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Review

The Role of Oxidative Stress in Autism Spectrum Disorder Pathophysiology, Diagnosis and Treatment

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Abstract: Autism spectrum disorder is an important health problem with no known single cause. There is a vast number of evidence showing the important role of oxidative stress in that disorder. The article's authors made the current literature research to summarize knowledge on that topic. In this paper the role of oxidative stress was studied in the context of its influence on pathogenesis, using oxidative stress biomarkers as diagnostic tools and use of antioxidants in ASD treatment. There is growing data on the involvement of oxidative stress in ASD pathogenesis as well as its direct effect and also by its interlay with inflammation and mitochondrial dysfunction. Oxidative stress biomarkers seem to have good potential to be used as diagnostic tools supporting early ASD diagnosis. Antioxidants show good potential in ASD-supportive treatment. In all described fields data support the importance of oxidative stress but also a need for further research.

Keywords: ASD; oxidative stress; oxidative stress biomarkers; antioxidants

1. Introduction

Autism spectrum disorder (ASD) is a global problem, sometimes it is called hidden disability. This is a neurodevelopmental disease described according to DSM V (Diagnostic and Statistical Manual of Mental Disorders 5th edition) criteria as a type of pervasive developmental disorder that is defined by: (a) persistent deficits in social communication and social interaction across multiple contexts,(b) restricted, repetitive patterns of behaviour, interests, or activities, (c)symptoms must be present in the early developmental period and cause impairment in different areas of functioning. The aetiology of the disorder is multifactorial. The prevalence of ASD has been increasing in the last 3 decades The median prevalence of 1/100 (range: 1.09/10,000 to 436.0/10,000) [1] The prevalence is higher in males with male-to-female ratio - 4.2 [2] The diagnosis is made by evaluation of the patient according to the behavioural and clinical criteria.

The average age of diagnosis is 3,5 years. [3], although some symptoms are usually apparent from the first year of life. Early diagnosis would enable the proper therapy to start, which may bring better outcomes. Until now, there is no single biomarker, neuroimaging finding or genetic mutation that has sufficient power in ASD diagnosis. That is because there is no known single main factor causing autism. Nowadays there is growing evidence of the important multimodal role of oxidative stress in ASD. The article aims to find how the knowledge of oxidative stress in ASD can be used in clinical practice.

There is vast evidence that [4–13] has indicated the presence of oxidative stress in individuals with ASD patients and some also in their parents [9].

Oxidative stress is described as an imbalance between reactive oxygen species (ROS) and reactive nitrogen species (RNS) and endogenous antioxidants. The function of antioxidant mechanisms is to balance and neutralise the effects of ROS and RNS [14]. ROS are mainly produced by mitochondria, during both physiological and pathological conditions, that is, $O_2^{\bullet -}$ can be formed by cellular respiration, by lipoxygenases (LOX) and cyclooxygenases (COX) during the arachidonic acid metabolism, and by endothelial and inflammatory cel [15] ls The excess concentrations of ROS and RNS can cause cell damage, influence energy metabolism and may have some impact on gene expression

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[7]. The brain is highly susceptible to oxidative stress due to its high energy consumption, in which a large amount of oxygen is utilized, secondary for its lipid-rich content with high proportions of polyunsaturated fatty acids (PUFAs) and due to its relatively low activity of antioxidant defense mechanisms[16]. Taking these factors into account, there is growing evidence that oxidative stress plays an important role in ASD as well as in other neuropsychiatric disorders.

In the following parts of this article the role of oxidative stress in ASD will be presented in the context of pathophysiology, use as an ASD biomarker and potential treatment target.

2. Materials and Methods

The author conducted a literature search of PubMed, Science Direct, and Google Scholar Databases to find actual articles on oxidative stress and ASD. Additionally, some cited articles were analyzed in preparation for this paper. The chosen articles were available in full versions and were written in English. The author aimed to present the current state of knowledge in the context of clinical practice.

In the following parts of this article the role of oxidative stress in ASD will be presented in the context of pathophysiology, use as a biomarker and potential treatment target.

3. Results

3.1. Role of Oxidative Stress in ASD Pathophysiology

Oxidative stress is an imbalance of ROS/RNS and the antioxidant protective system, causing multimodal pathogenic effects on numerous aspects of an organism's metabolic pathways and functions. Considering the importance of oxidative stress in that process, there is growing data on its causative function in ASD development.

Several genetic and environmental factors [17] may interact together promoting excessive ROS production, decreased antioxidant capacity, and mitochondrial dysfunction. These abnormalities happening in the prenatal and perinatal period promote oxidative stress that can influence epigenetic dysregulation, neuro-inflammation, cerebral injury, and neuronal dysfunction, which finally leads to ASD [8,17,18]. The most recognized genetic factors are interactions between GSTT1 and GSTP1 allele variants [19] and some specific gene polymorphisms [20] Environmental factors promoting oxidative stress are maternal neurotoxin exposure, prematurity, neonatal jaundice, use of medication during pregnancy [17,21].

As mentioned above it is widely described that oxidative stress influences many metabolic pathways. It affects cellular membranes, DNA strand breaking, protein synthesis and amino-acid side chains, post-translational changes, and neurotransmission [8] The cellular membrane is damaged due to lipid peroxidation. Lipids are the most susceptible to oxidative processes, especially polyunsaturated fatty acids (PUFA). The carbon-carbon double strands of PUFA are particularly prone to oxidant attack. The oxidation of PUFA induces cell membrane damage. These disturbances of membrane structure cause changes in membrane fluidity and permeability, thus negatively influencing its function. Peroxidation of PUFA leads to the formation of isoprostanes and generates reactive aldehydes, like 4-hydroxynonenal and malondialdehyde. The 4-hydroxynonenal binds to proteins and impairs their function. ROS by oxidizing amino acids causes the unfolding and misfolding of protein chains leading to inactivity and by [8] Numerous neurological studies underlined the important role of lipid peroxidation in the pathophysiology of autism spectrum disorder. Impaired metabolic pathways further enlarge the scale of oxidative stress. ROS and RNS also react with nucleic acids, causing DNA strand breaking, DNA-protein crosslinking, and modification of purine and pyridine-base structures, resulting in DNA mutations. Oxidation of RNA bases leads to the breakage of the nucleotide strand and causes ribosomal dysfunction [18,22,23]. The best-described oxidative stress-mediated post-translational modifications of proteins are 3-nitrotyrosine (3NT) and protein carbonyl formation that may change protein function [24]. In consequence, the processes described above impair protein synthesis and enable further cellular damage.

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A plethora of investigators describe numerous factors that influence brain metabolism, causing its increased vulnerability to oxidative stress, and underline specific abnormalities that are observed in ASD patients. Between them are the brain's high energy needs, high polyunsaturated fatty acids (PUFA) concentration in neuronal cell membranes, and high membrane ratio to cytoplasmic volume ratio. Another factor is the excitotoxic nature of one of the neurotransmitters- glutamate -its overaction is described in ASD patients. The next factor is the enhanced oxidation of neurotransmitters that promote ROS and quinones that reduce glutathione levels. Researchers observe reduced antioxidant defence mechanisms, particularly low catalase levels, glutathione peroxidase and vitamin E. In addition, excessive ROS directly downregulate proteins of tight junctions and indirectly activates matrix metalloproteinases (MMP) that contribute to unsealing the blood-brain barrier (BBB). Other studies show higher activation of microglia that causes greater ROS production and cytokines unsealing the blood-brain barrier unsealing the blood-brain barrier production in patients, a process similar to a vicious circle. To all above, the next underlined factors are disturbances in mitochondrial function that impair energy production and generate ROS [10,25].

The processes mentioned above are observed in ASD patients due to their increased vulnerability to oxidative stress, which is the result of multifactorial disturbances seen in that population [5]. Until now, there is still a debate about whether oxidative stress is a cause or effect of ASD. Below author tried to summarize factors connected to the presence of oxidative stress in ASD patients.

One of the most recognized factors is the impaired function of antioxidants. Between them is the abnormal metabolism of glutathione (GSH), which has a very important role in ASD pathophysiology. GSH is one of the most prevalent antioxidants in organisms. It also plays a regulatory role on glutamate receptors, and in that way, its disturbances may be linked with neural dysfunction [7,26].

Most researchers reported a decreased glutathione to glutathione disulfide (GSH/GSSG) ratio and decreased GSH levels in the brain, lymphoblastoid cells, and blood of ASD children. In the brain, a reduced glutathione redox ratio was found in the temporal cortex and cerebellum of autistic patients [27–30].

Additionally, widely described disturbances in ASD are related to the metabolism of sulfur amino acids [31]. Low methionine levels influence reduced protein synthesis. Low cysteine level in ASD patients results in decreased glutathione production in this disorder. The imbalances in sulfur amino acids metabolism impair methylation processes that further influence many metabolic pathways, including mitochondrial disturbances [8]. Another antioxidant impairments described in ASD patients are increased heme oxygenase-1 reported in the parietal and frontal lobes and the cerebellum [5].

There are also variations in superoxide dismutase (SOD) activity described, especially in plasma and erythrocytes, but data are not conclusive, probably age dependent. Although SOD is one of the most important antioxidant enzymes, comprehensive data on its activity in the brains of ASD patients are missing. There are attempts to measure SOD activity in human brain organoids that show no difference between ASD and healthy organoids [32]

Besides the above-described metabolic modifications caused by oxidative stress, it is necessary to underline its influence on synaptic plasticity. Because of its high energy needs, the brain utilizes large oxygen amounts that cause ROS production. Controlled ROS and RNS production provide the optimal redox state for the activation of transductional pathways involved in synaptic changes [33]. As it was already described in autistic patients the balance between antioxidative mechanisms and ROS production is disturbed. Due to that, oxidative stress that acts in the early stages of life may negatively influence synaptic plasticity in ASD. This process is caused either by neuroinflammation, genetic mutations such as CAPRIN1 haploinsufficiency that lead to neuronal tissue destruction, impaired calcium signalling with increased oxidative stress, and developmental/functional deficits of the neuronal network, including language deficits, attention deficit hyperactivity disorder, and ASD [34] as well as environmental factors such as high copper exposure or zinc deficiency that may lead to increased oxidative stress [35].

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Another important and widely described aspect of ASD that plays an important role in oxidative stress is inflammation [11]. There is still a debate on whether high ROS levels may cause inflammatory conditions or whether inflammation may induce oxidative stress. It is also a question of whether oxidative stress injury is a cause or a downstream effect of psychiatric disorders. Numerous researches show the importance of the link between neuroinflammation and oxidative stress. It is proven that prevalent oxidative stress supports chronic inflammation [36,37]

High concentrations of ROS can activate signalling pathways and induce high secretion of pro-inflammatory cytokines and chemokines, which promote further ROS production in a process remaining vicious circle [9]

There is growing evidence on the role of inflammation in ASD. In ASD, impairments associated with the innate and adaptive immune systems are described. These disturbances lead to immune system dysregulation and support the onset of pro-inflammatory conditions. These conditions may then lead to oxidative stress and, in the next step, promote chronic inflammation.

Recently Gevezova et al showed in their study that elevated TLR4/NOX2 signaling in B cells of ASD subjects could produce systemic oxidative inflammation, which may impact neuronal functions [38].

That is observed in addition to possibly altered metabolism in several cell types, particularly brain cells. [7].

Specifically, researchers identified abnormal alterations in microglial and astrocyte cell activation and atypical pro-inflammatory cytokine production [39,40], immune-related gene expression, and other inflammatory biomarkers [41], that occurs in the central neural system (CNS) [39,40]. Above all, those alterations may contribute to the pathophysiology of ASD.

Except for direct interest in the nervous system and considering the role of inflammation there is rising interest in the role of gastrointestinal tract disturbances and gut microbiota in many diseases including ASD [42,43].

Enteric bacteria interact directly with the intestinal epithelium, and in normal conditions, that interaction helps to maintain the integrity of the mucosal barrier, which has a protective function [42,44].

Additionally, it is important to note that some microbiota can produce neuroactive substances such as GABA and 5-HT and induce cytokine production. The next important fact is that fermentation of dietary fibers and resistant starch produces short-chain fatty acids (SCFAs), especially butyrate, propionate and acetate. Accumulation of propionate may influence neuroinflammation and cause gut disturbances [45]. There is rising evidence that ASD patients are more likely to experience gastrointestinal tract dysfunctions that include food allergies, dysbiosis, inflammatory bowel disease, and indigestion [46,47]. These problems are connected to oxidative stress and may induce mitochondrial dysfunctions [48] Maintaining the right balance of microbiota is vital for many processes in the organism. Numerous studies show the different composition of gut microbiota in ASD patients, but the direct consequences of this observation should be explored. More data on the existence and importance of the gut-brain axis and its probable role in the pathophysiology of ASD should be taken into consideration.

The next important factor that influences oxidative stress and has a role in the pathophysiology of ASD is mitochondrial dysfunction. Researchers found mitochondria dysfunction in different types of ASD objects from ASD animal models and cell lines (e.g., lymphocytes and granulocytes) derived from children with ASD. [49], to brain tissues of ASD patients [5,50] Increasing researches describe that there is an interaction between oxidative stress and mitochondrial function, which together affect the pathogenesis of ASD [51,52].

The mitochondrial electron transport chain is protected from damage caused by ROS by mitochondrial-specific superoxide dismutase and antioxidants such as glutathione (GSH). In ASD, the insufficiency of antioxidants, together with inflammatory processes, environmental factors and DNA and mtDNA mutations, influence mitochondrial dysfunction [5,53,54].

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Mitochondrial dysfunction may be detected by elevated ROS markers. The dysfunction has an important impact on neuronal metabolism and astrocytes. The oxidative stress influences Na+/K+-ATPase causing its malfunction that has multiple consequences on many metabolic pathways [55].

It is important to note that the mitochondrial electron transport chain is not only a source of free radicals but also a target of free radicals. Consequently, oxidative stress may damage mitochondrial function. Conversely, the abnormal mitochondrial function may cause further oxidative stress [51,56].

Figure 1. presents an outline of the causes and consequences of oxidative stress in ASD.

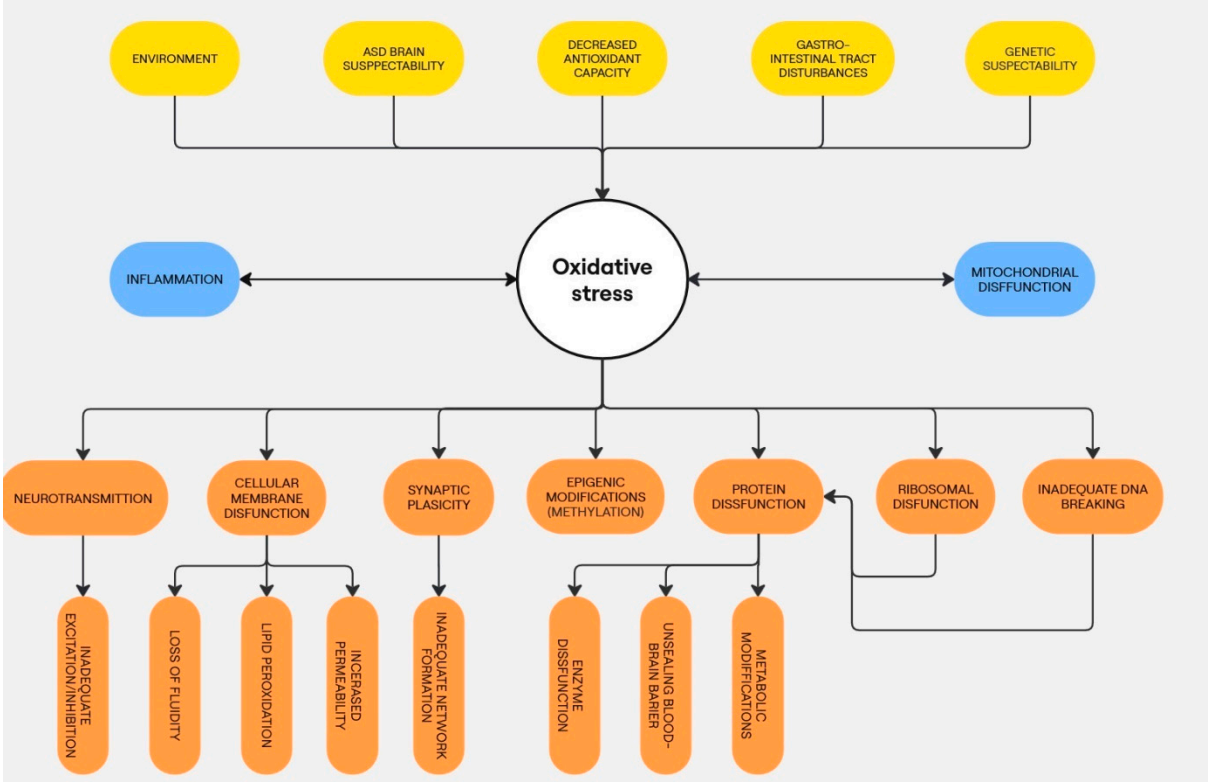

Figure 1. Causes and consequences of oxidative stress in ASD pathophysiology.

All the above mechanisms show the importance of oxidative stress in ASD. There is a great interplay between the described processes, showing that numerous disturbances can be simultaneously the cause and result of oxidative stress. Considering the extent of these metabolic disturbances, it is evident that oxidative stress has a role in ASD pathogenesis.

Specific biomarkers were developed and evaluated to detect the presence of oxidative stress. Oxidative stress biomarkers are being researched to find their utility in ASD diagnosis.

3.2. The Role of Oxidative Stress as an ASD Biomarker

Although ASD is a known problem, as in many other neurodevelopmental and neuropsychiatric diseases, the diagnosis is made by clinical criteria defined by ICD-10 or DSM V criteria. So far, there is no universal biomarker of ASD. Finding biomarkers would enable early diagnosis and shorten the time to begin proper therapeutic intervention. Numerous studies prove oxidative stress in autism by finding its biomarkers. Nowadays, the utility of oxidative stress biomarkers is explored in ASD timely diagnosis [10].

There were many attempts to find the right biomarkers- using blood, urine, and cerebrospinal fluid

samples. Direct ROS and RNS, such as superoxide, NO, and per-oxynitrite, are too reactive and/or have a short half-life, even shorter than 1 s. Therefore, such molecules cannot be isolated or measured directly in cells, tissues, and body fluids. On the other hand, molecular products formed from the reaction of RONS with various biomolecules are generally more stable than RONS

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themselves. Thus, in most cases, RONS have commonly been traced by measuring their oxidation target products or antioxidants [57]. The oxidative stress biomarkers can be divided by their origin as protein-, lipid-, or DNA-derived biomarkers and according to antioxidant type e.g. enzymes and thiols. The most prominent substances in ASD are superoxide dismutase, catalase, glutathione peroxidase, malondialdehyde (MDA), ceruloplasmin, and methionine.[6,10,36].

Glutathione (GSH) is the most abundant non-protein thiol; it plays crucial roles in the antioxidant defence system and the maintenance of redox homeostasis in neurons [28]. GSSG is a product of glutathione oxidation. According to numerous studies, one of the most pervasive markers in ASD was increased glutathione disulfide (GSSG) concentration. Also, GSH itself and the glutathione-to-glutathione disulfide ratio GSH/GSSG, which was reduced in the plasma of ASD patients, are significant biomarkers indicating oxidative stress in these patients [28,30,58–63].

Between other prominent blood-derived oxidative stress biomarkers MDA, homocysteine, SAH, nitric oxide, and copper concentrations were significantly increased. In contrast, S-Adenosyl *methionine*/ S-Adenosyl-L-*homocysteine* (SAM/SAH), methionine, cysteine, vitamins (B9, B12, D, and E), and calcium concentrations were significantly reduced in children with ASD. Due to the consistent and large ESs (effective size) for the associations between the above-mentioned biomarkers and ASD, they may be useful as diagnostic biomarkers for ASD. Therefore, future investigations into oxidative stress are necessary and have good potential for everyday clinical practice [64].

Considering diagnostic utility and possible use in clinical practice, biomarkers can be divided into two categories: blood-based and urine-based. Regarding the blood-based biomarkers, a subgroup study has shown the following results.

The next blood-based parameters were: Homocysteine (Hcy) [63] and Vitamin B6(folic acid), B9(folic acid), and B12 (cobalamin). Hcy is a non-protein amino acid derived from the methionine cycle required for activated methyl transfer and the trans-sulphuration pathway related to the synthesis of GSH [65] Vitamins B6, B9, and B12 play important roles in the development, differentiation, and functioning of the central nervous system [66]. They are involved in the methionine-homocysteine pathway and in that way are connected with oxidative stress [66,67]. Together, homocysteine, vitamin B6, B9, and B12 are described as important substances in ASD pathophysiology because of the metabolic abnormalities in ASD patients, their gastrointestinal disorders, and poor dietary habits [66]. However, assessing these parameters would be useful in detecting some additional disorders or nutritional deficiencies. The levels of these parameters, especially homocysteine, are too heterogeneous to be used as ASD biomarkers [4].

Other described blood-based biomarkers are isoprostanes, MDA and 4-Hydroxynonenal, which result from lipid peroxidation, a widely described process in ASD. The use of these parameters in clinical practice should be considered but more studies are necessary to introduce it as a diagnostic tool[8].

Other important parameters are 8-Hydroxy-2′-Deoxyguanosine and NRF2

Nuclear factor erythroid-2-related factor 2 (NRF2) is a cytoprotective transcription factor that regulates the expression of genes responsible for coding antioxidant, anti-inflammatory and detoxifying proteins and regulates cytoprotective genes\([68,69]). NRF2 concentration shows elevation in the serum of ASD patients [70].

8-Hydroxy-2′-Deoxyguanosine (8-OH-dg) is a product of oxidatively damaged DNA formed by hydroxy radicals, singlet oxygen and direct photodynamic actions [71]. 8-OH-dg is widely used as oxidative stress biomarker its elevation is described both in urine and plasma samples of ASD patients [72,73]

Because urine collection is easy, urine-based biomarkers have good potential for use in clinical practice. However, diagnostic methods have some limitations because urine production depends on kidney function and may be changed by kidney disorders.

Most investigations show the potential usefulness of the antioxidant capacity of blood and urine, as well as enzyme antioxidant activity and redox reaction intermediates. In other studies, homocysteine levels in blood and urine have been observed to be higher in children with ASD. Hcy levels in

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urine and blood in ASD patients are proven to be comparable, so detection in urine can be the preferred option [74,75].

Other biomarkers that can be measured in urine are isoprostanes and their indirect markers like phospholipase A2 [76,77]Isoprostanes are classified as reliable oxidative stress biomarkers. They are stable end products formed by the fermentation of hydroperoxides generated in lipid peroxidation [78].

Except for biochemical parameters, oxidative stress causes loss of cell membrane structure, including changes in its fluidity and permeability that can be measured [8,79,80].

As listed above, many biomarkers of oxidative stress can be detected in patients with ASD. More data are necessary to assess their selectivity and sensitivity to use their detection as a diagnostic tool for ASD [77] Another problem is diagnostic methods and their cost-effectiveness. Many authors underline that in future ASD diagnosis, using complex measurement methods with metabolomics techniques would be a good choice.

In Table 1. the author summarized biomarkers potentially useful in ASD diagnosis.

Table 1.

Oxidative stress biomarker	Medium	Characteristic of the biomarker in ASD patients	References
GSSG	blood	elevation	[28,30,58–63]
GSH/GSSG	blood	reduction	[28,30,58–63,81]
glutathione peroxidase	blood plasma, whole RBC	Inconclusive Elevation/reduction- more research is necessary	[82,83]
nitric oxide and its metabolites (nitrate and nitrite)	blood urine	elevation	[23,84]
superoxide dismutase SOD	Blood Plasma RBC	Inconclusive Elevation/reduction- more research necessary	[85–88]
SAH	blood	elevation	[81]
S-Adenosyl methionine/ S-Adenosyl-L-*homocysteine*(SAM/SAH),	blood	reduction	[81]
methionine	blood	reduction	[81,89]
cysteine	blood	reduction	[81]
homocysteine	blood/ urine	elevation	[89–91]
ceruloplasmin	blood (serum)	Elevation (inconclusive statistical difference)	[88,92]
copper concentrations	blood	elevation	[88,93]
calcium	blood	reduction	[94,95]
malondialdehyde (MDA)	urine blood	elevation	[64,85,96]
4-Hydroxynonenal	urine, plasma, RBC membranes	elevation	[72,97,98]
lipoprotein-associated phospholipase A2	Urine Blood (serum)	increase	[72,97,99]

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8-Hydroxy-2′-Deoxyguanosine	urine	elevation	[72,73]
NRF2	Blood (serum, monocytes)	Decreased/ increased in one study	[70,100,101]
vitamins (B9, B12, D, and E),	Blood Plasma, serum	reduction	[89,102–104]
loss of cell membrane structure	blood	changes in its fluidity and permeability that can be measured	[80,105,106]

3.3. The Role of Oxidative Stress as a Treatment Target

Nowadays, there is constant research of substances that could be used to cure autism, but there is no effective drug that can alleviate core symptoms of ASD.

Available pharmacological treatments are prescribed to correct comorbid symptoms of ASD, like sleep disorders, aggressiveness and irritability, hyperactivity and attention deficit [107]. In the United States FDA (Food and Drug Agency) approved drugs for autism are risperidone and aripiprazole to help with irritability and aggression [108].

Considering the role of oxidative stress as a pathogenic factor, there is growing evidence of potential therapies based on antioxidant effects in alleviating ASD symptoms. Antioxidants potentially useful in ASD treatment are summarized in Table 2 below.

In their reviews E. Zamberelli et al. and Bonomi et al. listed antioxidant substances potentially useful in ASD treatment: melatonin, tryptophan, Coenzyme Q10, L-Carnosine, luteolin and quercetin on sleep disturbances in children with ASD [107,109].

The study results showed that treatment with melatonin with doses varying from 2 to 10 mg/day was found to be well-tolerated and effective in shortening sleep-onset latency, reducing the number of awakenings per night and bedtime resistance and increasing total sleep time [109]. That is consistent with numerous primary studies on melatonin [110–112].

Additionally, improvements during treatment with melatonin were observed in reducing externalizing disruptive behaviours and parenting stress [113].

The authors underline the role of melatonin in multiple metabolic pathways not only as a circadian rhythm regulator but also as an antioxidant and anti-inflammatory agent. Additional studies on animal models show the relationship between sleep deprivation and oxidative stress [114,115]. These observations may explain its effectiveness in ASD patients [109,116].

The next well-described substance was tryptophan. As an essential amino acid and melatonin precursor, tryptophan was supplemented in the form of tryptophan-enriched cereals. In the actimetry study, patients were compared with those getting „control Cereals" without tryptophan. Studies revealed higher sleep efficiency, reduced sleep latency, and better total activity levels in those receiving enriched cereals [117].

Another study assessed the influence of L-carnosine [118,119] on sleep and showed a statistically significant reduction in sleep disturbances during that supplementation when assessed with sleep questionnaires and an autism severity scale [120,121].

The next well described antioxidant for autism treatment is coenzyme Q10 (Ubiquinone and Ubiquinol). Coenzyme Q10 is a lipid-soluble benzoquinone involved in oxidative phosphorylation as a cofactor for enzyme complexes in the mitochondrial membrane. It also has a recognized role as a free radical scavenger. Investigators showed improved sleep in patients receiving high doses (60mg/day) of ubiquinone when assessed with the Childhood Autism Rating Scale. Additionally, the authors observed some improvements in biochemical oxidative stress parameters [122].

Another assessed group of antioxidants were flavonoids luteolin and quercetin. Both substances were administered in combination as dietary supplementation, and authors described their positive

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effect on behaviour. Still, transient irritability and problems with sleep were listed as side effects of that therapy. Both described substances have additional anti-inflammatory action [123].

The next cited review on antioxidants' role in treating ASD assessed the use of cysteine-rich whey protein isolate (CRWP), a potent glutathione precursor, on behavioural problems. It was based on the results of other investigations that proved lower glutathione concentrations in children with ASD. The 90-day supplementation significantly improved glutathione levels and several aspects of behaviour associated with ASD. These aspects were socialization, adaptive behaviour, and internalizing and maladaptive behaviour. Overall, results in behavioural scales were comparable to the placebo group [124].

Another study of retrospective character, conducted by Cucinotta et al., gave preliminary results showing that so-called "metabolic support therapy" based on Q10 ubiquinol, vitamin E, and complex-B vitamins brought favourable outcomes in patients with neurodevelopmental disorders. The positive results were prominent in those with an intellectual disability. The greatest improvements were observed in cognition, adaptative functioning, and social motivation. The therapy was well-tolerated without any severe adverse events [125,126].

Considering the importance of glutathione in oxidative stress many authors underline the role of NAC (N-acetylcysteine) in ASD treatment. NAC is a synthetic derivative of the endogenous amino acid L-cysteine and a precursor of GSH (glutathione) [4,127,128].

The study of NAC treatments conducted by Lee T. et al. shows that this intervention can be an effective and well-tolerated option. The treatment results were assessed with the Aberrant Behavior Checklist and revealed improved hyperactivity and irritability and enhanced social awareness in patients with ASD. The authors conclude that more research on the NAC is necessary to recommend this treatment. Similar results are shown in the recent study made by Ramkumar Aishworiya et al. [129] In which the authors underlined the important role of NAC in regulating excitation/inhibition imbalance in ASD.

Another treatment option may be antioxidant-rich foods, including broccoli, camel milk, and dark chocolate for ASD, but the results are difficult to standardize [4].

Asadabadi et al. presented a line of therapy based on the theory of the interplay of inflammation with oxidative stress. The clinical trial was based on treatment consisting of risperidone in combination with celecoxib compared to risperidone plus placebo. The results of the trial were assessed using the Aberrant Behavior Checklist- Community Rating Scale and shoved superiority of the use of risperidone with celecoxib in treating irritability, social withdrawal and stereotypy [130].

The next group of substances with therapeutic potential in ASD are Nrf2 activators.

Nrf2 is a transcription factor in immunological dysregulation/inflammation, oxidative stress, and mitochondrial dysfunction. Nrf2, in case of oxidative stress, binds to specific DNA locus - antioxidant response elements (AREs). NRF2-ARE binding can regulate the expression of hundreds of cytoprotective genes, including antioxidant proteins and phase II enzymes [4,51]. NrF2 activators have anti-inflammatory and antioxidant effects. In this group most studies concerned on sulforaphane, resveratrol, naringenin, curcumin, and agmatine [131–135]. Yang J et. al. in their review study on Nrf2 activators, underline that the results of preclinical and few clinical studies are promising, but still more randomized trials are necessary to introduce this kind of treatment [136].

As described above numerous studies show that treatment with antioxidants may find its place in ASD management. Results of presented studies show different efficacy and durability of therapeutic effects. Usually, positive results were transient. That is probably caused by individual differences in genetics and environmental influence. Numerous research results were assessed with behavioural scales that use specially structured questionaries for caregivers or therapists and sometimes present the results in designed scales or only with the use of the caregiver's observation. That kind of study may have some bias as assessments are based on subjective opinions.

In future, it would be useful to find some specific biomarkers to make the assessment easier.

Above mentioned studies of antioxidants show promising results in improving sleep and some behavioural disturbances in children with ASD.

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However, more multicenter randomized trials are necessary to collect comprehensive data and introduce antioxidant therapy to clinical practice.

Table 2. Antioxidative substances potentially useful in ASD treatment.

Substance	Mechanism	effects	references
melatonin	circadian rhythm regulator but also as an antioxidant and anti-inflammatory agent	shortening sleep-onset latency, reducing the number of awakenings per night and bedtime resistance and increasing total sleep time, minimalizing disrupting behaviors, improving caregivers quality of life	[110–112,137]
tryptophan	essential amino acid and melatonin precursor	higher sleep efficiency reduced sleep latency and better total activity	[138]
L-carnosine	ameliorate cell energy metabolism, enhance immune response regulate the metabolism of RNS, modulate the glutamatergic system	statistically significant reduction in sleep disturbances	[118,119]
Q10 (Ubiquinone and Ubiquinol)...	Coenzyme Q10 is a lipid-soluble benzoquinone involved in oxidative phosphorylation as a cofactor for enzyme complexes in the mitochondrial membrane. It also has a recognised role as a free radical scavenger	Sleep improvement when using high doses (60mg/day) of ubiquinone	[139,140]
luteolin and quercetin	suppress oxidative damage and lipid peroxidation, and loss of antioxidant enzymes including catalase and SOD, possess the highest DNA-protective effect against H2O2 anti-inflammatory *http://dx.doi.org/10.20455/ros.2019.833*	Some positive effect on behaviour Have side effect described as transient irritability and problems.	[15,141,142]
cysteine-rich whey protein isolate (CRWP),	a potent glutathione precursor that increases glutathione concentration	The 90-day supplementation resulted in significantly improved socialization, adaptive behaviour and internalizing and maladaptive behaviour BUT overall results in behavioural scales were comparable to the placebo group [124].	[143]
"metabolic support therapy" based on Q10 ubiquinol, vitamin E, and complex-B vitamins	Enzymes cofactors	favourable outcomes in patients with neurodevelopmental disorders. The positive results were prominent in those improvements were observed in cognition, adaptative functioning, and social motivation. The therapy was well-tolerated without any severe adverse events [96,97].	[144]

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NAC (N-acetylcysteine)	a synthetic derivative of the endogenous amino acid L-cysteine and a precursor of GSH glutamatergic modulator	Improvement in hyperactivity and irritability and enhanced social awareness in Aberrant Behavior Checklist Scale	[145]
antioxidant-rich foods, including broccoli, camel milk, and dark chocolate	depending on substance	for ASD, but the results are difficult to standardize [4].	[24]
risperidone in combination with celecoxib	antiinflammatory	Reduction of irritability, social withdrawal and stereotypy	[146]
sulforaphane, resveratrol, naringenin, curcumin, agmatine	Nrf2 activators	improvement of irritability and hyperactivity symptoms	[136,147–149]

4. Discussion

The literature search results presented show the important role of oxidative stress in human organisms. It influences numerous metabolic pathways vital for energy metabolism, protein synthesis, defense mechanisms, DNA formation, transcription, neurotransmission, and more.

The presence of oxidative stress in ASD is well-proofed with the use of multiple biomarkers and functional studies. Taking into consideration the presence of this disturbance there are continuous attempts to use that knowledge in the management of ASD patients.

The pathogenesis of ASD is known to be multifactorial, the role of oxidative stress, despite its influence on numerous metabolic pathways must be taken into account. Data suggest that oxidative stress in ASD is a result of a combination of genetic and environmental factors e.g. diet, and inflammation. Conversely, oxidative stress influences metabolic pathways impacting genetic and inflammatory processes in the mechanism remaining vicious circle. Still, it is impossible to determine if oxidative stress is a cause or symptom of ASD. However, growing knowledge of the role of oxidative stress is very important in further research and may be useful in ASD management.

There are attempts to use oxidative stress biomarkers in early ASD diagnosis. Finding easy-to-detect biomarkers would be very helpful in shortening the time for the right diagnosis and the delay in introducing therapeutic interventions and also minimising the costs of differential diagnosis. Many studies have found oxidative stress biomarkers potentially helpful, but data on the sensitivity and selectivity of that diagnostic tool are still lacking. Most researchers compared ASD patient's results to those obtained from healthy controls. There can be bias when concerning patients e.g. with inflammatory disorders or energy deficits due to some genetic or/ and metabolic disorders. A complex biomarker assessment in combination with behavioural scales would probably be useful. As mentioned in the section above, probably the clinical utility of a panel consisting of glutathione, tGSH/GSSG ratio, isoprostanes, homocysteine and vitamin B can be the preferred option, but still there is a need for further studies.

Finally, the use of oxidative stress as a treatment target is another important field of research. There are many substances with antioxidant and anti-inflammatory functions with good safety profiles. The most promising results are described for melatonin, coenzyme Q-10, flavonoids, L-carnosine and antioxidant-rich diet. However, the efficacy of these interventions needs more detailed evaluation. Probably, due to the heterogeneity of ASD patients, the treatment interventions should be more individualized. During the antioxidant treatment, it is worth underlining that, to some extent oxidative stress has an important regulatory role in organisms [8,150]. It is involved in processes such as cell signalling during apoptosis, cell proliferation, gene expression, defence mechanisms generated in phagocytic cells, and others vital to maintaining cell homeostasis. This important role of oxidative stress should be considered during antioxidant treatment.

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5. Conclusions

This paper attempts to summarize the current knowledge on the role of oxidative stress in ASD. As described above, oxidative stress plays an important role in ASD pathogenesis through its direct influence on cells and its role in inflammation, mitochondrial dysfunction, and gene expression.

The growing data on oxidative stress and its role in autism is leading to attempts to introduce this knowledge into clinical practice. Many researchers are trying to find oxidative biomarkers that may be useful in early ASD diagnosis. However, data on their selectivity and sensitivity are still lacking. In numerous studies, liquid chromatography-tandem mass spectrometry, gas chromatography-mass spectrometry, and ELISA kits were used [4]. Probably, some dedicated panels that measure multiple parameters using metabolomic techniques will be used. There is still more research needed to use it as a diagnostic tool. Similarly, there are many attempts to use oxidative stress as a treatment target in ASD, with positive results for melatonin, tryptophan, flavonoids, NAC, and some combined treatment and dietary approaches. There are still more randomized, long-term trials to assess its safety and efficiency before introducing it as recommended management.

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