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Biomarkers in Autism

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Abstract: Prevalence of neurodevelopmental disorders is increasing now-a-days with the incidence of Autism spectrum disorder gaining momentum. It is a complex neurodevelopmental disorder classified as a pervasive developmental disorder of childhood characterized by behavioral abnormalities in social interaction; in communication; and restricted repetitive and stereotyped patterns of behaviors, interests and activities. Detection of autism becomes easier when we know the biomarkers. This review focuses on common biomarkers that can effectively and efficiently detect the occurrence of Autism Spectrum Disorder. Detection of this disorder at an early stage is possible by analyzing biochemical markers that provide inkling about the development of Autism spectrum disorder.

Keywords: Neurodevelopment disorder, Autism, Biomarkers.

Introduction:

"Neurodevelopmental disorders'' encompasses a large group of disorders in which the onset is during periods of ongoing maturation and development. They originate during fetal life and could be influenced by genetic, intrauterine and extrauterine factors that affect the fetal-maternal environment. Changes in development can produce alterations in neurogenesis, cell migration, and neuronal connectivity that are responsible for cognitive deficits in adults. Abnormalities in brain structure, resulting from perturbed development, are often associated with neurodevelopmental disorders [1]. Disturbances directed at the maternal host during pregnancy can lead to direct physiological changes in the fetal environment and negatively affect the normal course of early brain development in the offspring [2].

Fetal neurodevelopment depends on cell programs, developmental trajectories, synaptic plasticity, and oligodendrocyte maturation, which could be altered by stress, endocrine disruption, exposure to pesticides such as chlorpyrifos and to drugs such as terbutaline, maternal teratogenic alleles, and premature birth. Different neurodevelopmental disorders are developmental dyslexia, developmental dyscalculia, developmental coordination disorder, speech sound disorder, specific language impairment, attention deficit hyperactivity disorder, autistic spectrum disorder, Tourette syndrome, intellectual disability, Angelman syndrome, cerebral palsy, Cornelia de Lange syndrome, Cri du chat syndrome, Down syndrome, Duchenne muscular dystrophy, epilepsy, fetal alcohol syndrome, fragile X syndrome, galactosaemia, Klinefelter syndrome, Lesch-Nyhan syndrome, Lowe syndrome, Marfan syndrome, neurofibromatosis type 1, Noonan syndrome, phenylketonuria, Prader-Willi syndrome, Rett syndrome, Rubinstein-Taybi syndrome, trisomy 18, tuberous sclerosis, Turner syndrome, velocardiofacial syndrome, Williams syndrome, XXX and XYY [3].

One such neurodevelopmental disorder gaining momentum in the recent years is Autism Spectrum Disorder (ASD). Autism is a neurodevelopmental disorder beginning before the age of 3 years. It is classified as a pervasive developmental

disorder of childhood, characterized by behavioral abnormalities in social interaction; in communication; and restricted repetitive and stereotyped patterns of behaviors, interests and activities [4]. It is four times more common in boys than girls with an overall incidence of 5/10,000 [5]. The pathogenesis of autism is uncertain, but is thought to involve an interaction between multiple susceptibility genes and/or epigenetic effects and/or environmental factors. Currently, there appears to be a pronounced increase in the incidence of autism, with some authors suggesting as much as a 30-fold increase [6].

Susceptibility to autism is clearly attributable to genetic factor. The etiology of autism is not known but it has a strong genetic component [7] and exposure to teratogens appears to be a risk factor for the disorder. Thalidomide, valproic acid, and ethanol are some of the teratogens which can induce ASD.

Many areas of brain show abnormalities characterized by decreased Purkinje cell counts in cerebellar hemispheres, vermis, loss of granular cells and Purkinje cell atrophy. These abnormalities may be associated with disturbances of visuospatial-integration, impaired language, apraxia, and agnosia.

Biomarker selection:

Biomarkers should ideally be quantitative biological measures with an accurate indication of a specific mechanism and ideally are not invasive. Identifying biomarkers will almost certainly yield more successful genetic and epigenetic studies, leading to a better understanding of the pathogenesis required to design the most effective treatments for each type of autism. This is being exemplified in neurodevelopmental disorders such as Huntington's disease, Rett's Disorder and Fragile X, where the identification of a biomarker has led to the successful genetic identification of the cause of the disease and the evolution of new, targeted treatments. Thus, biomarkers can be used to identify a subgroup of autism; identify specific mechanisms of diseases or pathogenesis; and serve as an indicator of the efficacy of a treatment, as a surrogate, or correlate with improved behavioral outcomes with given treatments.

Inflammation:

The contribution of inflammatory processes to the etiology of individuals with autism is supported by an increasing number of studies [8]. The immune and nervous systems interact in health and disease, with both systems able to impact the development and function of the other. Abnormalities of the immune cells in brain - the microglia - and abnormalities in the peripheral immune system, including cytokines, leukocytes, and antibodies, have been reported in autism.

Cytokines:

Cytokines are protein messengers produced by immune cells that regulate immune responses and directly aid in immune protection. Cytokine production increases during immune activation, and various cytokines are known to have effects on the central nervous system (CNS). Cytokines can influence the development of the nervous system, ultimately affecting both cognitive and emotional processing [8]. Cytokines stimulate virtually every cell in the body, including neurons and glial cells in the brain. Cytokines are possible biomarker for evaluating inflammatory processes in autism. Many studies reveal increased levels of pro-inflammatory cytokines in brain, cerebrospinal fluid (CSF), and blood from children with autism.

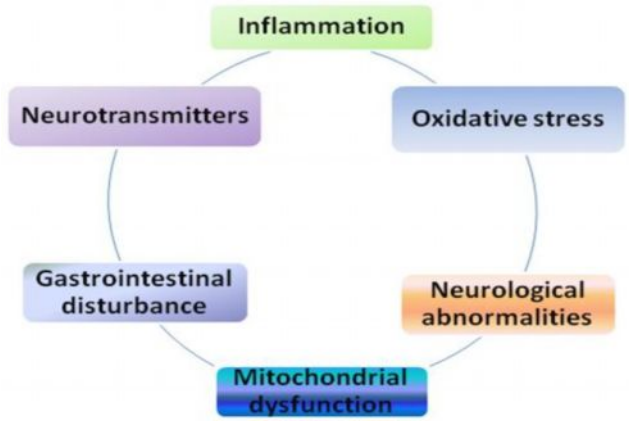

Fig 1: Different Biomarkers in autism

Inflammatory cytokines including tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-1β and IL-12 are elevated in the blood mononuclear cells, serum and plasma of autistic subjects. Recent studies have also demonstrated that transforming growth factor (TGF)-β1, macrophage chemoattractant protein (MCP)-1, IL-6, IL-8, IL-10, TNF-α and IFN-γ are increased in the frontal cortices of autistic brains. Furthermore, MCP-1, IL-6, IL-8, IFN-γ and TNF-α were found to be significantly increased in the CSF of autistic children. All this evidence suggests that inflammation and cytokines may play an important role in the pathogenesis of autism. The quantification of cytokines such as TNF-α, IFN-γ, IL-1β, IL-6, IL-8, IL-10 and IL-12 can be done by using human multiplexing bead immunoassays that are based on a sandwich immunoassay that utilize the Luminex fluorescent-bead-based technology.

Although cytokines are promising biomarkers for inflammation, many other inflammatory biomarkers must be considered, including lymphocytes (T cells, natural killer cells, and B cells) and autoantibodies, which have been found to be dysregulated in some children with autism.

In addition, a specific pattern of immune dysregulation that includes elevated T- lymphocyte production of TNF-α and IFN-γ and reduced levels of IL-10 was recently described in colonic, upper and lower small intestine mucosal samples, as well as plasma samples in children with autism and gastrointestinal (GI) symptoms [9]. In contrast to increased pro-inflammatory cytokine release, IL-10, an anti-inflammatory cytokine, was significantly reduced in children with autism and GI symptom. These results suggest that there is a type of autism associated with significant GI symptoms for which the balance between proinflammatory and anti-inflammatory cytokines may be used as biomarkers.

Neopterin:

Neopterin is a metabolite that is produced in high amounts by monocytes and macrophages during periods of immune activation, acute infections, autoimmune disease, and malignancies. Measures of neopterin in autistic children vary depending on the preparation of the sample. Neopterin levels were significantly decreased in the autistic group compared to control blood or urine with oxidative pretreatment. When no oxidative pretreatment was used, urinary neopterin measures were significantly increased in the autistic children. Neopterin and quinolinic acid were decreased and biopterin was increased in the autistic youth in the nonoxidized CSF.

Oxidative stress:

Oxidative stress occurs when there is excessive production of reactive oxygen species (ROS). It is suggested that autism may result from an interaction between genetic, environmental, and immunological factors, with oxidative stress as a mechanism linking these risk factors. Under normal conditions, a dynamic equilibrium exists between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cell. ROS includes superoxide (O2•−), hydroxyl, peroxyl, alkoxy, and nitric oxide (NO) free radicals. Stress and injury to cells occur when redox homeostasis is altered, and ROS generation overpowers the biochemical defenses of the cell. Lipid peroxidation reflects a chain reaction between polyunsaturated fatty acids and ROS producing lipid peroxides and hydrocarbon polymers that are both highly toxic to the cell. Superoxide is the first reduction product of molecular oxygen, and it is an important source of hydroperoxides and deleterious free radicals. Most toxic effects are due to hydroxyl radical formation, which also initiates lipid peroxidation. Some endogenous enzymes such as xanthine oxidase (XO), nitric oxide synthase (NOS), and monoamine oxidase (MAO) can directly produce ROS. Superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSHPx) are the primary enzymes involved in direct elimination of ROS, whereas glutathione reductase (GSH) and glucose-6-phosphate dehydrogenase are secondary antioxidant enzymes, which help in maintaining a steady concentration of glutathione and nicotineamide adenine dinucleotide phosphate (NADPH) necessary for optimal functioning of the primary antioxidant enzymes.

Increased lipid peroxidation in autism:

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. 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. Zoroglu et al. have reported increased thobarbituric acid reactive substances (TBARS) in erythrocytes of patients with autism as compared to normal controls [10]. MDA levels, referred as TBARS, can be measured from the method described by Wasowicz et al method [11].

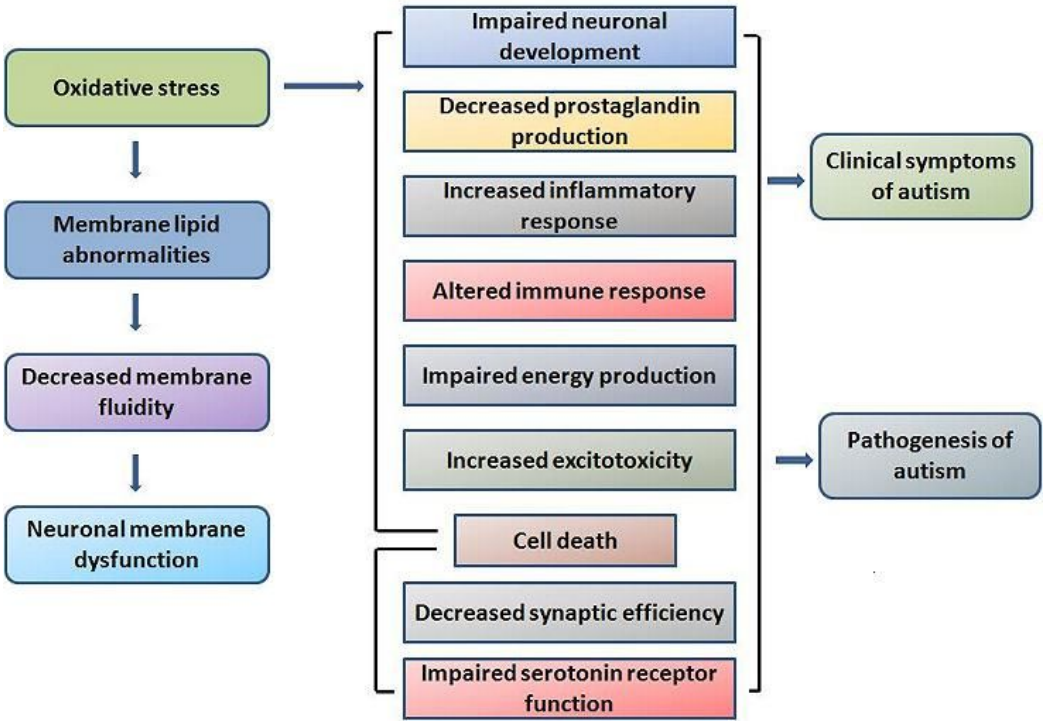

Fig 2: Schematic depiction of potential that may result in neuronal dysfunction and clinical symptoms in autism.

Alterations in antioxidant enzymes in autism:

Several studies have suggested alterations in the enzymes that play a vital role in the defense mechanism against damage by ROS in autism. Patients with autism showed decreased activity of GSHPx in plasma and in erythrocytes compared with the controls. Reduced levels of total glutathione and lower redox ratio of GSH to oxidized glutathione (GSSG) in plasma and decreased catalase and SOD activity in erythrocytes was also observed.

GSHPx activity in plasma and erythrocytes can be measured according to the method of Paglia and Valentine [12]. The levels of reduced and oxidized glutathione (GSH and GSSG) in plasma can estimated by method of Hissin and Hilf [13]. Catalase activity in erythrocytes can be measured by Aebi method [14]. SOD in erythrocytes can be assayed by using RANSOD kit U.K.

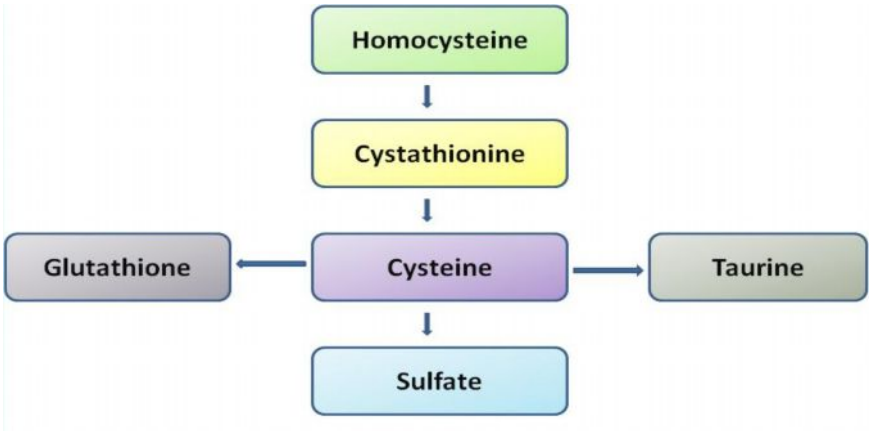

Fig 3: Transulfuration pathway

Increased nitric oxide in autism:

Nitric oxide is another toxic free radical that can react with superoxide anion and generate cytotoxic peroxynitrate anions (ONOO−). NO is known to affect the development and function of the CNS. The expression of inducible nitric oxide synthase (iNOS) and production of NO are also known to affect inflammatory processes. The induction of iNOS is mediated by the cytokines, namely IFN-γ, TNF-α and IL-1β.

Sogut et al., 2003 [15] have reported increased NO levels in erythrocytes of patients with autism and have suggested that NOS may be activated in autism. A positive correlation was observed between nitrates and IFN-γ levels in the autistic subjects, indicating that elevated plasma NO may be related to IFN-γ activity in autism. Decreased activity of receptors sensitive to NO or increased oxidative stress has also been reported in autism. The cholinergic receptors known to be sensitive to NO toxicity were decreased in the cortex of patients with autism. Additionally, treatment with cholinergic agonists improved behavioral abnormalities in autism. In other studies, gamma aminobutyric acid (GABA) receptors that are sensitive to oxidative stress were reduced in the hippocampus of patients with autism. Plasma NO levels can be measured using the Griess reagent as described by Moshage et al, 1995 [16].

Increased xanthine oxidase in autism:

Xanthine oxidase is an endogenous pro-oxidant that produces superoxide radicals during conversion of xanthine to uric acid. Increased XO activity has been reported in the erythrocytes of patients with autism. XO activity in erythrocytes can be determined by using Prajda and Weber's [17] method.

Abnormal iron and copper metabolism in autism:

Ceruloplasmin is a copper-transporting protein and transferrin is iron-transporting protein are major antioxidant proteins that are synthesized in several tissues including brain. Ceruloplasmin inhibits the peroxidation of membrane lipids catalyzed by metal ions, such as iron and copper. Transferrin acts as an antioxidant by reducing the concentration of free ferrous ion. Levels of ceruloplasmin and transferrin are reduced in the serum of children with autism as compared to their unaffected siblings [18]. Children with autism who had lost previously acquired language skills had reduced levels of ceruloplasmin and transferrin [18]. These results suggest that there is an altered regulation of transferrin and ceruloplasmin in a subset of children with autism. Such alterations may lead to abnormal iron and copper metabolism that may play a pathological role in autism. In fact, some preliminary studies have suggested altered serum Cu/Zn ratios in autism. Levels of ceruloplasmin can be estimated by colorimetric method by Karl et al [19]. Transferrin can be estimated by using Buglanov et al., method [20].

Neurological abnormalities:

Cerebellar anatomic abnormality in autism:

In postmortem studies of the brains of individuals with autism, the cerebellum has been the most consistently observed site of pathology. The most frequently reported pathology in these studies is a reduction in the normal number of Purkinje neurons, which has been reported when the cerebellum was, examined [21].

Purkinje cell loss in Autism:

One of the most consistent neurological abnormalities by histopathological post-mortem studies revealed Purkinje cell loss in the cerebellum and atrophy of the cerebellar folia in autism [22]. Research suggests Purkinje cells die relatively easily compared to other types of neurons [23]. Several studies have shown that Purkinje cell loss can result from insult, inflicted by ischemia; hypoxia; excitotoxicity; G protein dysfunction; viral infections; vitamin deficiencies; heavy metals (e.g. mercury, lead, bismuth); toxins (e.g. bilirubin, phenytoin, ethanol, alkaloids, toluene); as well as from chronic malabsorption syndrome (e.g. celiac disease, inflammatory bowel disease).

Increased Microglial and Astroglial Activation:

Neuroglial cells such as astrocytes and microglia, along with perivascular macrophages and endothelial cells play important roles in neuronal function and homeostasis. The neuroglial activation was particularly prominent in the granular cell layer and white matter of the cerebellum [24]. Analysis of the neuropathological changes in brain tissues of autistic patients showed extensive neuroglial responses characterized by microglial and astroglial activation. Reduction in the size of cerebellar regions such as the vermis, an increase in white matter volume, and reduction in the gray/white matter ratio are the most prominent changes observed in the cerebellum**.**

Amygdala abnormalities:

Abnormalities consisted of small neuronal size and increased cell packing density predominantly in the cortical, medial, and central nuclei of the amygdala, whereas the basolateral complex showed an intermediate degree of involvement [25].

Hippocampal abnormalities:

Relatively small and densely packed neurons were also observed in hippocampal fields CA1–CA4, the subiculum, entorhinal cortex, mammillary bodies, medial septal nucleus, and anterior cingulate gyrus of all the autistic brains [25]. Abnormalities in the ventricles, in particular enlargement of the left temporal horn are particularly found in autistic children.

Mitochondrial dysfunction:

Mitochondrial disorders often result in CNS dysfunction, leading to developmental regression, learning disability, and various behavioural disturbances. Autism can represent the main clinical presentation of a mitochondrial disease [26].

Blood ammonia:

Ammonia is derived from the deamination of the amine group of amino acids by gut bacteria or the liver. The process of detoxifying ammonia via the urea cycle is metabolically expensive and expends three valuable, high-energy adenosine triphosphate (ATP) molecules for every ammonia molecule processed. Hyperammonemia is more toxic for children than adults and can lead to permanent CNS damage. In several studies of individuals with autism, an increased level of ammonia has been reported. Ammonia levels can be estimated by Dienst SG method 1961 [27].

Lactate:

Lactate is a by-product of the anaerobic metabolism of glucose. Lactic acidosis has frequently been found in association with autism [28]. Lactic acidosis may be secondary to pyruvate dehydrogenase deficiency or mitochondrial respiratory chain defects including co-enzyme Q and cytochrome oxidase deficiency. Rett syndrome, a pervasive developmental disorder clinically similar to autism, has been associated with lactic acidosis and respiratory chain abnormalities. Marked increase in analogs of Krebs cycle metabolites were detected in the urine of autistic patients. These metabolites may be involved in disturbances of mitochondrial Krebs cycle function, producing defects in brain bioenergetic metabolism. Lactate can be assayed by the method of Everse [29].

Carnitine deficiency:

Carnitine deficiency is commonly found associated with autistic patients. Carnitine is essential for the utilization of fatty acids by the mitochondria, and deficient state results in impaired ATP production and decreased availability of high energy phosphate compounds. Carnitine levels can be measured by using Tenebrio bioassay [30].

Gastrointestinal Biomarkers:

Gastrointestinal inflammation has been described in many children with ASD [31]. Reflux, constipation, sensitivity to food, abnormal flora, abnormal stool pattern, frequent constipation, frequent vomiting, and frequent abdominal pain not associated with constipation are often encountered in autism.

GI ailments, including abdominal pain, diarrhea, and bloating, were examined extensively. Lymph nodules are encapsulated bodies lying within the submucosa of the intestinal wall, containing lymphocytes and neutrophils. The fluid absorbed from the intestinal lumen by the action of the absorptive epithelial cells is filtered through the lymph nodes. Endoscopy revealed that 10 of the 12 children displayed ileal lymphoid nodular hyperplasia (LNH). Of the 12 children, eight also displayed abnormalities in the mucosa, the region consisting of the absorptive epithelium, underlying connective tissue, and muscularis mucosae. Mucosal abnormalities included granularity, loss of vascular pattern, and patchy erythema (nonspecific colitis). Celiac disease also afflicts autism children, characterized by marked atrophy of the intestinal villi caused by a response of the intestinal immune system to gliadin, a peptide in gluten.

Neurotransmitters:

Serotonin (5-HT), glutamate, GABA, and dopamine (DA) are neurotransmitters that are critical for neurodevelopment, including cell proliferation, migration, differentiation, apoptosis, synaptogenesis, and synaptic plasticity [32]. Therefore, abnormalities of any of these neurotransmitters could be detrimental for neuronal development and potentially be associated with abnormal neuronal function in autism.

5-HT abnormalities:

Contradictory results have been reported on the involvement of the serotinergic system in autism [33]. Abnormalities of 5HT metabolism include 5HT transporter, tryptophan hydroxylase-2 (TPH2), tryptophan 2, 3 diooxygenase, 5HT receptor sensitivity and altered 5HT levels as a function of age were observed. The variable response to selective serotonin re-uptake inhibitors (SSRI) medications and atypical antipsychotic medications suggests a role for 5HT in at least some children with autism. 5-HT can be estimated by the method of Tagari et al [34]. Early studies of blood serotonin in autism consistently found hyperserotonemia in one-third of people with autism. Platelet 5-HT concentrations were determined by the spectrofluorimetric method Muck-Seler et al [35].

Dopamine:

In general, the dopaminergic system is thought to affect a wide range of behaviors and functions, including cognition, motor function, brain-stimulation reward mechanisms, eating, drinking behaviors, sexual behavior, neuroendocrine regulation, and selective attention. Once released from the neuron, central DA is broken down into homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC). These substances, as well as DA itself, can be measured in both blood and urine. Measurements of urinary excretion of DA and HVA in autism have been essentially equivocal. At this point, there does not appear to be any evidence of peripheral dopaminergic abnormalities of autism. Approximately 50% of subjects with autism exhibited significantly elevated levels of HVA in CSF [36]. HVA in CSF can be measured by using method by Sharman [37].

Glutamate:

The occurrence of seizures in many subjects with autism has suggested an imbalance of excitatory and inhibitory transmitters. Glutamate, the primary excitatory neurotransmitter, is elevated in plasma and CSF in many children with autism. Increased expression of the glutamate transporter and polymorphisms in genes encoding metabotropic and ionotropic glutamate receptors are also reported in children with autism. Abnormalities of glutamate could contribute to excitotoxicity, neuronal cell death and seizures. It has been suggested that the brain pathology in autism is consistent with excitotoxicity since there are reduced functional dendrites and decreased numbers of Purkinje cells. Glutamic acid can be estimated by the analytical method of Young and Lowry [38].

GABA:

Gamma aminobutyric acid function may also be abnormal. Metabolites of GABA, the primary inhibitory neurotransmitter, may be reduced in many children with autism [39]. Reduced GABA transmission has been associated with hyperkinesis, impaired sleep, seizures, mental retardation, impaired motor coordination, and hyperactivity, which are symptoms commonly observed in autism. However, contradictory data report elevated GABA in some children with autism that may be associated with a subgroup of affected individuals with catatonic symptoms [40]. GABA content can be estimated according to the method of Lowe et al [41].

Conclusion:

Autism is a neurodevelopmental disorder, characterized by behavioral abnormalities in social interaction; in communication; and restricted repetitive and stereotyped patterns of behaviors, interests and activities. An increasing prevalence of this neurodevelopmental disorder shows the importance of several biomarkers in the disease diagnosis and treatment. Alterations in neurotransmitters, oxidative damage, neuroinflammation, mitochondrial dysfunction, neurological abnormalities in brain and gastrointestinal disturbances play a promising in the pathology of disease. Periodic diagnosis of these biomarkers in biological samples will provide a basement for effective and efficient therapy.

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