Gastrointestinal and Metabolic System Biomarkers in Autism: A Review

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled “Gastrointestinal and Metabolic System Biomarkers in Autism: A Review a better approach to patient care in autism” submitted by Shpona Roy (16346031) of Summer, 2016 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelors of Pharmacy.

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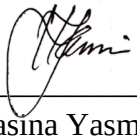

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Ethics Statement

This experiment has the ethical permission for conducting the research on animal (mice).

Abstract:

Autism spectrum disorders (ASDs) are heterogeneous and complex disorders that are growing dramatically in children. Currently, there are no objective methods to assess the disorder. However, subjective behavior can develop an objective understanding of the measurement of autism. Therefore, the objective of this study was to review various gastrointestinal (GI) and metabolic system biomarkers in autism. Different articles containing probable relevant biomarkers of autism were reviewed. All the case-control studies showed that β-endorphin, Casomorphine, Coproporphyrin, Dermorphin, Desmorphin along with vasoactive intestinal peptide levels of the GI system were increasing in the autistic subjects. Furthermore, the decreasing levels of Dipeptidyl peptidase IV were observed by researchers leading autism. Moreover, some oxidative stress biomarkers of the metabolic system such as glutathione-reduced/oxidized, methionine, cysteine, urine 8-OHdG, plasma F2t-isoprostanes and also transferrin with their abnormal levels that caused autism were also defined by the researchers.

Keywords: Autism; ASD; Gastrointestinal biomarkers; Metabolic biomarkers; Neurodevelopmental disorder.

Dedication

I want to dedicate this project to my respectable supervisor Dr. Sharmind Neelotpol, Associate Professor in Department of Pharmacy, Brac University for her continuous guidance throughout my project.

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ASD	Autism Spectrum Disorder
GABA	Gamma-Aminobutyric Acid
VPA	Valproic Acid
GI	Gastrointestinal Tract

1. Introduction

Autism is a complex, lifelong developmental disorder that usually begins in early childhood and may affect social skills, speech, relationships and self-regulation. A certain set of behaviors describe autism-like stereotypies, rituals, compulsions, obsessions, etc. It is a ‘’Spectrum disorder’’ that affects individuals differently and by other degrees (National Autism Society, 2018). They face difficulties in interpreting nonverbal communication, making and holding friends, maintaining the usual back and forth conversional style. Moreover, they also face problems with repeating sounds, repeating gestures, trouble with their routine, extremely controlled, strong desires, etc. Moreover, they also face trouble with severe and /or lower sensitivity to different sensory stimuli (National Autism Society, 2018). Autism is a heterogeneous neurodevelopmental disorder and analogous to broad syndromes such as mental retardation (Stagg & Belcher, 2019).

Researchers reported a median global prevalence of 17/10000 (approx. 1 in 588) for autism disability and 62/10000 (approx.1 in 161) for all prevalent developmental disorders; although indicating that changes in rates over time and variations between regions and countries may be due to diagnosis change, changing medical requirements, provision of care, the occurrence of neurological condition and the raising global autism awareness (Onaolapo, 2017).

Autism begins in infancy or the first 3 years of life (Kolvin, 1971). The boys are suffering more than girls from autism symptoms (Bryson and Smith, 1998). Some factors that lead to autism-like symptoms include genetic factors, environmental factors, immunological factors, etc. Some viral and bacterial infections, hypertension, gestational diabetes, smoking, proteinuria during pregnancy are considered as initiators for causing autism (Ratajczak, 2011). Neurotransmitters play a major role in the healthy development of the brain, memory, motor activity and behavior

regulation by creating connections between the neurons in the nervous system. The neurotransmitters are GABA (gamma-aminobutyric acid), glutamate, serotonin, catecholamine, acetylcholine, histamine, etc. When dysfunction of these neurotransmitters occur it is considered to be caused due to autism spectrum syndrome (ASD) by hampering neuronal cell migration, differentiation, synaptogenesis, and eventually developmental processes of the brain (Cetin et al., 2015).

Valproic acid (VPA) is a monocarboxylic acid and responsible for hampering the fetus during pregnancy. It is widely prescribed for epilepsy, bipolar disorder, migraine, Alzheimer's disease and recently for cancer and HIV polytherapy as well. Valproic acid is contraindicated with pregnancy as it is teratogenic and can potentially inflict damage upon the developing fetus (Lloyd, 2013).

The enzyme glutamic acid decarboxylase (GAD) converts glutamate into gamma-aminobutyric acid (GABA). Currently, valproate (VPA) and gabapentin (GBP) are known to have some effect on this enzyme and thereby enhance the synthesis of GABA, in addition to other potential mechanisms of action. VPA also blocks the neuronal sodium channel during rapid sustained repetitive firing (Lloyd, 2013).

Histamine is a nitrogenous compound and acts as a neurotransmitter which plays a role in both physiological conditions and psychological disorder e.g. schizophrenia. Moreover, in the brain histaminergic system, the histamine-N-methyltransferase enzyme is responsible for the metabolism of histamine in the central nervous system ((Eissa et al., 2018). As a result, it causes behavior changes in the children which leads to neurological disorders. Furthermore, when histamine binds with H_3 receptors, it causes an increase in GABA (gamma-aminobutyric acid), Dopamine, serotonin, glutamate, Acetylcholine neurotransmitter, etc. (Eissa et al., 2018). The

disorders that are caused by histamine H3 analogues can be treated with H_3 receptor antagonists. Therefore, the H_3 receptor antagonist, betahistine can be used to block the release of these neurotransmitters (Eissa et al., 2018).

Biomarkers of autism spectrum disorder refer to the irregularities in neurological and biological function in individuals diagnosed with autism. Biomarkers help to identify the autistic symptoms in children during the pre-symptomatic period. If any behavioral changes observes into these children, further separate them into some subtypes and predict treatment for this autistic group of children (Frye et al., 2019). There are no reliable biological markers to diagnose autism in an individual patient (Posey et al., 2008; Ecker et al., 2010).

However, there are biomarkers such as metabolic, genetic, neurologic, immunologic, hormonal, etc. that are related to ASD. When the normal level of these biomarkers are increased or decreased, it causes irregularities in the adaptive immune reactions of the ASD affected individuals. Studies show that knocking out the genes specific to these biomarkers incites seizures, hypertensive behavior, deficits in learning and memory, poor motor skills on a repetitive task, hyperactivity and other actions in transgenic experimental mice (Delorey et al., 1998).

Therefore, the aim of the study was to find out the change of biomarkers in the gastrointestinal and metabolic system in autism.

The objectives of this study were-

i. to find out the biomarkers of the gastrointestinal and metabolic systems.
ii. to identify the mechanism of action of these alterations in the gastrointestinal and metabolic systems in autism.

2. Methodology

Various published articles have been collected from valid and authentic sources. To recognize biomarker studies in ASD, we did a search on medical databases such as the NCBI and PubMed literature. Other sources of the information were also included like WHO, Researchgate which provides a precise and specific explanation on this topic. The studies also described any comparison between GI and metabolic biomarkers in autistic and control groups when they met certain conditions. Studies were being performed in diverse demographics including males, women, babies and children.

3. Discussion

Biochemical and pathological signs of autism are very apparent in early life. Newborns with elevated neurotransmitters play a crucial role to develop autism in their later age (Nelson et al, 2001). A significant target of our study is to classify various GI and Metabolic biomarkers of autism spectrum disorder (ASD). Therefore, the details of the biomarkers are explained below.

3.1. GI Biomarkers

A report has shown that autistic children almost always face GI problems including gaseousness, constipation, bloating, diarrhea, and discomfort in the bladder, reflux, etc. (Ratajczak, 2011). A few years back, esophagitis reflux was the most common identification in autistic children who underwent endoscopy of the upper GIT. The ASD affected individuals also suffer from actual GI abnormalities specially low disaccharides enzyme activity such as lactase, sucrose, maltase, palatinase, glucoamylase, etc. (Horvath and Perman, 2002)

Some clinical symptoms such as: chronic gastritis causes an increase in the level of lymphocytic infiltrate and lymphoid aggregates in the mucosa. Additionally, autistic cases showed higher Paneth cell numbers than the control groups (Horvath and Perman, 2002). The endogenous substances like xenobiotic and porphyrins were found at a greater level in the urine of autistic children (Nataf et al, 2006).

Furthermore, calcitonin, Dermorphin, Deltorphin II, and Desmorphin were also increasing in blood and urine to a greater extent which was documented from the urine sample of autistic patients (nelson et al, 2001). There was a hypothesis that opioids suppress the pain feeling in autistic subjects. The opioid theory in autism suggested that the endogenous opioid pathway hyper functions might be the source of most autism related symptoms in children. Greater

endogenous substances (opioid) β-endorphin was also noticed in the autistic subjects (Ratajczak, 2011).

On the contrary, some current studies had shown that opioid peptides didn't necessarily cause any abnormalities like painless sensation that were collected from autistic children's urine (Cass et al, 2008). Hence, the individual biomarkers that include in the GI system are explained below for better understanding.

Table 1: A list of gastrointestinal system biomarkers of autism

Biomarker	Increase Level in Autism	Decreases Level in Autism	Location
B-Endorphin	✔		MNC
Calcitonin gene-related Peptide	✔		B
Casomorphine	✔		U
Caproporphyrin	✔		U
Deltophin II	✔		U
Dermorphin	✔		U
Desmorphin	✔		U
Disaccharides Enzyme: Lactase, Maltase, Sucrose, Palatinase		✔	SI
Morphine modulating Peptide	✔		U

Vasoactive Intestinal peptide	✔		B
Dipeptidyl Peptidase IV		✔	U

B= Blood, MNC= Mononuclear cells, SI= Small intestine, U= Urine (Ratajczak, 2011).

3.1.1. β-endorphin

Many researchers found that individuals with autism had diminished pain control and shown a lack of nociceptive reflexes. There was also a report that over brain opioid function might clarify the surprising insensitivity to pain related to autism and contributed to understanding autism pathogenesis (Tordjman et al., 2009). From a different research, β-endorphin had enhanced extensively in autistic patients that decreased the sensitivity of the pain and elevated the pain threshold (Tordjman et al., 2009).

3.1.1.a. The Effects of High β-endorphin Levels

Due to high β-endorphin (BE) and pain insensitivity, autistic subjects exposed self-injurious and stereotyped behaviors. Numerous experiments had been performed to identify plasma BE levels in the autistic subject. The conflicting plasma BE data in autism might be partially explained by methodological difficulties, for example, scientific specificity in immunoassays and little sample size of autism (Tordjman et al., 2009).

Studies on core levels of opioids in autism had also incoherent results in a manner where BE levels in cerebrospinal fluid (CSF) were identified to be raised, declined or equivalent to the controls group. So it was very difficult to identify the actual cause of autism by analyzing the cerebrospinal fluid, but making up related pain trials in infants with cognitive impairment, psychiatric and behavioral retardation disorders provided for an investigative framework. Studies had been carried out to explain the issues of reactivity of discomfort and responsiveness in individuals with autism disorder and further examined potential improvements in plasma BE, investigated possible behavioral interactions with BE, and reactions of pain in physiology (Tordjman et al., 2009).

The reactivity of mental discomfort increased autistic illness which was studied in infants and adolescents by using many assessments of behavioral parameters in various observational conditions. The physiological reaction to pain was measured by evaluating the heart rate alterations; while changes of plasma BE levels were examined in a large group of autistic subjects (Tordjman et al., 2009).

The plasma BE calculation was conducted to better understand the possible role of the opioid response in autism and pain-related autistic behavior. Greater comprehension of the fundamental mechanisms might have major consequences for the diagnosis and care of people with autism (Tordjman et al., 2009).

3.1.1.b. Relationship between BE Levels with Autism

It was observed that the plasma of the autistic subject contained higher levels of opioid peptides and they showed pain insensitiveness to any pain stimuli. BE itself acted like an opioid peptide. These peptides had greater affinity to bind with mu and delta opioid receptors. When more number of BE bound with these receptors, larger pain insensitivity occurred in autistic children. As a result, they showed repetitive utterance including movements and self-injurious behaviors. As pain insensitivity was directly related with autism, a table (table 2) with some data provided for proper understanding (Tordjman et al., 2009).

3.1.1.c. Observational Condition

In that study, the researchers evaluated the behavioral pain reactivity in 3 separate observational conditions. They had identified five different types of behavioral pain reactivity such as paradoxical, absent, hyporeactivity, normal and hyperreactivity. Paradoxical pain reactivity refers to apparent enjoyment responses such as laughing or smiling to a painful stimulus. Absence of pain reactivity refers to the patient not feeling any pain when their body is going to burn or given to injection. They don't contain any nociceptive reflexes. Hyporeactivity to pain means when the patient gets any pain, response to it with delayed time. Normal pain reactivity refers to the fact that after getting pain the patient cries, moans, screams, responses when someone touches their injured area etc. is observed. Furthermore, hyperreactivity to pain refers that the response after the painful stimuli is higher than the normal (Tordjman et al., 2009).

The researchers found out the pain reactivity of autistic subjects in three separate conditions like caregiver, parental and phlebotomist. Different types of pain reactivity levels are given below for comparison.

Table 2: Evaluation of the different types of behavioral pain reactivity in 3 separate observational conditions

Types of behavioral pain reactivity					
	Type 1	Type 2	Type 3	Type 4	Type 5
Observational condition	Paradoxical	Absent	Hyporeactivity	Normal	Hyperreactivity
Parental evaluation in individuals with autism (n=73)					
Overall pain reactivity	1 (1.4%)	2 (2.8%)	48 (65.8%)	20 (27.4%)	2 (2.8%)
Reaction to burning self	3 (4.1%)	0 (0%)	11 (15%)	57 (78.1%)	2 (2.7%)
Reaction to internal pain (tooth pain, ear infection etc.)	0 (0%)	2 (2.8%)	34 (47.2%)	33 (45.8%)	3 (4.2%)
Reaction to other pain (accident, banging self- etc.)	1 (1.4%)	1 (1.4%)	46 (64.8%)	19 (26.8%)	4 (5.6%)
Caregiver evaluation of individuals with autism (n=73)					
Overall pain reactivity	2 (2.7%)	3 (4.1%)	22 (30.1%)	43 (58.9%)	3 (4.1%)
Psychiatrist/Nurse evaluation during venepuncture					
Individuals with autism (n=63)	4 (6.3%)	26 (41%)	9 (14.3%)	14 (22%)	10 (15.9%)
Normal controls (n=115)	1 (0.9%)	10 (9%)	25 (30.4%)	67 (58.3%)	1 (1.7%)

Results are given as number of individuals (and % of group) appointed for each pain reactivity classification (Tordjman et al., 2009).

Different types of behavioral pain reactivity were observed in the 3 observational conditions that were introduced in the above table (Table 2). A significant percentage of individuals with autism had poor/lacking pain reactivity depending on the appraisal of the phlebotomist, caregiver and parents (55.6%, 34.2% and 68.6% respectively).

Higher BE levels were lowering pain reactivity reactions to the autistic children along with impairment to the social communication and repetitive utterance of the GI system. Comparing the behavioral pain reactivity in the venipuncture between the control and autistic group, there was a considerable and important difference in the distribution of pain reactivity reactions (Tordjman et al., 2009).

Twenty two percent autistic subjects had demonstrated natural venipuncture pain reactivity while 15.9 percent had reported hyperreactivity. In comparison, 60.3% of autistic subjects exhibited some autism-related actions directly after the venipuncture such as aggressiveness towards others, SIB, social withdrawal and repetitive attitudes were observed in 23.8%, 9.5%, 38.1% and 34.9% of patients respectively. Caregivers and parents had also identified related behaviors after unpleasant stimuli like aggressiveness to others, SIB, repetitive behaviors and social withdrawal. Further, caregivers and parents had confirmed that 9 of the 22 verbal autistic patients communicated verbal grievances after a painful stimulation but could not identify the painful region correctly (Tordjman et al., 2009).

There was no substantial connection between ratings in the two cases, based on the contingency study of caregiver and parental measurements of the overall behavioral pain reactivity form. Likewise, the nurse/psychiatrist tested for the general behavioral pain reactivity was not found to

be strongly linked during the phlebotomist. In three observational cases, we specifically showed that in people with low to medium autistic disorders, irregular behavioral responses to unpleasant stimuli were particularly prevalent. Therefore, we can say the autistic subject shows hyporeactive to any painful stimuli or lacking pain signals (Tordjman et al., 2009).

3.1.2. Casomorphin

The opioid-like casomorphin was noticed to be increased in autistic patient's urine because they had very low or null amounts of dipeptidyl peptidase-IV enzyme. The function of this enzyme is to break down the casomorphin. When there is a lack of this peptidase enzyme, casomorphin can't be broken down properly. On the contrary, the level of the enzyme was normal in the control groups of neurotypicals (Ratajczak, 2011).

Chronically high levels of casomorphin might modulate opioid and other neurotransmission systems directly in the brain, contributing to ASD growth (Sokolov et al., 2014). Several conditions including autism, postpartum hysteria, and schizophrenia were thought to be related to an inability to properly absorb gluten and casein. Such insufficient digestive components casomorphin and gliadorphin could be found in autism patients' urine and cerebrospinal fluid (Receptor et al., 2013).

Theoretically, they cross both brain and intestinal barriers and later attach to endogenous opioid receptors causing signal transmission interference. Casomorphin and Gliadorphin were reported to have an adverse pharmacological impact on concentration, learning, brain maturation and social experiences (Receptor et al., 2013).

3.1.2.a. Observational Condition

As casein related to causing autism, the researchers found that taking the casein free diet was more effective than prescribed pharmaceuticals for autistic patients. For these reasons some researchers did two small trials.

The first trial had included 10 participants where they had reported that casein free diet decreased the autistic like symptoms such as bizarre behavior and social detachment only at 12 months of age. In the second trial had included a total of 15 participants in where the autistic subjects had taken casein containing diet. The researchers had reported that the results between the control group and dieting group were not substantially different in terms of language and communication ability, motor ability, cognitive skills at their 12 months of age. So, we can say as casein or casomorphin can cause autism, by taking a casein free diet can also reduce the autistic like disorders (Receptor et al., 2013).

3.1.3. Coproporphyrin

Porphyrinuria is an alternative, non-invasive approach for the diagnosis of environmental toxicity in children with ASD. The origin of porphyrins is from the derivatives of the heme synthesis pathway allows independent adverse exposure measurement (Brewster, 1988). The development of heme happens most commonly in the kidney, liver, and erythroid cells. The synthesis of heme takes place in two stages. At first succinyl-CoA along with glycine make uroporphyrinogen. And further in another step by coproporphyrinogen and pentacarboxyporphyrinogen make heme (Nataf et al., 2006). The porphyrin derivatives that derived from the heme synthesis are given below.

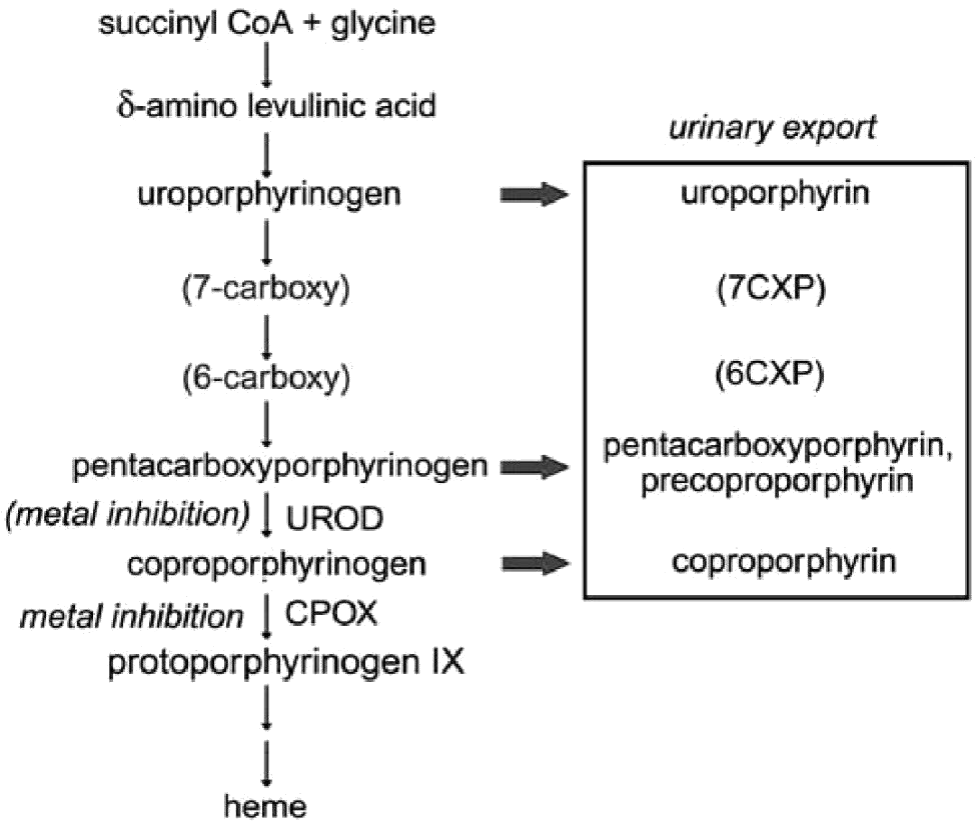

Figure 1: Porphyrin derivatives from heme synthesis (Nataf et al., 2006).

Excess porphyrinogenic metabolites were excreted as oxidized porphyrins in the urine especially coproporphyrin and uroporphyrin, representing the most enormous molecules of kidneys in rats. Porphyrins were the highest water-soluble in mid-pathway of heme synthesis and mainly appeared in the urine, while protoporphyrin was hydrophobic and appeared in feces and bile for the most part (Nataf et al., 2006).

Porphyrin Levels in Autistic Subjects:

The excess porphyrin levels of the urine might play a contributing function in autism disorder behavioral manifestation. The higher blood levels of both precursor molecule 5-aminolevulinic acid and porphyrins were consistent with porphyrinuria. Such metabolites targeted the receptors

of benzodiazepines in the brain and had been associated with epilepsy, autistic like syndromes and neurologic disturbances. Elevated levels of these compounds could lead to abnormal behavior and brain function in some autistic subjects (Nataf et al., 2006).

3.1.3.a. Effects of Excess Porphyrin Levels

Excess urinary excretion of porphyrin resulted from the blockage of essential enzymatic steps like heme synthesis enzyme. The heavy metal porphyrins were released into urine at high levels in both humans and laboratory animals. The most common heavy-metal inhibition targets were coproporphyrinogen oxidase and uroporphyrin decarboxylase reactions leading to common coproporphyrin and pentacarboxyporphyrin elevations in the urine (Nataf et al., 2006).

The causal link between porphyrinuria and heavy metal inhibition had been demonstrated; both in human exposed to heavy metal and in rats exposed to mercury removal with chelating agents (DMPS, EDTA, tetra acetic acid ethylenediamine, etc) decreased urinary porphyrin levels to regular values. Although nonmetal agents that targeted the heme pathway could also increase levels of urinary porphyrin. Precoproporphyrin was synthesized through in vivo alteration of pentacarboxyporphyrinogen under pressure of heavy-metal interference (Heyer et al., 2006), provided a specific heavy-metal toxicity (mercury) porphyrin marker (Nataf et al., 2006).

3.1.3.a. Observational Condition

To counter the heavy metal load of ASD children in the large community of children with autism or other neurodevelopmental conditions, a retrospective study of rates of common urinary porphyrins had been carried out. The study was performed about urinary markers of hemicycles inhibition, including coproporphyrin, and the particular marker of precoproporphyrin, heavy metal toxicity (Nataf et al., 2006).

To identify the potential environmental toxicity in autism etiology the researchers experimented by conducting about 269 children who had neurodevelopmental disorder or related problems. Further, the researchers separated the children into different groups for examining the urinary porphyrin levels and autistic symptoms.

Table 3: The comparison of different types of neurodevelopmental disorder and autistic disorder values in autistic and control subjects

Condition	Male (M)	Female (F)	Total	Mean age (year)	M/F	% total	% ASD group	
Allergy	5	3	8	7.3	1.67	3		
Asperger	10	1	11	10	10	4.1	5.8	
Attention deficit	2	7	9	9.4	0.29	2.3		
Autism (autistic disorder)	79	27	106	6.4	2.9	39	55.5	ASD= 71% of total sample (M/F= 3.34)
Autism + epilepsy	7	2	9	9.3	3.5	3.3	4.7	
Cerebral palsy	6	6	12	8.3	1	4.4		
Epilepsy	2	0	2	10	NA	0.7		
Hyperreactivity	27	2	29	9.1	13.5	10.7		
MR + epilepsy	1	1	2	6	1	0.7		
PDD-NOS	51	12	63	6.6	4.3	23.4	33	
Psychomotor retardation	1	3	4	7.3	0.33	1.5		
Rett	0	2	2	2.5	0	0.7	1	
Control group	7	5	12	10.3	1.4	4.4		
Total	198	71	269	7.4	2.8			

MR= Mental retardation, PDD-NOS= Pervasive developmental disorder not otherwise specified; subthreshold symptomatology, Rett= Rett's disorder (Nataf et al., 2006).

In this study the age of the experimental children were approximately 1 to 16 and specifically of the autistic children were 2 to 15. The researchers reported that among 269 children 71 percent of these children were diagnosed with autistic spectrum disorder. Most of these subgroups about 56 percent were diagnosed with autistic disorder or autism in children. Other diagnosis classes throughout the report included attention deficit, hyperactivity, Asperger, PDD-NOS, cerebral palsy and the independent type of autism condition associated with epilepsy. In this study both male and female samples were taken for further investigation to find out the autistic symptoms and to identify the comparison between them (Nataf et al., 2006).

The mean values for urinary coproporphyrin (COPRO) and uroporphyrin were compared between control groups and with diagnostic categories. The researchers showed that there was no substantial alteration in urinary uroporphyrin levels in any disorder examined. On the contrary, there were clear reports of coproporphyrin excess levels in two disorders like in autism and autism with epilepsy (figure 2). The mean values of coproporphyrin levels had surpassed the mean value of control group along with two times higher standard deviation (Figure 2) (Nataf et al., 2006).

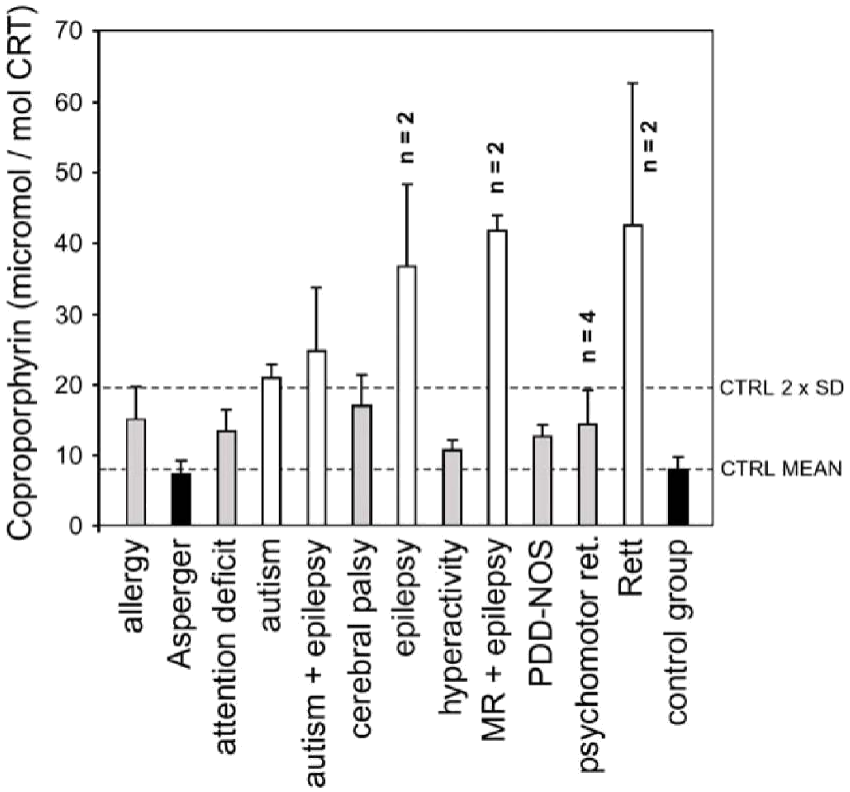

Figure 2: Urine coproporphyrin levels in children with neurodevelopmental and associated disorders. Error bars were standard error of mean. The mean of the control group and the mean plus 2 × standard deviation were shown in the horizontal dashed line. N values are defined for classes of less than eight subjects (Nataf et al., 2006).

However, the researchers recorded porphyrinuria in a broad community of children with autistic like symptoms. They had provided evidence of higher levels of coproporphyrin in their experimented children who had neurodevelopmental disorder. Unexpectedly, the researchers had shown that porphyrin levels in terms of Asperger's syndrome were inseparable from the control group. Moreover, they had identified that the mean value of coproporphyrin was significantly higher in case of autistic symptoms than any other disorders mean value (Nataf et al., 2006). Hence, we agree that the excess levels of coproporphyrin can cause autism spectrum disorder.

3.1.4. Dermorphin

Dermorphin is known as one source of brain neuronal disorders. It is a toxic material structure of opiate 30 to 40 times stronger than morphine. The theory of dermorphin peptide autism indicated that certain autistic symptoms could be related to opioid peptides that were produced by the incomplete breakdown of casein and gluten foods (Amin & Abdou, 2011).

Increased intestinal permeability was often known as a leaky intestinal syndrome that enabled such peptides to move through the intestinal membrane, then reached the bloodstream and later crossed the blood-brain barrier, impacting the endogenous opiate system and internal nervous system neurotransmission (Amin & Abdou, 2011).

3.1.4.a Potential Therapy

Until this, gluten and casein-free diet seems to have justification for its use and are still available as a form of therapy to children with an autism spectrum disorder that was claimed by the greatest scientist. Because there's no proven treatment, the focus was on early detection and action intending to maximize ASD affected children's quality of living. This research speculated that autism symptoms might have a notable connection with excessive opioid as well as an incomplete breakdown of gluten and casein. Thus, casein and gluten removal can cause improving standards for autism (Amin & Abdou, 2011).

3.1.4.b. Observational Condition

For this study, a group of autism spectrum disorder children (42 numbers) and another group of normal children (36 numbers) were taken under some conditions. The results showed that after six months of dietary removal, there was a statistically significant decrease in the mean overall

CARS (Childhood autism ranking scale) ranking in patients with autism or children with and without dermorphin intervention. For better understanding, a table is given below (Table 4) (Amin & Abdou, 2011).

Table 4: Comparison between control and autistic group in terms of serum dermorphin like action

Autistic group (42)					Control group (36)				
	No.	%	Mean	SD	No.	%	Mean	SD	P
Total	18	42.85	1.89	0.76	14	38.89	1.71	0.76	0.60
+1	6	33.33			6	42.85			
+2	8	44.44			6	42.85			
+3	4	22.23			2	14.30			

SD= Standard deviation (Amin & Abdou, 2011).

A total of 42 children had met autism spectrum disorder eligibility requirements along with average age 50.60 ± 11.38 months. As a healthy control 36 children were taken along with average age 54 months ± 10.55. Table 4 showed a positive dermorphin in 18 (42.85 percent) autistic subjects as compared to 14 (38.89 percent) the control group. The study identified that there were no substantial differences in both group's mean levels of dermorphin peptide (Amin & Abdou, 2011).

Table 5: Comparison between autistic children with positive and negative dermorphin

Autistic Children with Positive Dermorphin		
	Mean	**SD**
0 Month	45.17	7.20
3 Months	40.67	7.89
P Value	0.083	
0 Month	45.17	7.20
6 Months	34.83	8.4
P Value	0.000	

SD= Standard deviation (Amin & Abdou, 2011).

Autistic Children with Negative Dermorphin		
	Mean	**SD**
0 Month	46.29	6.95
3 Months	42.78	7.01
P Value	0.088	
0 Month	46.29	6.95
6 Months	40.15	6.73
P Value	0.003	

SD= Standard deviation (Amin & Abdou, 2011).

Table 5 indicated that the overall cumulative decrease was statistically significant CARS (childhood autism rating scale) score in autistic subjects after 6 months of dietary exclusion, with and without dermorphin.

Improving GIT symptoms with dermorphin action in autistic children (60 percent) was more than in the control group action of dermorphin (50 percent) after six months of dietary elimination. A table (table 6) is provided here for knowing the actual data.

Table 6: Before and after diet removal, impact on GIT symptoms monitored autistic children against

	Prior to Diet Elimination		Improvement Upon Elimination of Diet	
	No	%	No	%
Symptoms of GIT with dermorphin action in children of Autism 18 (42.9 percent)	15	100	9	60
Symptoms of GIT with dermorphin action in control group 14 (38.9)	6	100	3	50

SD= Standard deviation (Amin & Abdou, 2011).

Furthermore, table 6 showed that a large number of autistic subjects with dermorphin action improved in GIT symptoms than the control group after maintaining diet (Amin & Abdou, 2011).

This research confirmed that dermorphin peptides had been noticed in autistic children and in control children serum. In addition, the study had given importance to the excessiveness of autistic symptoms in autistic children with and without dermorphin action and did not find any big differences. This result of dermorphin peptide action in autistic subjects could be considered

as non-specific effects. But a scientist Millward had suggested that casein and gluten peptides might play a special role in autism origins and the psychology along with physiology of autism could be explained by excessive activity of opioids associated with these peptides (Amin & Abdou, 2011).

Besides, the study focused that if proper diet control was continued, the intensity of autistic symptoms could be improved in both dermorphin negative and positive autistic children. This finding had indicated that autistic children had an advantage in diet removal. Knivsber mentioned that diet exclusion had contributed to autistic activity decreases and the reappearance of autistic characteristics after diet breakage (Amin & Abdou, 2011).

3.1.5. Dipeptidyl Peptidase IV

Casein is the protein present in milk products and certain pharmaceutical items like some stomach tablets and some pills. Proteins in milk produce large amounts of different casein antibodies which cause delayed or immediate inflammatory reactions. After investigating, patients turned up either very soon or up to multiple days or even weeks after taking the dairy items. The most severe signs were respiratory, cutaneous eczema, and gastrointestinal disorders (El-Alameey et al., 2018).

Dipeptidyl peptidase-IV (DPP-IV) is a proteolytic-digesting enzyme, found in the cells that lined the tiny bowel villi. This enzyme plays a major role in the digestion of foods including casein. Lack of the DPP-IV enzyme might induce maldigestion of these dietary proteins and generate tiny peptides that later attach to opioid receptors in the brain resulting in interference with cognitive function and lead to symptoms in autistic children. DPP-IV was also derived from T-lymphocytes (El-Alameey et al., 2018).

CD26 expression involved a very important down-regulation on lymphocytes in certain individuals with autism who suffered from allergic casein reaction. Dietary peptides (casein) bind to DPP-IV, which contributed to the development and regulation of autoantibodies, immune response, and inflammation in autistic children (El-Alameey et al., 2018).

So far, precise GI symptom control medications for children with ASD are unknown. It was difficult to maintain a diet free from casein optimally because there was a high chance of consuming casein in daily foods and products. Autistic children's treatment was targeted specifically at managing symptoms. Bashir and AL-Ayadhi presumed that DPP-IV supplementation might require protein for slow digestion and may be effective in reducing or eliminating the casein-related inflammatory response, minimizing the need for a highly restricted dietary regimen. This research therefore aimed at measuring the reaction of an antibody to casein protein by examining serum rates of casein antibody and DPP-IV enzyme activity among autistic children and examining their association with GI symptoms and feeding habits of these babies (El-Alameey et al., 2018).

3.1.6. Disaccharides Enzyme

The presence of some digestive disaccharides enzymes such as sucrase, maltase, lactase, glucoamylase, and palatinase played a very important role in the digestion of food, and low levels indicated autistic-like symptoms in the children. Many researchers had reported that autistic children had a lack of a digestive enzyme. Researchers also recorded that children with gastrointestinal disorders and ASD showed low digestive enzymes particularly lactase (Saad et al., 2015).

Lack of lactose could lead to abdominal irritation, pain, and aberrant behavior in autistic subjects. Clinical assessment did not classify most autistic children with lactose sensitivity. The community who provided digestive enzyme therapy for three months had substantial changes in 4 dimensions according to GBRS and CARS in a double-blind placebo-controlled study of digestive enzymes in children with ASD. After treatment of these ASD patients with digestive enzymes, a calculation suggested that emotional reaction, overall perception, general actions, and gastrointestinal symptoms dramatically improved (Saad et al., 2015).

3.1.7. Morphine Modulating Peptide

Some morphine modulating peptides can cause autism in children. Many specific neuronal transmitters modulate social behavior at a neuronal level which either enhances or impedes social interactions. Some of the former are well-known participants in the recompense mechanism like serotonin, cannabinoids, noradrenaline, sulfur oxytocin which are opiates and were previously described as motivating or rewarding reinforcement mediators for the misuse of medicines (Pellissier et al., 2018).

In comparison, glucocorticoids, 5-HT, and neuropeptides including the corticotropin release components, substance p, arginine vasopressin, or cholecystokinin had been identified as the neurobiological substrates of social avoidance. Anti-social and pro-social neuromodulators were thereby fighting for the development of appropriate social behavior (Pellissier et al., 2018).

3.1.7.a. Important Role of μ Receptor

The μ receptor plays a vital role as a neurobiological substrate of social behavior. Opioid receptors are part of the broad spectrum of GPCRs (G-protein couple receptors) and are composed of three members such as μ, δ, and k opioid receptors which have opioid peptides, endorphins, dynorphins, and enkephalins as their main endogenous ligands respectively. The opioid pathway was a widely known pain modulator and opiate opioid receptor exogenous ligands had been used as pain relief agents for thousands of years. Nevertheless, the medical usage of opioids had also contributed to their identification of addictive characteristics, a second major role for opioid physiology shedding light on the modulation of reward (Pellissier et al., 2018).

The discovery and enhancement of opioid peptides, their receptors and their corresponding genes had subsequently improved the assessment of their mode of action in pain and reward regulation as well as many other roles in the field of respiration, stress, food consumption, endocrine, gastrointestinal transit and immune processes (Plein and Rittner, 2017). Panksepp and colleagues had proposed their “Brain Opioid Theory of Social Attachment” whereby bonding and affiliating behavior, focused in particular on compelling correlations of social association and drug abuse, was highly contingent on the amount of endogenous opioid peptides (Pellissier et al., 2018).

This hypothesis assumed that social deprivation led to social distress and contact searches for the insufficient tone of opioids. Social contact had alleviated this negative effect through the release

of opiates (endorphins). After that time, studies had shown that μ receptors predominantly engage in the pro-social effects of opioids that were not only found in social distress conditions but more favorable and even optimistic social circumstances (Pellissier et al., 2018).

3.1.7.b. Prolonged and Excessive Opioid Stimulation of μ Receptor Agonists (Morphine)

Surprisingly, many experiments had shown that μ receptor agonists delivered at moderate to high doses or at persistent low doses can suppress social activities such as socio-sexual activity, maternal comportments, and the period of direct social experimentation, irrespective of social background. Ironically, former drug-dependent individuals in hospitals had social experiences with defects in their memory, experienced the sense of unrelatedness, and treated themselves autistically (chronically small dose) (Pellissier et al., 2018).

In other experiments a large dose of an acute or persistent μ receptor agonist decreased socio-sexual behavior, group interaction, and was harmful to socio animal long-term memory. However, long-term social activity in mice subjected to the doses of morphine or cocaine had serious deficits of social contact before seven weeks after the exposed to large doses of opiates therapy termination (Pellissier et al., 2018).

Noticeably, in abstinent animal's brain production of opioid peptides decreased after 4 weeks, indicating that prolonged optimum activation contributed to long-lasting down regulation of adaptive peptides. Accordingly, the reduced concentration levels of β-endorphin were distributed in opiate-abstinent patients without adequate therapy and a decreased in prefrontal cognitive response suggesting social activity deficits. Such results suggested together that μ receptor stimulation had deleteriously affected social activity when it was intense or sustained (Pellissier et al., 2018).

3.1.8. Vasoactive Intestinal Peptide

Vasoactive intestinal peptide (VIP) is the essential 28 amino acid peptide bound to the G protein-coupled receptor (GPCRs) that belongs to the class II group. It was commonly found in the human body and played a significant role in other biochemical functionalities. The vasoactive intestinal peptide works by three separate GPCRs such as PAC1, VPAC1, and VPAC2 that were found in several tissues, including the brain, heart, gastrointestinal system, kidney, linguistic tongue, and cells such as lymphocytes and macrophages that were immune-competent (Impagnatiello et al., 2000).

VIP transmission and signaling were gradually altered in various neurological diseases. VIP and its receptor might be therapeutic loci to cure many central nervous systems pathological condition.

The anatomy of many significant neurological diseases was discussed in this analysis along with the possible pharmacotherapeutic function of VIP and their receptors in therapies of autism spectrum disorders (Impagnatiello et al., 2000).

Although there was no clear common cause, a variety of striking variations from typical subjects had been seen in physiological and neuroanatomical studies of autistic patients. In the plasma, newborns later diagnosed with mental retardation or ASD had greater neuropeptide rates such as VIP, brain-derived neurotrophic factor, calcitonin-gene controlling peptide, and neurotrophin 4/5. A further correlation between ASD and VIP was also seen in a community of ASD individuals with inflammation of the intestine as VIP is an essential immunomodulatory and intestinal peptide factor (Impagnatiello et al., 2000).

3.1.8.a. Mechanism of Action of VIP

It had also been shown to be correlated with the gastrointestinal and stereotypical actions of autism patients in the upstream area of the VPAC2 receptor gene. The great scientists Asano and colleagues studied modifications to the VPAC2 gene in a limited group of autistic individuals in 2001and identified ten different polymorphisms. The most common variabile was a polymorphic single-nucleotide with no alteration found in amino acids. Preliminary results found that in the upstream portion of the gene in particular, three polymorphisms might have a function in autism (Impagnatiello et al., 2000).

However, the levels of VPAC2 polymorphisms in ASD patients with different control patients did not show a substantial variance. A social interaction deficiency was identified in mice after a VIP blockage during neural tube shutdown. This was close to the time when inappropriate neural growth contributing to ASD was believed to arise in human development (Impagnatiello et al., 2000).

The close connections between ASD and VIP also indicated this pluripotent peptide could have pharmacotherapy potential. Although analogs of VIP were not presently used for the treatment of ASD, this peptide might be a viable goal in potential drug production (Impagnatiello et al., 2000).

3.2. Metabolic Biomarkers

So far, there are no specific metabolic biomarkers that define autism. When ASD-related metabolic biomarkers are being analyzed, some possible metabolic changes can be observed over time. Individual children may have various metabolic disorders, nutritional shortages, and toxicity exposures associated with SNPs (single nucleotide polymorphisms). The presence of metabolic biomarkers such as biotinidase deficiency, purine metabolism disorders, phenylketonuria, creatine deficiency, excess propionic acid and lack of cerebral folate is responsible for causing autism disorders (Goldani et al., 2014).

A new study reviewed the literature on ASD-related physiological disorders. Several authors had classified these metabolic biomarkers into four categories like oxidative stress, immunologic, mitochondrial abnormalities, and environmental toxicants. Furthermore, the authors had collected reports on microglial activation, lipid, and microbiome and had shown how they played a role in producing biomarkers that create autism-like disorders (Goldani et al., 2014).

The biomarkers are interconnected to each other. When one biomarker becomes defective, it impacts on other biomarkers and shows its opposite activities. Many metabolic disorders can result in endpoints like methylation impairment, detoxification, sulfuration, and lack of nutrition.

The factors like metabolic imbalances, mitochondrial dysfunction, genetic sensibility, and environmental risk factors can direct oxidative stress which further causes autoimmunity, methylation impairment, destruction of cell membrane, inflammation, and neurological shortage (Goldani et al., 2014).

In children, particularly during their early development, the brain is highly susceptible to oxidative stress. As the above factors affect oxidative stress, they can immediately affect gene expression. Various studies had established altered selection volumes of substances relative to ASD subjects in body-based fluids. These reports represented two major mechanisms that cause disease; one is CNS disorder detected peripherally which include sulfate, serotonin, low level of oxytocin, and low level of gamma-aminobutyric acid. Another mechanism is a systemic abnormality that affects the brain (Goldani et al., 2014).

The metabolic biomarkers are described in detail below.

3.2.1. Oxidative Stress Biomarkers

These biomarkers can be identified by analyzing antioxidant status such as protein oxidation, lipid peroxidation, antioxidant enzymes, all of them have been noticed to be increased in autistic children. Various subgroups of ASD kids have distinct redox defects from different origins (Goldani et al., 2014).

Table 7: Oxidative stress biomarkers in autism spectrum disorder (ASD)

Biomarkers	Increased level	Decreased level	Location
Glutathione-reduced/oxidized		✔	Urine
Methionine		✔	Urine
Cysteine		✔	Urine
Organic acid test-alpha hydroxybutyrate, pyroglutamate and sulfate		✔	Urine
Plasma F2t-isoprostanes	✔		Urine
Urine8-OHdG	✔		Urine
Transferrin		✔	Serum
Ceruloplasmin		✔	Serum
Plasma 3-chlortyrosine (3CT)	✔		Plasma
3-Nitrotyrosine (3NT)	✔		Plasma

3NT= 3-nitrotyrosine, 3CT= 3-chlortyrosine (Goldani et al., 2014).

3.2.1.1. Glutathione-reduced/ oxidized

Oxidative stress occurs as antioxidant resistance mechanisms fail to efficiently prevent reactive oxygen species from exogenous or endogenous sources. Glutathione is the principal antioxidant that is responsible for the maintenance of reducing the intracellular microenvironment that is crucial to normal cellular viability and activity. Many oxidative stress markers in children with autism were previously reported in blood, including reductions in activities of antioxidant

enzymes, accelerated peroxidation of lipids and advanced glycemic result aggregation (Rose et al., 2012).

Kids with autism in three separate cases of irregular plasma concentration of metabolites were demonstrated in the glutathione redox synthesis pathway. In this research, the mean level of reduced glutathione (GSH), redox buffer, and principal intracellular antioxidant was found to have decreased significantly, while the oxidized glutathione disulfide (GSSG) was elevated significantly, resulting in reduction in the glutathione redox ratio (GSH/GSSG) in autistic children immune cells and plasma. Taken together, growing data indicated that autistic children had more oxidized intracellular and extracellular immune cell microenvironment than aged, normal regulated children (Rose et al., 2012).

The antioxidant level released into the urine was observed to be considerably smaller in the autistic subject than normal children. These results were relevant to the extent of the ASD (Goldani et al., 2014).

Furthermore, the study also discussed aconitase activity along with glutathione in different brain regions like cerebellum (CB) and Brodmann area 22 (BA22) that are related with autism. Irregular results in the brain superior temporal gyrus (STG) were considered to cause autism because it played a significant role in sound and speech production. The STG is made up of Brodmann area 22 (BA22) which corresponds to the area of Wernicke in the left hemisphere including sound processing (Rose et al., 2012).

3.2.1.1.a. Observational Condition

The aconitase and redox-sensitive enzyme had been calculated using an Aconitase Assay kit according to the guidance of the manufacturer for the assessment of a practical effect of

oxidative stress. The experiment was based on transforming citrate into isocitrate which further gets converted into ketoglutarate and results in NADPH production (Rose et al., 2012). The assay tested the rise in absorbance controlled at 340 nm correlated with NADH formation which is further proportional to lacotonase activity. In this study 12 control and 12 autism tissues from BA22 including 15 controls and 15 autism tissues from cerebellar cortex were examined (Rose et al., 2012).

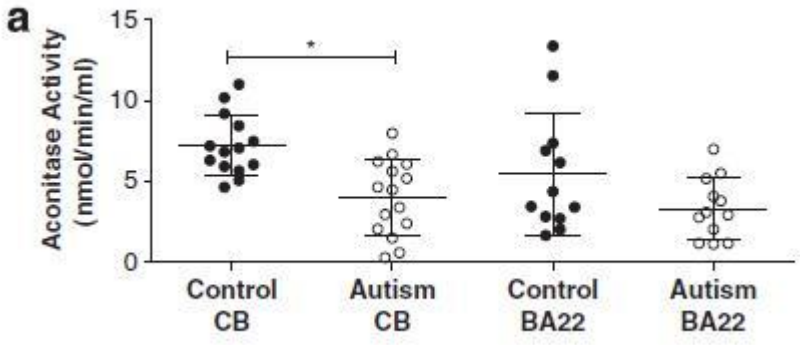

Figure 3: Aconitase activity in the cerebellum (CB) and Brodmann area 22 (BA22) in both autism and control group (Rose et al., 2012).

Figure 3 represented Aconitase activities ($nmolmin^{-1}ml^{-1}$) that were measured in both BA22 and autism samples. In autism cases, the mean cerebral aconitase activity was slightly less than in control cerebellum tissue ($P< 0.01$). There was a pattern in autism relative to controls in BA22 in terms of reduced aconitase activity; however, statistical relevance was not reached ($P= 0.1$). A positive relation between GSH/GSSG had been observed in the control and combined case located both in BA22 ($P= 0.03$) and cerebellum ($P= 0.01$) (Rose et al., 2012).

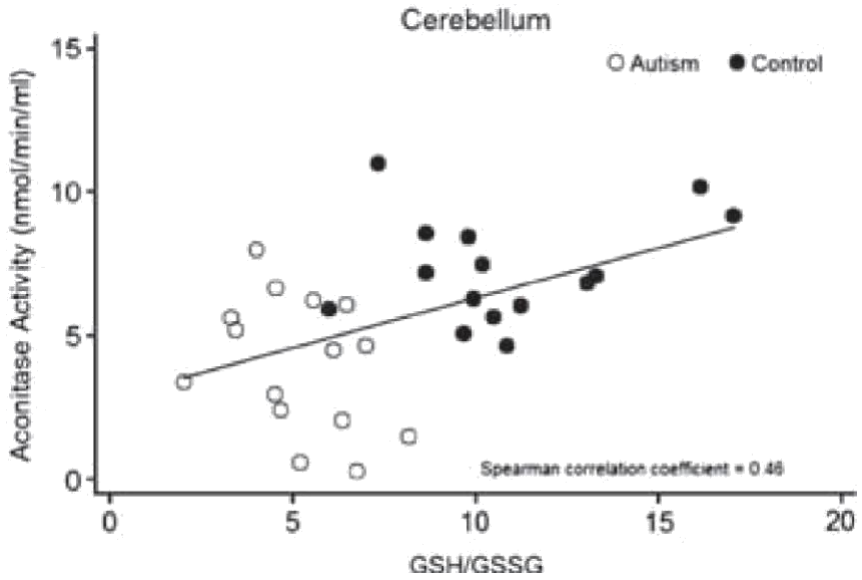

Figure 4: Acontinase activity was substantially linked to GSH/ GSSG (P = 0.01) in the control group and combined case of CB samples (Rose et al., 2012).

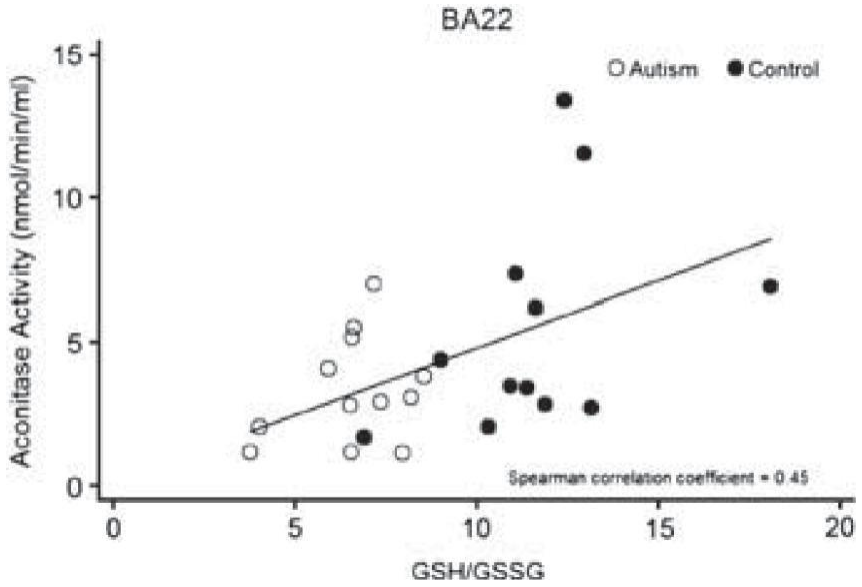

Figure 5: Aconitase function was equally significantly related to GSH/ GSSG within BA22 in the control samples and combined case (P= 0.03) (Rose et al., 2012).

Figure 4 and 5 indicated that autism cases were aggregated in the lower-left quadrant of the charts along with reduced aconitase activity and GSH/ GSSG relative to controls (Rose et al., 2012).

In the current investigation, this study demonstrated that decline in potential antioxidant/ glutathione-mediated redox previously observed in immune cells and plasma from autistic children had also been substantially decreased in two brain areas BA22 and cerebellum. This research results had indicated that in these two brain areas, oxidative damage markers in subjects with autism had been increased (Rose et al., 2012). So our findings will be that decreased levels of glutathione redox ratio (GSH/ GSSG) can lead to autistic disorders.

3.2.1.1.b. Glutathione Enzyme

Excessive reactive oxygen species (ROS) cause oxidative stress. The defense mechanism of antioxidants can be enzymatic (superoxide dismutase, glutathione peroxidase) or nonenzymatic (selenium, reduced glutathione, carotenoids, etc.) air for ROS detoxification. ROS may adversely affect biological molecules such as proteins, lipids, and DNA, resulting in cell dysfunction. Accumulation of ROS can result in the generation of anti-inflammatory and pro-inflammatory cell cytokines (Oshodi et al., 2017).

Glutathione S transferases (GST) are various functional enzymes that detoxify the toxic compounds caused by carcinogens, electrophilic components, and oxidative stress materials. They are associated with glutathione phase II metabolism of xenobiotics. There are eight categories of GSTs in the human body. Glutathione S transferase Theta, pi, and mu are located on chromosome 11q13, 22q11.2 and 1p13.3. They play an important role in the defense of cells from oxidative stress toxic materials. Polymorphisms of GSTT1 and GSTM1 emerge from gene

deletion resulting in a loss of enzyme activity in persons with no genotype GSTT1 and GSTM1. GST polymorphism was associated with a variety of oxidative stress-related disorders such as lung cell cancer, asthma, and Type 2 diabetes mellitus (Oshodi et al., 2017).

While autism had a clear genetic history, there was prevalent evidence that epigenetic, genetic and environmental influences were involved. The levels of these genetic differences had shown little variability of symptoms in the form of ASD. Single-nucleotide polymorphisms (SNP) had been investigated as genetic biomarkers that are used by researchers on genes connected with complex diseases. SNP was used to determine whether an individual had a single disease or a similar disease subtype. Models for SNP diagnosis were developed for many diseases that were useful for the detection of conditions such as asthma and hypertension. Some scientists had proposed that SNPs would have a precise description of the severity of the ASD symptoms (Oshodi et al., 2017).

3.2.1.2. F2t-Isoprostanes

To measure the exact oxidative stress levels in clinical populations were complicated because of some factors. Firstly, this study had tested plasma F2t-Isoprostanes (F_2-IsoPs) from the different techniques for quantifying metabolic dysfunction and oxidative stress. Isoprostanes are prostaglandin molecules made in vivo by arachidonic acid's non-enzymatic free radical and oxidation process. F2-IsoPs are biologically inactive and survive a long time (Gorrindo et al., 2013).

The biomarkers of oxidative stress analysis were commissioned by the NIEHS and had been found to be the most important measures for redox dysfunction and to be a gold standard oxidative stress test. F2-IsoPs were increased across different organ systems of the autistic

subject in a range of disorders. To gain sufficient statistical capacity to resolve the possible variability and variances between comparative categories, the researchers had collected data on a larger sample size to date (Gorrindo et al., 2013).

Furthermore, the researchers had involved three clinical groups such as ASD only, GID (gastrointestinal dysfunction) only, and ASD-GID and one control group without intimacy which allowed the role of functional stratification. This study also noted that despite the high levels of oxidative stress in all three categories, a previously unidentified extreme subgroup of children may be found within the ASD-GID group, specifically highly in risk for metabolic disorders (Gorrindo et al., 2013).

3.2.1.3. Urine 8-hydroxy-2-deoxyguanosine (8-OHdG)

8-OHdG is one of the oxidized metabolites most commonly studied and was considered to be an oxidative damage biomarker of DNA. Kasai and Nishimura first reported the formation of 8 OHdGs by oxygen radicals in 1984. The association with the nuclear bases on the DNA chain, such as guanine contributed to the production of 8-OHdG by the hydroxyl radical which was the most powerful oxygen-free radical. Some diseases like obstructive chronic pulmonary diseases, cardiovascular and autism-like disorders had been linked to excessive levels of 8-oHdG. These markers were often elevated because of obesity, smoking, chemical, physical and biological factors (Graille et al., 2020).

Recent research proposed that 8-OHdG was the most appropriate biomarker for oxidative stress in spot urine samples with high-intraclass association coefficients for reproductive measurements and low variance coefficients. Urinary oxidative stress marker levels had been suggested as

effective biomarkers to study population's exhibiting xenobiotics such as particles, most recently developed nanomaterials and oxidizing agents (Graille et al., 2020).

There were some benefits to measuring the urinary 8-OHdG as they were very stable and noninvasive in urine and its excretion process is similar to oxidative DNA damage reflection. It could be measured by the two following methods; one was a mass-based process like GC, LC and another was immunological methods. Urinary 8-OHdG also could be obtained from another source which was DNA polymerase-dependent addition of 8-oxodGTP from the nucleotide pool. Chromatographic procedures were known as the gold standard method; however, less expensive and long-term immunologic approaches had been commonly used since enzyme-related immunosorbent assay kits had been created to easily detect and quantify 8-OHdG (Graille et al., 2020).

The levels of 8-OHdG were tested and reported in different research for autistic subjects and healthy peoples. However, this study revealed a broader spectrum of values, making it difficult to define background-cutting values (Graille et al., 2020).

3.2.1.4. Ceruloplasmin

Ceruloplasmin is the most important carrier for copper. They are the ferroxidase enzyme. It is an enzyme in the liver that contains six copper atoms in its form. When the level of the ceruloplasmin decreases, copper concentration increases in the serum. The extra copper in the serum can develop free radical production and lead to DNA breakage, mitochondrial damage, and neuronal injury. Copper is a part of a variety of metalloenzymes related to dopamine synthesis, which involve antagonism in the processing of dopamine or a mechanism of disintegration in biochemical forms (El-Baz et al., 2018).

Since dopamine is associated with autism, copper homeostasis can be especially important. The presence of excess copper can cause dopamine dysregulation. The recent study had shown that copper was statistically higher in autistic children than in the control group. Their research also concluded that the copper level might be connected with autism severity of the infant (El-Baz et al., 2018).

3.2.1.5. 3-Nitrotyrosine (3NT)

Although the pathogenesis of neuropsychiatric and neurodegenerative diseases includes oxidative stress with a wide body of search, comparatively few structural studies recorded shifts in oxidative stressors, especially concerning protein modifications. Particularly, 3-NT is an oxidative stress biomarker that damages proteins. Increased 3-NT brain concentrations had been recorded with immunohistochemical procedures in Parkinson's and Alzheimer's disease (Sajdel-Sulkowska et al., 2008).

Although the latest research suggested a relative growth in the brain content of 3-NT in Alzheimer's disease, measurable levels of the oxidative stress biomarkers in people were not documented. The concentration of 3-NT in the mouse liver was recorded in 0.17-0.3 pmol/mg of protein. Therefore, this study included new findings on 3-NT levels in the brain and proved that 3-NT levels increase in the brain of the autistic subject (Sajdel-Sulkowska et al., 2008).

An increase in protein nitration had been reported in multiple diseases. In the proteogenomic method, unique target proteins had been identified in Alzheimer patients' hippocampus. In a recent report, 32 specific nitrotyrosine sites were found in 29 proteins, of which more than half were implicated in Alzheimer's, Parkinson's disease and others. In terms of the nitration of

various brain proteins, synaptophysin, three-glial fibrillary acid protein (GFAP) and superoxide dismutase (SOD) may be especially connected to autism (Sajdel-Sulkowska et al., 2008).

3.2.1.5.a. Observational Condition

GFAP level was increased in the brain CSF fluids of the autistic children. Nitration of GFAP had occurred in the mice's brain that was defined by the proteomics method. In autistic subjects, a decreased number of synaptophysin-expressing synaptic vehicles were found. The researchers identified that reduction in the level of synaptophysin in the cerebella indicates autistic-like disorders. Decreasing in synaptophysin expression can lead to alteration in behaviors related to autistic subjects since there was a substantial decrease in the synaptophysin levels in animals with spatial learning deficiency (Sajdel-Sulkowska et al., 2008).

After the infusion of amyloid beta-protein, synaptophysin was the primary component of nitration in the rat hippocampus. SOD is an endogenous superoxide anion scavenger. Declining SOD levels in plasma were found in autism. SOD nitration was found in the aging heart and under oxidative stress conditions in mice. Thus, the rise in the average amount of nitration in the cerebella of autism subjects indicated oxidative stress-related protein modifications specific to autistic pathology. Altered protein levels along with their oxidative alteration may greatly influence the development of the cerebellar, contributing to autism pathology (Sajdel-Sulkowska et al., 2008).

3.2.1.6. Serotonin and Oxytocin

Excessive blood serotonin concentrations or hyperserotonemia (5-hydroxytryptamine; 5-HT) was present in 25-35 percent of children with ASD. Entire blood 5-HT was almost completely found in platelets that picked up 5-HT through the serotonin carrier and traveled into the enteric

bloodstream. In this system, 5-HT was produced and released by the cells of enterochromaffin. The blood levels of 5-HT were associated with ASD children and their families. 5-HT concentrations in large inbred population research were noticed to have a heritability of ~1.0 (Hoda Badr, et al., 2011).

Anomalies in the brain serotonin system were also recorded in ASD including reports of impaired serotonin production and binding of receptor and one observation of serotonergic dystrophic axons. Thus, the general research included the 5-HT mechanism in ASD. However, attempts to correlate heritable and robust entire blood concentrations of 5-HT with clinical symptoms were contradictory, with sporadic reports of stereotyped or self-injurious conduct (Hoda Badr, et al., 2011).

3.2.1.6.a. Different Observational Condition of Oxytocin

The levels of blood oxytocin were also included in ASD studies. The autistic kids had lower overall blood Oxytocin (OT) levels compared to normal children. An analysis of adults with ASD indicates that they had a higher level of OT at baseline. The value of the oxytocin mechanism in ASD was recognized in social mammalian behavior including social understanding, attachment and family behavior. Without the oxytocin receptor gene, mice had shown decreased social recognition, low reversal learning, increased susceptibility to epilepsy, and a laboratory model of cognitive versatility (Hoda Badr, et al., 2011).

Genetic studies indicated a mild synthesis of the gene of oxytocin receptors with ASD. Allelic difference in CD38, as well as key signaling molecules, a positive OT release regulator also had shown a connection with ASD. Based on genetic and biomarker evidence, as well as the impact of OT on species-wide associate behavior, the possible application of OT in ASD is of great

concern. Initial trials of intranasal or intravenous OT were positive 75-79; however, there was still inadequate evidence available to determine whether this results in lasting gain or was acceptable for long-term use in children or adults (Hoda Badr, et al., 2011).

3.2.1.6.b. Mechanism of Action of Serotonin and Oxytocin

The mechanism of oxytocin and serotonin communicated in the brain throughout infancy and in adulthood. When voles or rats were exposed to a non-selective serotonin receptor agonist, it led to lower social contact, less affiliating activity and fewer oxytocin cells in the hypothalamus paraventricular nucleus. In comparison, early exposure of pups to exogenous OT contributed to greater serotonin axons in many regions of the brain. In humans, the 5-HT mechanism controlled the release of OT in adults and proved that the higher level of OT was treated with 3, 4-methylenedioxymethamphetamine that helped to release the 5-HT. Also, in the adult mouse, OT functioned as an antidepressant by oxytocin receptors in the serotonin neurons (Hoda Badr, et al., 2011).

The different proof lines of blood 5-HT and oxytocin tested as biomarkers in ASD, together with proof of interactions between the respective brain structures. These observational biomarkers research with an experimental model in mice that lacks an oxytocin receptor gene, examining whether oxytocin system genetic modulation might influence the peripheral 5-HT whole blood biomarker (Hoda Badr, et al., 2011).

4. Conclusion

ASD (autism spectrum disorder) is not a single disorder. It comes with multiple disorders along with abnormalities of various biomarkers that are described in our whole review. A single biomarker cannot be able to identify autism spectrum disorder conditions. However, synthesis of

many biomarker arrays may differentiate between various autism spectrum disorders with the use of biostatistics and bioinformatics (Ratajczak, 2011).

The increasing or decreasing levels of the biomarkers of the gastrointestinal (GI) and metabolic system that lead to autism are described in this study. Further, the abnormal levels of these biomarkers are directly related with neurodevelopmental disorders which may later define as autism like disorders like speech problems, disconnected from the social interaction etc. In other words, if individually irrelevant biomarkers are examined together, they produce a clinically relevant trend such as diagnosis, serious stage or treatment response (Goldani et al., 2014).

In this study, the data were included which contains statistically important results ($P < 0.05$) relative to neurotypically age and sex compared persons. However, it cannot be clarified without more study how much a difference in biomarker expression suggests autism. Therefore, more analysis of GI and metabolic biomarkers are needed with huge samples to determine the cause of autism (Ratajczak, 2011).

5. Limitations of the study

This study had a couple of limitations.

1. In this study, all of the individual information of the gastrointestinal (GI) and metabolic system biomarkers were not included because some of them were still unknown with their proper mechanism.
2. In the original article the specific values of separate biomarkers were not contained that caused autism. It was another limitation of our study. More laboratory works and analysis were required to establish this review.

6. Future Research Plan

Gray and white matter for whole brain MRI scans using the computer algorithm program (super vector machine), developments in proteomic and chromatographic techniques along with multiplex immunoassays can facilitate easier examination of various biomarkers specific to autism. A review of these methods can also be conducted in the future with a wider view of these biomarkers and their mechanisms.

7. References

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