

A Prospective Study of Oxidative Stress Biomarkers in Autistic Disorders

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Abstract

The aim of this study was to evaluate oxidative stress (OS) biomarkers in a prospective, blinded cohort study of participants diagnosed with autism spectrum disorders (ASDs). OS biomarkers, including: blood glutathione (GSH), urine lipid peroxide, blood superoxidase dismutase (SOD), and blood GSH peroxidase (GPx) among participants diagnosed with ASDs ($n=28$) were evaluated in comparison to laboratory provided reference ranges. Testing was conducted using Genova Diagnostics (CLIA-approved). Participants diagnosed with ASDs had significantly ($p<.005$) decreased blood GSH and GPx relative to laboratory reference ranges. By contrast, participants diagnosed with ASDs had significantly ($p<.000$) increased urine lipid peroxide levels relative to laboratory reference ranges. A bimodal distribution of significant differences from the laboratory reference for blood SOD levels were observed (high=10.7%, low=14.3%). Finally, a significant ($p=.05$) inverse correlation was observed between blood GSH levels and ASD severity using Childhood Autism Rating Scale scores. The present observations are compatible with increased OS and a decreased detoxification capacity, particularly of mercury, in patients diagnosed with ASDs. Patients diagnosed with ASDs should be routinely tested to evaluate OS biomarkers and potential treatment protocols should be evaluated to potentially correct the OS abnormalities observed.

Keywords: *Heavy metal; Metabolic; Endophenotype; Sulfation; Sulfur*

Introduction

Autism spectrum disorders (ASDs) are prevalent neurodevelopmental disorders that affect an estimated 1 in 150 children in the US (Austin, 2008). ASDs are characterized by severe impairments in socialization, communication and behavior. Children diagnosed with an ASD may display a range of problem behaviors such as hyperactivity, poor attention, impulsivity, aggression, self-injury and tantrums. In addition, these children often display unusual responses to sensory stimuli such as hypersensitivities to light or certain sounds, colours, smells or touch and have a high threshold for pain

(Austin, 2008). Finally, common co-morbidity conditions often associated with ASDs include gastrointestinal disease and dysbiosis (White, 2003), autoimmune disease (Sweeten, Bowyer, Posey, Halberstadt, & McDougle, 2003), and mental retardation (Bolte & Poustak, 2002).

In attempting to understand the underlying pathogenesis of ASDs a considerable body of research has been conducted to evaluate potential candidate causal genes. Genetic studies, to date, have not uncovered genes of strong effect. It was postulated that increasing rates of ASDs and less than 100% monozygotic concordance of ASDs support a more inclusive reframing of ASDs as a multi-system disorder with genetic influence and environmental contributors (Herbert et al., 2006). Investigators suggested that ASDs may result from an interaction between genetic, environmental, and immunological factors, with oxidative stress as a mechanism linking these risk factors (James et al., 2006).

Given the well-established fact that mercury (Hg) is known to significantly increase oxidative stress and that fetuses and infants are routinely exposed to Hg from environmental sources (i.e. fish, dental amalgams, vaccines, etc.), investigators have described that many ASDs may result from a combination of genetic/biochemical susceptibility, specifically a reduced ability to excrete Hg, and exposure to Hg at critical developmental periods (Geier, King, Sykes, & Geier, 2008). Further, it was reported that Hg can cause immune, sensory, neurological, motor, and behavioural dysfunctions similar to traits defining/associated with ASDs, and that these similarities extend to neuroanatomy, neurotransmitters, and biochemistry. Also, it was reported when reviewing the molecular mechanisms of Hg intoxication that it can induce death, disorganization and/or damage to selected neurons in the brain similar to that seen in recent ASD brain pathology studies, and this alteration may likely produce the symptoms by which ASDs are diagnosed (Geier et al., 2008).

Under normal conditions, a dynamic equilibrium exists between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cell (Granot & Kohen, 2004; Stohs, 1995). ROS includes superoxide, hydroxyl, peroxy, alkoxy, and nitric oxide (NO) free radicals (Stohs, 1995). Superoxide is the first reduction product of molecular oxygen, and it is an important source of hydroperoxides and deleterious free radicals (Fridovich, 1986). Hydrogen peroxide (H_2O_2) reacts with reduced transition metals such as iron, via the Fenton reaction, to produce the highly reactive hydroxyl radical (McCord & Day, 1978). Most toxic effects are due to hydroxyl radical formation, which also initiates lipid peroxidation (McCord & Day, 1978). Some endogenous enzymes such as xanthine oxidase (XO), NO synthase, and monoamine oxidase (MAO) can directly produce ROS (Granot & Kohen, 2004; Kellogg & Fridovich, 1975; Stohs, 1995). Normally, the ROS within the cells are neutralized by antioxidant defense mechanisms. Superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) are the primary enzymes involved in direct elimination of ROS, whereas glutathione reductase and glucose-6-phosphate dehydrogenase are secondary antioxidant enzymes, which help in maintaining a steady concentration of glutathione (GSH) and NADPH necessary for optimal functioning of the primary antioxidant enzymes (Chance, 1954; Gutteridge, 1977; Maddipati & Marnett, 1987; Vendemiale, Grattagliano, & Altomare, 1999). These enzymes require micronutrients as cofactors such as selenium, iron, copper, zinc, and manganese for optimal catalytic activity and effective antioxidative defense mechanism (Halliwell & Gutteridge, 1992). Additionally, GSH, iron-binding transferrin, copper-binding ceruloplasmin, tocopherol (Vitamin E), carotenoids, and ascorbic acid (Vitamin C) are also involved in the anti-ROS defense system (Erden-Inal, Sunal, & Kanbak, 2002; Gutteridge, Richmond, Halliwell, 1980; Loeffler et al., 1995). GSH is the most important antioxidant for detoxification and is important for the elimination of environmental toxins. Oxidative stress occurs when ROS levels exceed the antioxidant capacity of a cell. These ROS are highly toxic and react with lipids, proteins and nucleic acids, and lead to cell death via apoptosis or necrosis (Kannan & Jain, 2000).

The brain is highly vulnerable to oxidative stress due to its limited antioxidant capacity, higher energy requirement, and higher amounts of lipids and iron (Juurlink & Paterson, 1998). The brain makes up about 2% of body mass but consumes 20% of metabolic oxygen. The vast majority of energy is used by the neurons (Shulman, Rothman, Behar, & Hyder, 2004). Due to the lack of GSH-producing capacity by neurons, the brain has a limited capacity to detoxify ROS. Therefore, neurons are the first cells to be affected by the increase in ROS and shortage of antioxidants and, as a result, are most susceptible to oxidative stress.

Antioxidants are required for neuronal survival during the early critical period (Perry, Norman, Litzburg, & Gelbard, 2004). Children are more vulnerable than adults to oxidative stress because of their naturally low GSH levels from conception through infancy (Erden-Inal, Sunal & Kanbak, 2002; Ono, Sakamoto, & Sakura, 2001). The risk created by this natural deficit in detoxification capacity in infants is increased by the fact that some environmental factors that induce oxidative stress are found at higher concentrations in developing infants than in their mothers, and accumulate in the placenta. Taken together, these studies suggest that the brain is highly vulnerable to oxidative stress, particularly during the early part of development that may result in neurodevelopmental disorders such as ASDs.

The purpose of the present study was to provide greater mechanistic insight into ASD associated disease pathology by evaluating biomarkers of oxidative stress in a cohort of participants diagnosed with ASDs. The overall hypothesis of the present study was that there would be increased clinically identifiable biomarkers of oxidative stress and susceptibility to oxidative stress in a cohort of participants diagnosed with ASDs. Further, it was hypothesized that susceptibility factors associated with increased oxidative stress would significantly correlate with the clinical severity of the participants diagnosed with ASDs examined.

Method

The study was conducted at the Autism Treatment Center (ATC; Dallas, Texas). Phlebotomy took place at Medical Center Plano, Outpatient Phlebotomy (Plano, Texas).

The study protocol received Institutional Review Board (IRB) approval from Liberty IRB, Inc. (Deland, Florida). All parents signed a consent and Health Insurance Portability and Accountability Act (HIPAA) form and all received a copy. Children were in the presence of one or both parents at all times during the study.

Participants

The present study looked at qualifying participants ($n = 28$) who were prospectively recruited from the community of Dallas/Fort Worth, Texas area. All of the children had a diagnosis of autism or pervasive developmental disorder (PDD). Children included in the present study were between 2 – 16 years of age and had an initial Childhood Autism Rating Scale (CARS) score ≥ 30 . A child with a CARS score ≥ 30 is considered to have autism (Schopler, Reichler, DeVellis & Daly, 1980). This study excluded children who had a history of Fragile X disorder, tuberous sclerosis, phenylketonuria (PKU), Lesch-Nyhan syndrome, fetal alcohol syndrome, or history of maternal illicit drug

use. In addition, this study excluded any children who had a history of chelation therapy.

Clinical Evaluation

As a baseline, the researchers obtained information regarding demographics, formal diagnosis, age at diagnosis, age of apparent onset, information regarding delay or regression, any current medical issues, medications, and allergies on each child. A baseline CARS evaluation was performed by Dr. Kern, who was trained in the use of CARS, and has 12 years experience in using the CARS to evaluate more than 300 persons with an ASD diagnosis. Dr. Kern interviewed the parents and observed each child. Table 1 summarizes the pertinent demographics of the participants included in the present study.

Table 1: A summary of the participants with ASD included in the present study

Descriptive Information	
Sex / Age	
Male / Female (ratio)	23 / 5 (4.6 : 1)
Mean Age in Years \pm Std (range)	5.8 \pm 2.6 (2 – 13)
Race (n)	
Caucasian	60.7% (17)
Hispanic	10.7% (3)
Black	10.7% (3)
Asian	10.7% (3)
Mixed	7.1% (2)
Autistic Disorder Characteristics	
Mean CARS Score \pm Std (range)	38.9 \pm 6.4 (30 – 51)
Regressive (n) ¹	60.7% (17)
Non-Regressive (n)	39.3% (11)
Autism (n)	67.9% (19)
Autism Spectrum Disorders (n) ²	32.1% (9)
Previous Treatments	
Supplements (n)	32.1% (9)
Chelation (n)	0% (0)
Supplements + Chelation (n)	0% (0)

Note: Std = standard deviation; All participants examined in the present study were living in the state of Texas:

¹ Includes participants that had a regressive event in development at any time following birth.

² Autism spectrum disorders include participants diagnosed with pervasive developmental disorder – not otherwise specified (PDD-NOS) and Asperger's disorder.

Lab Evaluation

Following the intake evaluation, each participant in the present study had blood samples collected. The laboratory specimens were all collected in the morning following an overnight fast. Specimens were immediately taken to and processed at LabCorp in Medical City Hospital (Dallas, Texas) and then shipped

to Genova Diagnostics (Asheville, North Carolina). The lab used in the present study was blinded and received no information regarding the clinical status of the participants examined or their CARS scores prior to their testing of each sample.

Participants were tested for the following at Genova Diagnostics (all CLIA-approved): blood GSH, blood SOD, blood GPx, and urine lipid peroxides.

Controls

In order to evaluate each of the oxidative stress biomarkers measured among the study participants diagnosed with ASDs examined in the present study, reference ranges for each test from Genova Diagnostics were utilized.

Statistical Analyses

In the present study, the statistical package contained in Microsoft Excel 2002 and StatsDirect (Version 2.7.2) were utilized. For each study participant, their biomarkers of oxidative stress were evaluated in relation to the mean level from the reference range for each test, so as to convert each patient's measured test values into a percent of the mean value ([patient's laboratory value / mean level from the reference range] \times 100 = percent of the pertinent mean). For each type of biomarker of oxidative stress examined, the individual results were then averaged to compute an overall average percent of the pertinent means, and the standard deviations for each attribute were calculated. Using the two-sample heteroscedastic t-test statistic, these "normalized" results from the study participants diagnosed with an ASD were then statistically compared to the corresponding data from the normal control populations that comprised the laboratory reference ranges. The null hypothesis was that there should be no difference in the normalized means between the study participants diagnosed with an ASD for each biomarker of oxidative stress examined and the corresponding means from the control populations (derived from laboratory reference ranges). Further, the blood GSH values obtained for each study participants diagnosed with an ASD were evaluated for their correlation with the severity of the disorder as derived from the CARS scoring conducted on each study participants using the unweighted least squares test statistic. The null hypothesis was that the slope of the line would be equal to zero. For all the statistical tests in the present study, a two-tailed p -value \leq .05 was considered statistically significant.

Results

Table 2 summarizes an assessment of biomarkers of oxidative stress among the study subjects with ASD in comparison to the laboratory reference ranges. Overall, the ASD group means for the biomarkers of oxidative stress did not fall outside the laboratory reference

Table 2. An assessment of oxidative stress biomarkers among the participants diagnosed with an ASD in comparison to laboratory reference ranges

Lab Test	Mean \pm Std (% of Pertinent Mean \pm SEM)	Laboratory Reference Range Limits	<i>P</i> -value ¹	% > Reference Range Upper Limit	% < Reference Range Lower Limit
Urine Lipid Peroxides ($\mu\text{mol} / \text{g}$ creatinine)	8.7 \pm 2.4 (174 \pm 9.1)	0 – 10	<.00	32.1 (9)	0 (0)
Blood Glutathione ($\mu\text{mol} / \text{L}$)	1,004 \pm 320 (82 \pm 4.9)	669 – 1,793	<.00	3.6 (1)	7.1 (2)
Blood Glutathione Peroxidase (U / g Hb)	22.5 \pm 5.2 (78 \pm 3.4)	20 – 38	<.00	0 (0)	35.7 (10)
Blood Superoxidase Dismutase (U / g Hb)	9,767 \pm 4,375 (89 \pm 7.6)	5,275 – 16,662	.20	10.7 (3)	14.3 (4)

Note: Std = standard deviation; SEM = standard error of the mean

¹ The unpaired *t*-test statistic was utilized.

ranges (even though a sizeable percentage of individual scores did so). It was observed that the study subjects with an ASD had significantly decreased levels of blood GSH and GPx. By contrast, the study subjects with an ASD had significantly increased levels of urine lipid peroxides. No overall significant differences were observed for the blood level of SOD among study subjects with an ASD and the laboratory reference ranges, but a bimodal distribution of significant differences from the laboratory reference for blood SOD levels were observed (high=10.7%, low=14.3%). The abnormalities observed were greatest for urine lipid peroxides (ASD mean was 174% of the control mean), followed by blood GPx and blood GSH (ASD means were 78% and 82% of the control means, respectively). Additionally, as show in Figure 1, a significant inverse correlation was observed between GSH levels and ASD severity.

Discussion

The overall results of the present study showed significant abnormalities in the biomarkers of oxidative stress in a cohort of study participants diagnosed with ASDs relative to laboratory provided reference ranges. Further, the results suggest that there was a significant inverse correlation between blood GSH levels and ASD severity measured using CARS scoring. The oxidative stress in autism may be caused by an imbalance between the generation of ROS by endogenous/exogenous pro-oxidants and the defense mechanism against ROS by antioxidants.

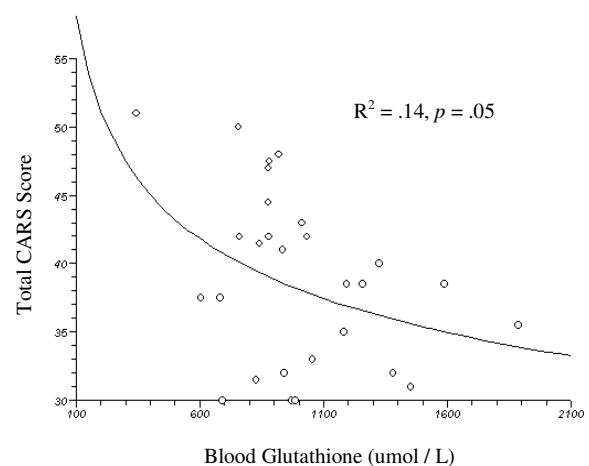


Figure 1. A summary of the correlation between blood glutathione and ASD severity

Note: The unweighted least squares statistic was used to evaluate the relationship between blood glutathione levels and ASD severity. ASD severity was measured blinded to the level of blood glutathione using Childhood Autism Rating Scale (CARS) scoring.

Investigators have previously reported that lipid peroxidation is increased in the plasma of children with autism as compared to their developmentally normal, non-autistic siblings (Chauhan, Chauhan, Brown, & Cohen, 2004). Lipid peroxidation is a chain reaction between polyunsaturated fatty acids and ROS, and it produces lipid peroxides and hydrocarbon polymers that are both highly toxic to the cell (Horton &

Fairhurst, 1987). Malonyldialdehyde (MDA) is an end product of peroxidation of polyunsaturated fatty acids and related esters, and is, therefore, used as a marker of lipid peroxidation (Jain, 1984). The plasma MDA contents measured by reaction with thiobarbituric acid (TBA) were higher in 13 of 15 (87%) of autistic subjects (Chauhan et al., 2004).

Recent reports also indicate increased levels of other lipid peroxidation markers in autism, thus confirming an increased oxidative stress in autism. For instance, Zoroglu et al. (2004) have reported increased TBA-reactive substances in erythrocytes of patients with autism as compared to normal controls. Ming et al. (2005) reported increased excretion of 8-isoprostane-F2alpha in the urine of children with autism. Isoprostanes are produced from the free radical oxidation of arachidonic acid through non-enzymatic oxidation of cell membrane lipids. Evans et al. (2008) evaluated the oxidative stress metabolites of carboxyethyl pyrrole (CEP) and iso[4]levuglandin (iso[4]LG) E2-protein adducts in cortical brain tissues in subjects diagnosed with autism. Significant immunoreactivity toward all these markers of oxidative damage in the white matter and often extending well into the grey matter of axons was found in every case of autism examined. These investigators reported that the striking thread-like pattern appears to be a hallmark of the autistic brain as it was not seen in any control brain, young or aged, used as controls for the oxidative assays. In another study, the density of lipofuscin, a matrix of oxidized lipid and cross-linked protein that forms as a result of oxidative injury in the tissues, was observed to be greater in cortical brain areas concerned with communication in subjects diagnosed with autism (Lopez-Hurtado & Prieto, 2008). Lipofuscin was previously demonstrated to be a depot for mercury in human brain autopsy specimens from mercury intoxicated patients (Opitz, Schweinsberg, Grossman, Wendt-Gallitelli, & Meyermann, 1996). Finally, and perhaps most importantly, Sajdel-Sulkowska, Lipinski, Windom, Audhya, and McGinnis (2008), evaluated cerebellar levels of the oxidative stress marker 3-nitrotyrosine (3-NT), mercury, and the antioxidant selenium levels between subjects diagnosed with autism in comparison to controls. It was observed that there were significant increases in the mean cerebellar levels of 3-NT and the ratio of mercury/selenium in the brains of subjects diagnosed with autism in comparison to controls. It was also observed that there was a significant positive correlation between cerebellar 3-NT and mercury levels.

Several studies have suggested alterations in the enzymes that play a vital role in the defense mechanism against damage by ROS in autism. For instance, compared to controls, patients with autism showed decreased activity of GPx in plasma and in erythrocytes (Yorbik, Sayal, Akay, Akbiyik, & Sohmen, 2002), reduced levels of total GSH and lower redox ratio of

reduced GSH to oxidized glutathione (GSSG) in plasma (Geier & Geier, 2006; Geier & Geier 2007; Geier et al., 2009a; James et al., 2006), and decreased catalase (Zoroglu et al., 2004) and SOD (Yorbik et al., 2002) activity in erythrocytes. In contrast, Sogut et al. (2003) reported unchanged plasma SOD activity and increased GPx activity in autism.

The significantly decreased blood GSH levels among the participants diagnosed with ASDs in the present study is of concern. GSH is a tripeptide of cysteine, glycine, and glutamate that is synthesized in every cell of the body. The essential intracellular reducing environment is maintained by the high ratio of reduced GSH to GSSG (Schafer & Buettner, 2001). The GSH redox equilibrium regulates a wide range of functions that include nitrogen and oxygen free radical scavenger (Dickinson et al., 2003), protein redox status and enzyme activity (Klatt & Lamas, 2000), cell membrane integrity and signal transduction (Dickson & Forman, 2002; Sagrista, Garcia, Africa De Madariaga, & Mora, 2002), transcription factor binding and gene expression (Deplancke & Gaskins, 2002), phase II detoxification (Pastore, Federici, Bertini, & Piemonte, 2003), and apoptosis (Hall, 1999).

Consistent with low total GSH levels and increased oxidative stress, autistic children would be expected to have difficulty resisting infection, resolving inflammation, and detoxifying environmental contaminants. Indeed, patients diagnosed with ASDs were reported to suffer from recurrent infections (Konstantareas & Homatidis, 1987), neuroinflammation (Zimmerman et al., 2005), gastrointestinal inflammation (Horvath & Perman, 2002; Jyonouchi, Geng, Ruby, & Zimmerman-Bier, 2005), and impaired antioxidant and detoxification capacity (Chauhan et al., 2004; Geier & Geier, 2006; Geier & Geier 2007; Geier et al., 2009a; James et al., 2006; Yorbik et al., 2002; Zoroglu et al., 2004).

Further, an important relationship between GSH availability and mercury excretion has been found (Ballatori & Clarkson, 1985). Bile is the main route of elimination for many metals, and the rate of secretion of methyl and inorganic mercury into bile was low in suckling rats but rapidly increased to adult rates soon after weaning. These changes closely paralleled similar developmental changes in the biliary secretion of reduced GSH. It was observed that when reduced GSH secretion into bile was completely inhibited, without changing hepatic levels of reduced GSH or mercury, mercury secretion was also completely blocked. These researchers concluded that their results indicated a close correspondence between the secretion of mercury and reduced GSH.

Because GSH is essential for effective detoxification, the effects of a lack of availability total GSH on detoxification are far-reaching. Exposure to toxins in children with compromised detoxification capability has an even greater potential to disrupt critical

developmental processes and result in developmental neurotoxicity (Rice & Barone, 2000).

Lack of availability of GSH may be only one part of the issue. Examination of the effects of heavy metals reveals that the presence of heavy metals, e.g., mercury, can disrupt the very processes needed to excrete the metals. Evidence shows that metal ions disrupt methionine synthetase which then, results in the inhibition of GSH production (Mutter, Naumann, Schneider, Walach, & Haley, 2005). In addition, the presence of metals causes oxidative stress, and since GSH has the dual function of both reducing of oxidative stress and detoxifying heavy metals, GSH may be become rapidly depleted as a result of demand. This situation may be further compounded in ASDs due to the significant reduction in GPx (which further helps to reduce oxidative stress).

Also, consistent with the results observed in the present study, Pasca et al. (in press) observed a significant inverse relationship between blood GSH levels and diagnosed autism severity. These investigators concluded that variations in GSH abnormalities across the autism spectrum suggests the possibility that it might be functionally significant in subjects with mercury intoxication. Further, another recent study extended the observation to observe that the severity of ASDs were significantly positively correlated with biomarkers of mercury intoxication, and subjects diagnosed with an ASD were observed to have a significant positive correlation between increasing biomarkers of mercury intoxication and increased plasma GSSG levels (Geier et al., 2009b). Taken collectively, the present results, and those observed in previous studies (Geier et al., 2009b; Pasca et al. in press), indicate that GSH levels play a particularly important functional role in determining the severity of an ASD diagnosis, when mercury plays a causal role.

Strengths and Limitations

The present study has number of potential strengths that help to support the observations made. First, the design of the present study, as a prospective, blinded study, helps to minimize the chance for selection bias of study participants. In addition, the blinded nature of the study ensured that biasing factors regarding clinical or lab assessments of individual participants were minimized because neither group was aware of the other's results.

Second, since the present study was conducted at the ATC, a non-biomedical intervention center, the patients examined in the present study were *a priori* not skewed toward those seeking biomedical interventions at a physician's office. The participants examined in the present study were selected from community contacts.

Third, and most importantly, the consistency and specificity of the results observed were strengths of the present study. It was observed that for each biomarker of oxidative stress, with the exception of blood SOD,

there were significant overall differences relative to the laboratory provided reference ranges.

Finally, since two-tailed *p*-values were used, and the directions of the significant effects observed were in the biologically plausible directions, with the mere chance occurrence of observing the results found in the present study being minimal. Furthermore, since < 20 total statistical tests were generated in the present study, a two-tailed *p*-value $\leq .05$ was considered significant, and most of the *p*-values calculated were < .01, it is reasonable to conclude that the results observed were not due to statistical chance.

In considering the potential limitations of the present study, the number of study participants was of moderate size. Despite this potential limitation of the present study, it was observed that there were consistent statistical effects observed. It would be worthwhile to evaluate the consistency of the results observed here with those in different and expanded cohorts of individuals diagnosed with ASDs. In addition, the present study did not examine a recruited cohort of neurotypical children, but instead utilized the laboratory reference ranges. It would be worthwhile to evaluate the consistency of the results observed here with a cohort of neurotypical children, but the results observed in the present study were consistent with observations made by other researchers in previous study (Geier et al., 2008). Another potential limitation of the present study was that the biomarkers analyzed were from peripheral sources, and not directly measured in the brain of the patients examined. In future studies, it would be of interest to evaluate the correlation between peripheral biomarkers with those measured in the brain of patients diagnosed with an ASD. Finally, in the present study, data was not evaluated concerning other biomarkers of oxidative stress present in the study participants examined. It would be of value in future studies to examine if there was a potential correlation between other biomarkers of oxidative stress among individuals diagnosed with ASDs, and to evaluate their potential correlations with ASD severity.

Conclusion

The present study is a novel prospective study conducted to evaluate biomarkers of oxidative stress in a cohort of patients diagnosed with ASDs using routinely available clinical lab testing. For the study participants examined, this study found that they had significant evidence of decreased blood GSH and GPx. By contrast, it was found that they had significant evidence of increased levels of urine lipid peroxides. Finally, it was also observed that blood SOD levels were found to be significantly outside of the laboratory reference range in a bimodal fashion. We recommend that future studies should focus on further evaluating biomarkers of oxidative stress in an expanded cohort of individuals diagnosed with ASDs, and potential treatment protocols be evaluated to potentially correct

the oxidative stress abnormalities observed in the present study. Additionally, we suggest that future studies of individuals diagnosed with ASDs should examine further biomarkers of oxidative stress. Finally, we recommend, since the lab testing employed in the present study for examining biomarkers of oxidative stress are clinically available, relatively inexpensive, and relatively noninvasive, that patients diagnosed with ASDs be routinely tested to evaluate them.

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Conflict of Interest

David Geier, Janet Kern, and Mark Geier have been involved in vaccine/biologic litigation. David Geier and Mark Geier have a patent pending for the treatment of autistic disorders. The authors have no financial relationship with the laboratory utilized in the present study.

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Research Profile

Mark Geier has a M.D. and PhD. in genetics. He is both board certified in genetics by the American Board of Medical Genetics and a Fellow of the American College of Epidemiology. In clinical practice for more than 29 years, Dr. Geier is a founder, and presently the medical director, of ASD Centers, LLC. Dr. Geier has authored more than 20 peer-reviewed medical studies on patients diagnosed with an autism spectrum disorder. Dr. Geier's research has specifically focused on environmental exposures linked with autistic disorders, as well as the underlying biochemistry of autistic disorders. Over 600 patients diagnosed with an autism spectrum disorder have been evaluated and treated with the clinical protocols Dr. Geier has developed. Dr. Geier is the co-holder of a patent pending for the treatment of patients diagnosed with an autism spectrum disorder.

David Geier is a founder of the non-profit 501(c)3 institute of Chronic Illnesses, Inc. as well CoMeD, Inc., also a 501(c) 3. Studying and publishing on the relationship of genetic, biochemical and hormonal changes in autism David's research has resulted in new insights to the causes and treatment of autism and other chronic illnesses. Overall, David has authored more than 20 peer-reviewed medical studies on patients diagnosed with autistic disorders. David is a co-holder of a patent-pending for the treatment of patients diagnosed with an autism spectrum disorder.

Janet Kern has an MS in Neuroscience and a PhD in Human Development. She is an RN with 28 years of experience in medicine. For the last 14 years Dr. Kern has been doing research in autism. She specializes in autism clinical research with a current focus of biomedical vulnerability, toxicity, biomarkers, and treatment efficacy. Dr. Kern has 21 peer-reviewed publications in autism, 17 of which she is first author. She began her post-doctorate in 1999 at the University of Texas Southwestern Medical Center (UTSW) and was promoted to assistant professor. Dr. Kern became an independent research investigator in 2006 and remains an adjunct assistant professor at UTSW.