group.* ***Conclusions:*** *This study provides a pioneering discovery of the biochemical markers GSH, GST, GST:GSH ratio and Co Q10 in autism, as mitochondrial dysfunction, oxidative stress and electron transporters are the most important chemical causes of autism.*

Keywords: Autism Spectrum Disorder, Glutathione, Glutathione S Transferase, GST:GSH Ratio, Co Enzyme Q10.

Article Information

Received: April 24, 2026; Revised: May 27, 2026; Online: June 2026

INTRODUCTION

Autism is defined as a neurodevelopmental disorder characterized by impaired communication and social skills along with restricted interests and repetitive behaviors [1]. Clinical symptoms are usually manifested by 3 years old; however, deficits in communication, social responsiveness, and play may be present as early as 6–12 months of age [2].The etiology of ASD likely involves environmental factors triggering physiological abnormalities in genetically sensitive individuals. Despite this fact, the exact cause still is unknown, ASD is a multifactorial disease [3]. One of these major physiological abnormalities is mitochondrial dysfunction, that may affect a significant subset of children with ASD [4]. Mitochondrial dysfunction can

https://doi.org/10.46966/msjar.v7i2402

be classified as either primary or secondary [5]. Primary mitochondrial dysfunction generally caused by a defect in a gene directly involved in the function of mitochondrial systems responsible for producing ATP [6]. Secondary mitochondrial dysfunction refers to other metabolic or genetic abnormalities that impair the ability of mitochondria to produce ATP [7]. For example, metabolites produced by toxic substances (such as environmental toxicants) or by the dysfunction of other metabolic systems that are not specifically involved in producing ATP (such as increased oxidative stress because of dysfunctional antioxidant pathways such as glutathione and glutathione S transferase) [8].

Several recent studies had shown that the oxidative stress may play a key role in supporting the pathogenesis and the severity of ASD [9]. Oxidative stress exists when there is an imbalance between oxidative and antioxidative substances, producing reactive oxygen species (ROS). Glutathione and Glutathione S Transferase, the major endogenous antioxidant, are the body's primary defense against damage from ROS. ROS are produced through natural aerobic metabolism, and removed by glutathione and glutathione S transferase [10]. Within the cells, mitochondria are the major source of ROS, most of them originating at the electron transport chain (ETC) [11]. During mitochondrial respiration some leakiness, or partial reduction reactions occur, mainly from complexes I and III even under physiologic conditions. This leakiness causes the release of superoxide and hydrogen peroxide mostly to the mitochondrial matrix [12]. Therefore, mitochondria need constant protection from the toxic action of ROS. Low molecular weight antioxidants, such as GSH and GST defense systems, are responsible for providing this protection [13]. The role of GSH and GST in ASD is emerging as a major topic, due to its role in the maintenance of the intracellular redox balance. Several studies have implicated glutathione redox imbalance as a leading factor in ASD[14]. Glutathione metabolism, through its impact on redox environment or redox- independent mechanisms, interferes with multiple mechanisms involved in ASD pathogenesis [15].

Coenzyme Q10 (CoQ_{10}) also known as ubiquinone, is a naturally occurring coenzyme and an antioxidant produced by the human body [16]. CoQ_{10} plays a role in mitochondrial oxidative phosphorylation [17] , aiding in the production of (ATP) [18], which is involved in energy transfer within cells, and is a life- sustaining cofactor for the three complexes (complex I, complex II, and complex III) of the ETC in the mitochondria [19]. CoQ_{10} has a role in the transport
of protons across lysosomal membranes to regulate pH in lysosome functions [20]. Recent research on CoQ10 in relation to ASD has shown promising results, particularly in addressing mitochondrial dysfunction and oxidative stress, both of which are commonly observed in individuals with ASD[21].

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. Patients who suffer from other 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 less than 0.05 which the set of statistical significance.

RESULTS

Table 1, was shown that patients with ASD had significant (P value < 0.05) high levels of GSH and GSTS, GST:GSH ratio and Co Q10.

Table 1: Baselines 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
GSH (ng/ml)		12.650± 4.907	1.318± 0.646	0.0001
GSTS (ng/ml)		14.894± 9.107	0.894± 0.483	0.0001
GST:GSH ratio		1.344± 1.291	0.806± 0.592	0.004
CoQ10 (ng/ml)		11.773± 20.583	0.963± 0.460	0.0001

As was shown in Table 2, the results revealed no significant statistical differences between males' group and females' group for all of the parameters (P values > 0.05).

Table 2: Comparison of biochemical parameters in patients according to sex.

Variables	ASD -Males (n=61) 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
GSH (ng/ml)	12.450±5.214	13.414±3.535	0.488
GSTS (ng/ml)	15.282±10.060	13.417±3.534	0.470
GST:GSH ratio	1.434±1.439	1.000±0.001	0.234
CoQ10(ng/ml)	13.289±22.533	5.993±8.397	0.209

Table 3 showed A receiver-operating characteristic (ROC) curve plotting and corresponding area under the curve (AUC) calculations of accuracy parameters of each of the variables revealed that were GSH, GST, GST: GSH ratio and Co Q10 significant identifies of ASD.

Table 3 A receiver-operating characteristic (ROC) curve and area under the curve (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
GSH (ng/ml)	1.000	0.0001	4.860	100	100	100
GSTS (ng/ml)	1.000	0.0001	4.995	100	100	100
GST:GSH ratio	0.692	0.055	0.995	92.2	66.4	79.3
CoQ10(ng/ml)	0.971	0.0001	1.505	90.9	88.5	89.7

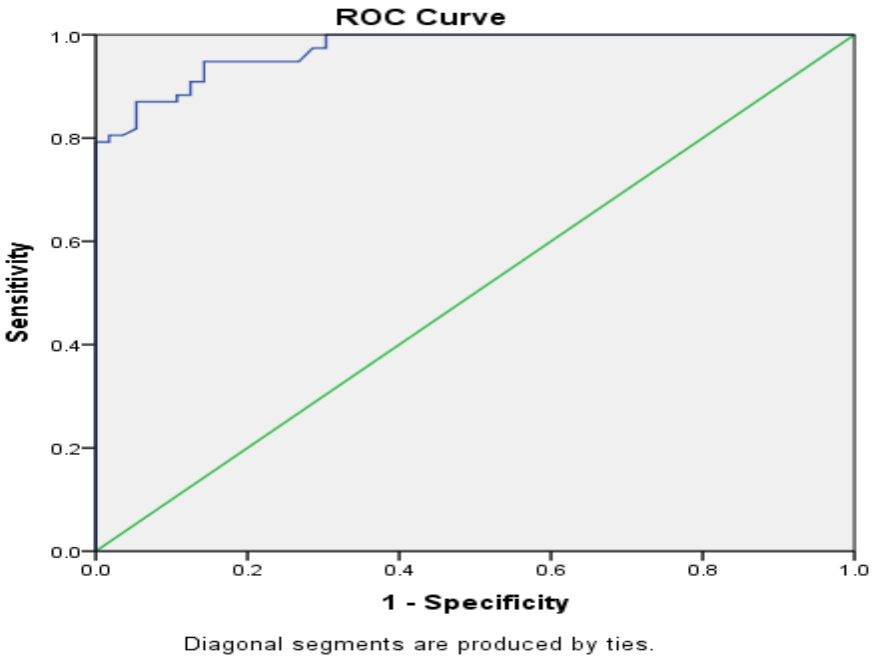

Fig. 1 Receiver operational curves for Co Q10

Table 4 was shown the Correlations among the variables for ASD patients.

Table 4 Correlations among the parameters for patients.

Variables		RBS	Urea	CK	LDH	CoQ10	GSH	GSTSH	Ratio
Hb	R*	0.042	0.035	0.414	0.059	-0.103	-0.089	0.007	0.145
	P value	0.715	0.765	0.001	0.612	0.374	0.441	0.949	0.207
RBS	R		0.217	-0.029	0.050	-0.101	0.016	-0.017	-0.072
	P value		0.058	0.799	0.667	0.384	0.891	0.882	0.535
Urea	R			-0.100	-0.186	-0.058	-0.161	0.001	0.109
	P value			0.388	0.106	0.614	0.163	0.991	0.344

Variables		RBS	Urea	CK	LDH	CoQ10	GSH	GSTSH	Ratio
CK	R				0.300	-0.038	-0.006	0.142	0.046
	P value				0.008	0.742	0.960	0.218	0.689
LDH	R					-0.068	0.044	-0.023	-0.093
	P value					0.559	0.705	0.842	0.421
CoQ10	R						0.024	0.266	0.204
	P value						0.839	0.019	0.074
GSH	R							0.543	-0.268
	P value							0.001	0.019
GSTSH	R								0.469
	P value								0.001

**Spearman's correlation coefficient.*

DISCUSSION

It has been stated that glutathione is one among important antioxidants for healing [22]. It is a vital factor that enables the elimination of toxicants and sustaining of the cellular redox balance [23]. Children with ASD had significant (P value < 0.05) high levels of GSH, GSTS, GST:GSH ratio and Co Q10. A previous study, showed that children with ASD had elevated total glutathione and glutathione S-transferase levels in their blood [24]. This finding indicated that there was higher requirement for deactivating the oxidative stress. Cell signaling, which has been considered as autisms etiopathogenic origin, is an unfavorable level of reactive oxygen species in relation to the ability to neutralize them. This condition leads to what is referred to as oxidative stress [25]. This shifts the link's balance and finally brings about oxidative stress, which can be attributed to the depletion of antioxidants such as glutathione [26].

Rogge and Janssen (2019), report that autistic children have low GSH and high GSSG, which are the body's antioxidant systems that are useful in counteracting oxidative stress resulting from mitochondrial disorders and neuro inflammation as an indication of ASD [27].

CoQ10 influenced synthesis of cellular energy on the presumption of its involvement in the electron transport chain in mitochondria [28]. It also acts as an antioxidant and unpacks the cells from the effects it gains from oxidative stress levels [29]. Higher levels of CoQ10 have been reported in ASD children as well as in other several illnesses such as coronary heart disease [30]. This may be a compensatory response to mitochondrial disease or oxidative stress; both of which are known to be present in this group [31]. In particular mitochondrial dysfunction is believed to play a role in ASD because it decreases energy generation and cellular viability while increasing oxidative stress thus overall cell pathology [32]. Therefore, to override extra harm and to sustain the functionality of the mitochondria CoQ10 synthesis might augment due to increased oxidative stress in the body.

The multifaceted reaction of the body to various physiological loads can be addressed

to increased level of CoQ10 glutathione and glutathione S-transferase in children with autism. This type includes the mature stress which includes; neurological changes, oxidative stress, mitochondrial dysfunction and the demand of a higher detoxification mechanism. Maybe these biomarkers are not pathophysiological but are probably physiological processes that should preserving the body from the common issue in autism spectrum disorder [33]. An example of this will be an increase in Co Q10 and glutathione indicating are trying to combat for futility, oxidative stress and mitochondrial dysfunction.

This homeostasis attempt to counteract the adverse impact of mitochondrial dysfunction on the generation of free radicals and antioxidants is clearly seen by the increase in CoQ10 [34], GSH: GST in both male's and female's autistic children by fold. In accordance with the results presented by Filipek *et al*., the content of these antioxidants is higher in both sexes and confirm that mitochondrial dysfunction is one of the turning points in the development of ASD, which equally affects boys and girls. Studies supporting this finding are almost yet non-existent.These biomarkers participate in such processes associated with ASD as energy metabolism and oxidative stress [35], thus providing useful insights into the ASD etiology.

CoQ10, had significant positive correlation with GSH. Since, CoQ10 is involved in reducing oxidative stress, its high levels might correlate positively with GSH and GST [36]. This could reflect a strong antioxidant defense in ASD patients.

Area under the curve from the ROC curve provided a numerical value that summarizes the overall diagnostic ability of the test or biomarker [37]. 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 [38], there are high efficiencies of CoQ10, practically GSH (100) and GSTS (100) in diagnosis of ASD and identify children with the condition at the earliest stage of their complaint.

CONCLUSIONS

There is a significant increase in the vital biomarker; GSH, GST, GST:GSH ratio and Co Q10. The study successfully identified essential biochemical markers (GSH and GSTS) that could be used for diagnosing and early detection of autism spectrum disorders.

Acknowledgements

for all participants in this study.

Conflict of Interest

There are no conflicts of interest disclosed by any of the authors.

Ethical Consideration

The author obtained consent from each person participating in this study in accordance with international ethical research standards.

Source of Support and Finding

Self-financed.

Authorship

Hamid Jaddoa Abbas, worked together on the manuscript's conception, composition, data analysis, assessment, submission, revisions, and final proofreading. Data collection, writing, and analysis were carried out by Noor Ahmed Hameed. Shrouk Abdulrazak Hassan, was in charge of coming up with the concept and gathering the data.

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