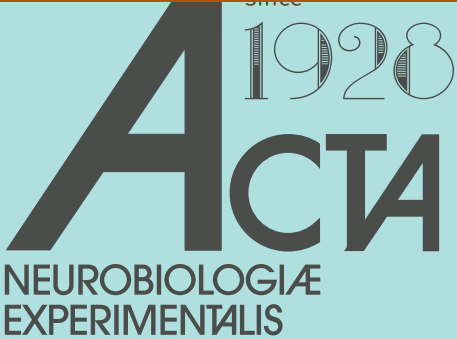


REVIEW

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 The logo for Acta Neurobiologiae Experimentalis features a large, stylized 'A' on the left. To its right, the year '1928' is written in a decorative, outlined font. Below the 'A' and '1928', the word 'ACTA' is written in a large, bold, sans-serif font. Underneath 'ACTA', the words 'NEUROBIOLOGIAE' and 'EXPERIMENTALIS' are stacked in a smaller, all-caps, sans-serif font.

Putative shared mechanisms in autism spectrum disorders and attention deficit hyperactivity disorder, a systematic review of the role of oxidative stress

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Oxidative stress is now believed to play a crucial role for neurodevelopment disorders such as autism spectrum disorders (ASD) and attention deficit hyperactivity disorder (ADHD). To review the most recent literature regarding the role of oxidative stress for the pathophysiology of ASD and ADHD, we conducted a systematic search of the relevant literature and further discuss the clinical and research implications of this knowledge. A systematic search in PubMed database retrieved 132 articles, of which 33 were included in the review. This review found relevant evidence concerning the role of oxidative status in ASD and ADHD, albeit with some contradictory findings. In order to overcome the incongruities found, more studies are needed in the study of neurodevelopmental disorders, with more thorough study designs and particular attention to the accuracy of the diagnostic tools used for the cases included.

Key words: autism spectrum disorder, attention deficit disorder with hyperactivity, oxidative stress, child psychiatry

INTRODUCTION

Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental disorders characterized by impairments in communication and in reciprocal social interaction along with restrictive and repetitive behaviours (American Psychiatric Association, 2013). Questions regarding the etiopathological mechanism of this complex disorder remain unanswered (Lai et al., 2014; Happe et al., 2006; Antshel and Russo, 2019).

Attention deficit hyperactivity disorder (ADHD) is a frequent and debilitating disorder diagnosed on the basis of persistent and developmentally inappropriate levels of over activity, inattention and impulsivity (American Psychiatric Association, 2013). The prevalence of both disorders has continued to rise for the

past decades (CDC, 2018; Lai et al., 2014), despite the systematic use of DSM-5 criteria for diagnostic purposes. Although this rise is probably most due to improved awareness and recognition, an increase in risk factors for both disorders cannot be overruled.

Evidence suggests that the two disorders are comorbid in a high percentage of cases, with percentages of co-occurrence varying from 28%-44% (Rommelse et al., 2010; Lai et al., 2014; Musser et al., 2014; Oerlemans et al., 2015). Clinicians frequently find a high proportion of siblings of ASD patients who are diagnosed with ADHD, and *vice versa*, which raises the suspicion for common aetiological ground for both disorders.

There are several hypotheses concerning ADHD and ASD aetiology, albeit questions regarding some of the etiopathological mechanism of these complex disor-

ders remain unanswered. The current line of evidence suggests that neurobiological, neurochemical and genetic factors are crucial in the pathogenesis of ASD and ADHD. The patient neurocognitive dysfunction, its severity and pervasiveness into multiple developmental areas point to the involvement of a diverse and widely distributed group of neuronal systems.

A number of risk factors have been identified for both disorders, but until now none has proven to necessary or sufficient on its own for the disorder to develop (Lai et al., 2014; Sciberras et al., 2017). The most common factors implied in the epidemiology of neurodevelopmental disorders are familiar traits of the disorder, complications during pregnancy, preterm delivery, male gender and low birth weight (Wang et al., 2017; Sciberras et al., 2017). Advanced maternal and paternal ages were associated with increased risk for autism spectrum disorders (Hultman et al., 2011; Sandin et al., 2012). Additionally, iodine deficiency, even at a sub-clinical level, appear to contribute to an increased risk for both disorders (Blazewicz et al., 2016; Abel et al., 2017). Exposure to environmental pollutants and chemicals has also been suggested to increase the risk of autism and hyperactivity disorder (Dickerson et al., 2015; Tran and Miyake, 2017).

Oxidative stress markers have been found in autism and ADHD, suggesting that increased oxidative stress may contribute to the development of the disease.

A series of primary defences exist to protect against free radicals and oxidative damage but may not be completely effective in this fight. (Davies, 1995; Dalle-Donne et al., 2006). Reactive oxygen species (ROS) play a fundamental part in human physiological and pathophysiological processes. ROS react readily with proteins, lipids, carbohydrates and nucleic acids. Overproduction of ROS induce can alter or even destroy cellular function, and human antioxidant defenses are not always sufficient to maintain a proper ROS balance (Brieger et al., 2012). ROS balance is affected by mitochondrial activity, and electron transport chain alterations, or respiratory chain alterations lead to disruptions in the redox balance in the mitochondrial matrix (Angelova and Abramov, 2018).

Due to its high oxygen utilization and generation of free-radicals by-products, its modest antioxidant defenses and its rich lipid constitution, the brain is then particularly prone to oxidative damage (Halliwell, 2006; Ng et al., 2008). Additionally, the brain is also susceptible to secondary and self-perpetuating damage from oxidative cellular injury or necrosis, via the neurotoxic effects of released excitatory amines and iron, and the activated inflammatory response (Halliwell, 2006; Ng et al., 2008). Oxidative stress may therefore be a plausible pathogenic candidate for psychiatric disorders

and play a crucial role for neurodevelopmental disorders such as ASD and ADHD (Chauhan et al., 2004).

Several markers of oxidative stress are available, including quantification of ROS and molecular products formed from redox and methylation metabolism, pro-oxidant and lipid peroxidation markers and DNA damage markers. Antioxidant pathways markers and the availability of antioxidant compounds can also configure a way to measure the presence of antioxidant defenses (or the lack of them).

This review addresses the most recent literature regarding the role of oxidative stress in the pathophysiology of ASD and ADHD.

Oxidative stress in neurodevelopmental disorders ASD and ADHD

Studies of oxidative stress parameters in ASD and ADHD written in English were systematically searched with Medline (PubMed, National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda, Maryland), until September 2019. The search strategy was to combine free radicals, oxidative stress and reactive oxygen species Medical Subject Headings (MeSH) terms with keywords describing clinical outcomes (“autistic disorder”, “autism spectrum disorders” (MeSH term introduced in 2016 in PubMed) and “attention deficit disorder with hyperactivity”). This was supplemented by a thorough search of references of the selected articles. Observational case control studies including children or adult patients diagnosed with autism spectrum disorders or with attention deficit hyperactivity disorder were eligible for the review. Exclusion criteria were: the studies did not present data on included oxidative stress parameters; the records were review, editorial or opinion articles; the records were animal studies; or the studies were not published in English.

After removal of duplicates, 128 records were assessed based on title and abstract. The full-text of 78 of these records was retrieved for further assessment. Of these, 45 did not meet the inclusion criteria for qualitative analysis. The records retrieved approached either the search for oxidative markers in these two populations, as well as possible alterations in the antioxidant pathways and respective markers. Summary of all articles included can be found in Tables I and II.

ROS, redox and methylation metabolism

In four of the studies included, nitric oxide (NO) was measured, two in ASD populations and two in ADHD

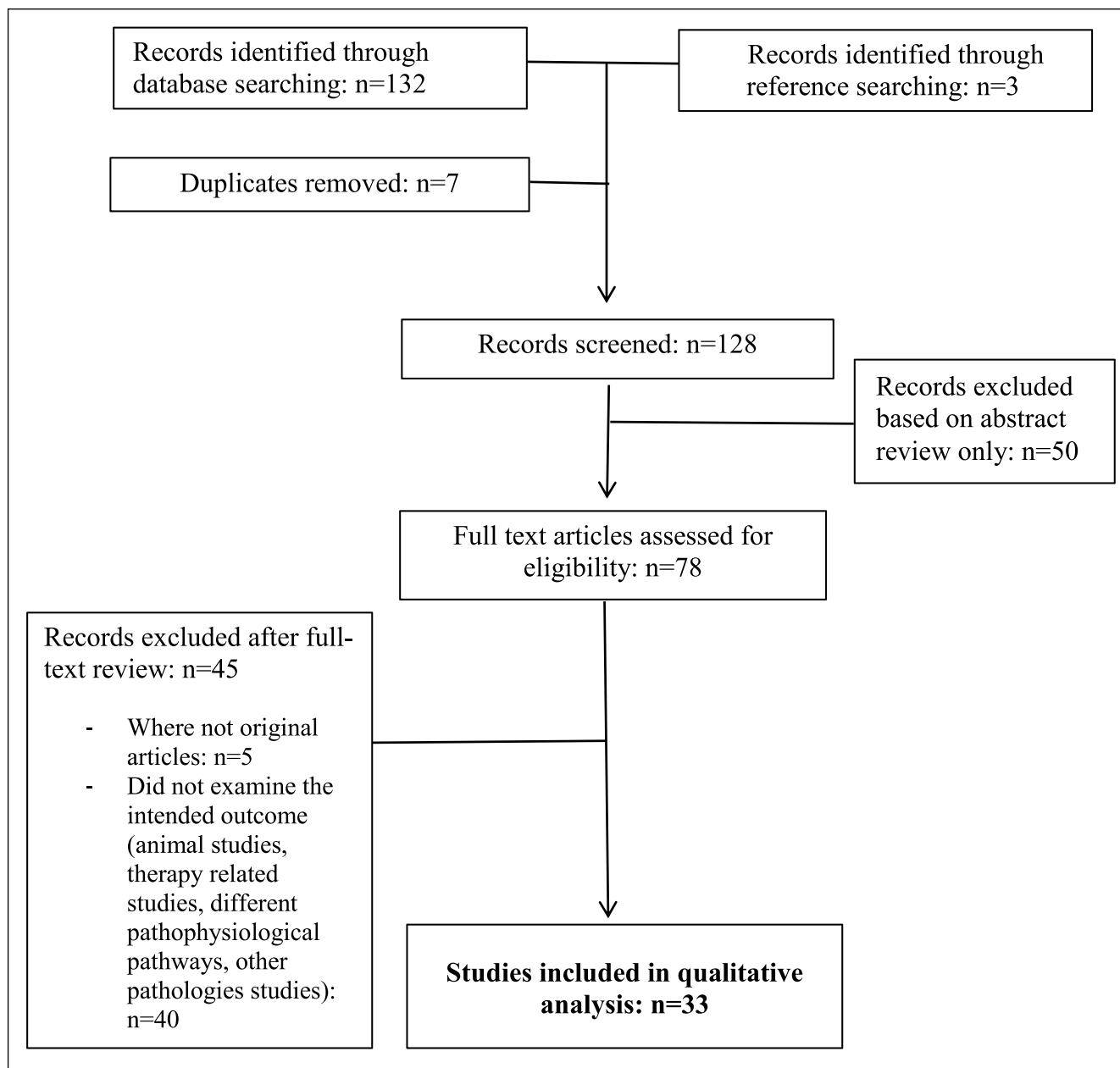


Fig. 1. Summary of the paper selection process as recommended by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.

populations. All of them found NO to be significantly increased in cases when compared to controls (Sogut et al., 2003; Selek et al., 2008; Ceylan et al., 2010; Lakshmi Priya and Geetha, 2011). One study measured NO₂ and NO₃ as surrogate markers to assess the activity of nitric oxide synthase (NOS) in a small sample of adults with ADHD diagnosis but found no significant differences in cases when compared to controls (Kittel-Schneider et al., 2015). However NOS was found to be increased in another study regarding ADHD in a paediatric population (Ceylan et al., 2012).

Reduced glutathione (GSH) was found to be decreased in seven studies regarding ASD patients (James et al., 2004; 2006; Al-Gadani et al., 2009; Lakshmi Priya and Geetha, 2011; Rose et al., 2012; Chauhan et al., 2012; Hodgson et al., 2014). One recent study measured of GSH in ADHD patients, which were significantly higher in patients when compared with controls (Verlaet et al., 2019). Glutathione peroxidase (GSH-Px) was significantly lower in four studies of ASD patients (Yorbik et al., 2002; Mostafa et al., 2010; Meguid et al., 2011; Lakshmi Priya and Geetha, 2011), and significantly increased

Table I: Summary of articles included regarding ASD.

Reference	Age mean/range (years)	Diagnostic Method	Biological Specimen	Patients/controls (n)	Biomarkers of interest	
					Oxidative stress markers	Antioxidant defences markers
Yenkoyan, 2018	4-10	DSM-5 ADI-R ADOS	plasma urine	10/10	8-OHdG↑	SOD↓, CAT↑, carbonyl
Ranjbar A, 2014	6-12	DSM-IV-TR	urine	29/22	Thiol molecules↓,	CAT↑, Fe3+ to Fe2+ (uTAC)↓
Hodgson NW, 2014	5,3	CARS	plasma	27/27	GSH↓, Homocysteine↑, Cystathionine=, cistine↑, cysteine=, Methionine=, SAM=, SAH↑, Homocysteine=, GSSH=	
Pecorelli A, 2013	12	DSM-IV-TR ADOS CARS	plasma	20/18	NPBI↑, 4-HNE PA's↑	
Ciccoli L, 2013	6-26	DSM-5 ADOS ABC	RBC	15/15	NPBI↑, F2-ISOP's↑, 4-HNE PAs↑	
Rose S, 2012	unknown	ADI-R	cerebellar cortex/ BA22 tissue	15/12	GSH↓, GSSG↑, 3-NT↑, 8-oxodG↑	
Melnyk S, 2012	3-10	CARS	plasma	68/54	Methionine↓, SAM↓, SAH↑, adenosine↑, homocysteine=, cysteine↓, Cystine↑, 3-NT↑, 3-CT↑, 8-oxodG↑.	
Al-Ayadhi L, 2012	3-9	CARS	plasma	44/40		SHH↑, BDNF↑
Chauhan A, 2012	12.6	DSM-IV ADI-R, ADOS, VABS, BSID-II *	cerebellum/ temporal	7-10/9-10	GSH↓, GSSG↑, GSH/GSSG↓	
Lakshmi Priya, 2011	4-12	CARS	urine	45/50	TBARS↑, LHP↑, 4-HNE↑	
Meguid NA, 2011	3-10	CARS	plasma	20/25	SOD↓, GSH-Px↓, MDA↑	
Lakshmi Priya, 2011	4-12	CARS	hair and nails	45/45	TBARS↑, NO↑, GSH↓, SOD↓, GSH-Px↓	vit A↓, vit C↓,
Mostafa G, 2010	3,5-12	DSM-IV	plasma	44/44	F2-ISOP's↑, GSH-Px↓	
El-Ansary A, 2010	3-15	DSM-IV	plasma	30/30	MDA↑	
Al-Gadani Y, 2009	3-15	DSM-IV	plasma	30/30	MDA↑, GSH↓, GSH-Px↑, SOD↑	vitamin E↓, Vitamin C=, CAT=
Sadjel-Sulkowska E, 2009	5-32	NOT DESCRIBED	cerebellar tissue	3/3	8-OHdG=, 3-NT↑	
James S, 2006	3-14	DSM-IV ADOS CARS	plasma	80/73	SAM↓, SAH↑, cysteine↓, GSH↓	
Ming X, 2005	4-17	ADI-R ADOS-G DSM-IV	urine	33/29	8-OHdG=, 8-iso-PGF2α↑	

Reference	Age mean/range (years)	Diagnostic Method	Biological Specimen	Patients/controls (n)	Biomarkers of interest	
					Oxidative stress markers	Antioxidant defences markers
James SJ, 2004	6.4	DSM-IV	plasma	20/33	Methionine↓, SAM↓, SAH↑, adenosine↑, homocysteine↓, cystathionine↓, cysteine↓, GSH↓	
Chauhan A, 2004	4.4/6.0	ADI-R ADOS-G	plasma	30/30	MDA↑, ceruloplasmin↓, transferrin↓	
Zoroglu S., 2004	4.7	CARS	RBC	27/26	TBARS↑, SOD↑, CAT↓, ADA=, XO↑	
Sogut S, 2003	2-12	CARS	RBC	27/30	SOD=, GSH-Px↑, TBARS=, NO↑	
Yorbik O, 2002	4-12	DSM-IV	plasma/RBC	45/41	GSH-Px↓, SOD↓	

AB: autism behavior; ADA: adenosine deaminase; ADI-R: autism diagnostic interview – revised; ADOS: autism diagnostic observation scale; ADOS-G: autism diagnostic observation scale – generic; ASD: autism spectrum disorder; BA22: Brodmann area 22; BDNF: brain-derived neurotrophic factor; BSID-II: Bayley scales for infant development-II; CARS: Childhood autism rating scale; CAT: catalase activity; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders-IV; DSM-IV-TR: Diagnostic and Statistical Manual of Mental Disorders-IV text revision; F2-IsoPs: F2-Isoprostanes; GSH: reduced glutathione; GSH-Px: glutathione peroxidase; GSSG: glutathione disulfide; GSSH: glutathione persulfide; LHP: lipid hydroperoxides; MDA: malondialdehyde; NO: nitric oxide; NPBI: non protein-bound iron; PA's: protein adducts; RBC: red blood cells; ROS: reactive oxygen species; SAH: S-adenosylhomocysteine; SAM: S-adenosylmethionine; SHH: sonic hedgehog; SOD: superoxide dismutase; TBARS: thiobarbituric acid reactive substances; uTAC: urinary total antioxidant concentration; VABS: Vineland adaptive behavioural scale; XO: xanthine oxidase; 3-CT: 3-chlorotyrosine; 3-NT: 3-nitrotyrosine; 4-HNE: 4-hydroxynonenal; 8-OHdG: 8-hydroxy-2-deoxyguanosine; 8-oxo-dG: 8-oxo-deoxyguanosine.

Table II. Summary of articles included regarding ADHD.

Reference	Age mean/range (years)	Diagnostic Method	Biological specimen	Patients/controls (n)	Biomarkers of interest	
					Oxidative stress markers	Antioxidant defences markers
Verlaet A, 2018	6-12	DSM-IV/DSM-5	plasma urine	57/69	MDA↑ 8-OHdG=	GSH↑ α-tocopherol= γ-tocopherol= β-carotene= retinol= retinyl palmitate↑, coQ10=
Guney E, 2015	9.28	CTRS	plasma	36/52	TAS↓, TOS↑, THIOL↓	PON=, SPON=, ARES=,
Kittel-Schneider S, 2015	34.3	DSM-IV	plasma	14/33	NO ₂ =, NO ₃ =	
Bulut M, 2013	25.3	DSM-IV	plasma	35/29	MDA↑	PON↓, ARES↓
Selek S, 2012	24.7	DSM-IV	plasma	50/31	TOS↑, OSI↑	TAS↑
Ceylan M, 2012	7-15	DSM-IV CTRS	plasma	35/35	NOS↑, XO↑, GST↓, ADA↑	PON-1↓
Oztop D, 2012	6-12	DSM-IV	plasma	30/30	MDA↓, 8-OHdG↓, AOPP=, Thiol=	PON-1=
Kawatani M, 2011	10	DSM-IV	urine	10/73	Acrolein-lysine↑	
Ceylan M, 2010	7-15	DSM-IV	plasma	35/35	NO↑, MDA↑, SOD=, GSH-Px↓	CAT=
Selek S, 2008	27.95	DSM-IV	blood	20/21	SOD↓, NO↑	

ADA: adenosine deaminase; ADHD: attention deficit hyperactivity disorder; AOPP: advanced oxidation protein products; ARES: arylesterase; CAT: catalase activity; CTRS: Conners' Teacher Rating Scale; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders-IV; GSH-Px: Glutathione peroxidase; GST: glutathione S-transferase; MDA: malondialdehyde; NO: nitric oxide; NOS: nitric oxide synthase; OSI: oxidative stress index; SOD: superoxide dismutase; PON: paraoxonase; SPON: stimulated paraoxonase; TAS: total antioxidative status; TOS: total oxidative status; XO: xanthine oxidase; 8-OHdG: 8-hydroxy-2-deoxyguanosine.

in two other studies (Sogut et al., 2003; Al-Gadani et al., 2009). One study measured GSH-Px in ADHD and found it to be significantly decreased in those patients (Ceylan et al., 2010).

Superoxide dismutase (SOD) was assessed in eight of the included records, seven regarding ASD patients (four of them found it to be decreased (Yorbik et al., 2002; Meguid et al., 2011; Lakshmi Priya and Geetha, 2011; Yenkovyan et al., 2018), one didn't find significant differences between groups (Sogut et al., 2003) and two found it to be significantly increased (Zoroglu et al., 2004; Al-Gadani et al., 2009), and one regarding adult ADHD patients, in which SOD was significantly decreased compared to controls (Selek et al., 2008).

Methylation metabolism was also assessed in ASD patients by methionine (James et al., 2004; Melnyk et al., 2012; Hodgson et al., 2014), S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) (James et al., 2004; 2006; Melnyk et al., 2012; Hodgson et al., 2014), adenosine (James et al., 2004; Melnyk et al., 2012), homocysteine (James et al., 2004; Melnyk et al., 2012; Hodgson et al., 2014), cysteine (James et al., 2004; 2006; Melnyk et al., 2012; Hodgson et al., 2014), cystathionine (James et al., 2004; Hodgson et al., 2014), GSSG (Chauhan et al., 2012; Rose et al., 2012; Hodgson et al., 2014), homocystine (Hodgson et al., 2014), xanthine oxidase (XO) (Zoroglu et al., 2004) and adenosine deaminase (ADA) (Zoroglu et al., 2004). Globally, all of these records point towards a decreased methylation capacity in the samples studied.

Pro-oxidant/ lipid peroxidation markers

Malondialdehyde (MDA), a common marker for lipid peroxidation, was assessed in four studies regarding ASD populations, all of them found this marker to be significantly increased when compared to controls (Chauhan et al., 2004; Al-Gadani et al., 2009; El-Ansary et al., 2010; Meguid et al., 2011). Similar results were found in three articles regarding ADHD patients (Ceylan et al., 2010; Bulut et al., 2013; Verlaet et al., 2019). One study found MDA to be significantly decreased in ADHD patients when compared to controls (Oztop et al., 2012). Thiobarbituric acid reactive substances (TBARS) were measured in four articles studying ASD patients (three found an increased level of this marker (Zoroglu et al., 2004; Damodaran and Arumugam, 2011; Lakshmi Priya and Geetha, 2011) and in one no significant differences were found between groups (Sogut et al., 2003). One record included measured total thiol molecules who were significantly decreased in ASD patients when compared to controls (Ranjbar et al., 2014) and

one study found similar results in ADHD patients and controls (Oztop et al., 2012).

Five articles measuring pro-oxidant and lipid peroxidation markers in ASD populations, namely non protein-bound iron (NBPI) (Ciccoli et al., 2013; Pecorelli et al., 2013), 4-hydroxynonenal (4-HNE) (Damodaran and Arumugam, 2011; Ciccoli et al., 2013; Pecorelli et al., 2013), F₂-Isoprostanes (F₂-IsoP's) (Mostafa et al., 2010; Ciccoli et al., 2013) and lipid hydroperoxides (LHP) (Damodaran and Arumugam, 2011) were also reviewed. All of them showed higher levels of this markers of oxidative stress when compared to controls. One record measured total antioxidant capacity in ASD patients, who was significantly lower in the patients group (Ranjbar et al., 2014). Total oxidative status (TOS) was found to be significantly increased in two studies including ADHD populations (Selek et al., 2012; Guney et al., 2015). These same studies measured total antioxidative status (TAS) with conflicting results. Guney et al. (2015) found TOS to be significantly increased in patients when compared to controls levels, while Selek et al. (2012) found TAS in patients to be significantly higher than in controls. Acrolein-lysine levels were assessed as a reflexion of lipid peroxidation in ADHD cases and were found to be significantly increased when compared to controls (Kawatani et al., 2011).

Oxidative protein/DNA damage markers

In ASD patients, three records included measured 3-nitrotyrosine (3-NT) (Sajdel-Sulkowska et al., 2009; Melnyk et al., 2012; Rose et al., 2012) and one record measured 3-chlorotyrosine (3-CT) (Melnyk et al., 2012), all of them found DNA damage markers to be significantly increased compared to controls. Measurement of 8-hydroxy-2-deoxyguanosine (8-OHDG) was found to be significantly increased in patients compared with their siblings (Yenkovyan et al., 2018), but two previous studies in ASD didn't find significant differences between groups (Ming et al., 2005; Sajdel-Sulkowska et al., 2009). Two studies measured 8-oxo-deoxyguanosine (8-oxo-dG) and found significant increases between groups (Melnyk et al., 2012; Rose et al., 2012). One study found 8-OHDG to be significant decreased in ADHD patients compared to controls (Oztop et al., 2012), while one study reports a trend for higher urinary levels of 8-OHDG in patients with ADHD (Verlaet et al., 2019).

Antioxidant compounds/antioxidant pathways markers

Vitamins A and C were significantly decreased in one population of ASD patients (Damodaran and Aru-

mugam, 2011), with another record showing a significant decrease in vitamin E in ASD patients but failing to show significant differences in vitamin C levels (Al-Gadani et al., 2009).

In ASD patients, catalase activity was shown to be decreased intraerythrocytic (Zoroglu et al., 2004) and increased in another study (Yenkoyan et al., 2018), and one record shows no significant differences in plasma between cases and controls (Al-Gadani et al., 2009). Catalase activity was also been shown to be increased in urine (Ranjbar et al., 2014). In ADHD, one record measured catalase activity in plasma of patients, showing no significant differences between groups (Ceylan et al., 2010).

One study measured brain-derived neurotrophic factor (BDNF) and sonic hedgehog (SHH), which were significantly increased in ASD patients (Al-Ayadhi, 2012). Paraoxonase (PON) were significantly decreased in two ADHD populations (Ceylan et al., 2012; Bulut et al., 2013), although one study failed to find any differences between cases and controls (Oztop et al., 2012). Aryles-terase (ARES) was significantly decreased in one ADHD population (Bulut et al., 2013), and no significant differences between cases and controls were found in another study (Guney et al., 2015).

In summary, the majority of the studies included point towards a pro-oxidative environment and/or a reduced antioxidant capacity in patients with ASD and ADHD, resulting in a negative imbalance that subsequently leads to oxidative damage, confirming the authors hypothesis that negative oxidative imbalance could be one of the shared factors between these two neurodevelopmental disorders.

This review found a considerable amount of evidence concerning oxidative status in ASD and ADHD. However, contradictory findings were detected and some of the analyzed studies presented limitations, such as inaccurate ratio of cases versus controls, failure to pair cases and controls by age, selection of controls among non-diagnosed siblings (which constitutes a risk, taking in account that some of them may have subclinical traits of the disorders) and most important, poor assessment and use of diagnostic methods.

The biologic basis of autism involves gene-environment interactions during critical development periods. Methionine trans methylation and trans sulfuration pathways are some of the metabolic pathways important for the regulation and modulation of cellular methylation, DNA synthesis and redox status (Melnik et al., 2012). Methionine is an essential amino acid that is trans methylated into homocysteine via SAM, the major cellular methyl donor. Both methionine and SAM were found to be decreased by several authors in ASD. Additionally, methylation inhibitors SAH and ade-

nosine were found to be increased in these patients. To date, there are no reports regarding trans methylation metabolites in patients with ADHD.

Both 3-NT and 3-CT are stable oxidative post-translational modifications of protein tyrosine residues and both were significantly increased in plasma from children with autism, compared to controls. In nuclear and mitochondrial DNA, 8-OHdG is a free radical-induced oxidative lesion that is widely used as a biomarker of oxidative damage. There are reports on 8-OHdG both in ADHD patients and ASD patients. As expected, 8-OHdG seem to be increased in plasma of ASD children, but no significant levels were found in urine samples. Oztop et al. (2012) found significant lower levels of this marker in ADHD children, compared to healthy children. The authors explain that this can be early findings and that, being a neurodevelopmental disorder, the levels may rise in older populations, as it has been suggested for neurodegenerative disorders. The age factor doesn't explain, however, how some authors found significant higher levels of 8-OHdG in younger populations of ASD patients as those studied by Ming et al. (2005) and Sajdel-Sulkowska et al. (2009), in which adults were also included.

Another point in favor of the contribution of oxidative stress to the etiology of neuropsychiatric disorders of the infancy, as is the case of ASD and ADHD, is the depleted level of antioxidant enzymes like SOD and GSH-Px found in several of the articles included (Yorbik et al., 2002; Sogut et al., 2003; Selek et al., 2008; Al-Gadani et al., 2009; Mostafa et al., 2010; Ceylan et al., 2010; Lakshmi Priya and Geetha, 2011; Meguid et al., 2011). Contradictory findings, however, are also reported in both disorders, which make this topic relevant for further investigation.

Some of the studies showed a rise in the antioxidant defenses in the populations studied, as is the case in the study by Al-Ayadhi et al. (2012) who showed a significant increase of SHH and BDNF in an autistic population. These results could be at first sight considered a paradox, since the same studies also report a significant rise in free radicals production. This is however an important finding that can be explained as a protective mechanism secondary to increase oxidative stress in these patients. This was the first time a correlation between higher levels of oxygen free radicals and SHH and BDNF was reported (Al-Ayadhi, 2012). The authors fail however to explain the reason why they found a positive correlation between clinical severity and SHH and an absence of correlation between clinical severity and levels of oxygen free radicals. On the other hand, how the degrees of severity of the disorder were determined was not reported, since the cases were selected based on a screening test (CARS) and not on an evaluation by a child psychiatrist nor gold standard measures like

ADIR or ADOS. In fact, the diagnostic methods used are one of the major limitations in most of the studies included, since only few used the gold-standard methods for the diagnosis of ASD or ADHD. The methods used to determine diagnosis are detailed in Tables I and II.

Oxidative stress is also evidenced in several articles by the depleted blood levels of enzymatic and non-enzymatic antioxidants. Glutathione, found to be decreased in several ASD populations studied, is a potent antioxidant which could react and counteract with both ROS and RNS to neutralize them during which GSH is converted to GSSG. The regeneration of reduced glutathione needs the cooperation of vitamins C and E which are the free radical scavenging agents. Another powerful antioxidant, considered essential for growth and development of young children is vitamin A. All of these vitamins were found to be decreased in at least one study (Al-Gadani et al., 2009; Damodaran and Arumugam, 2011). Whether those deficiencies are due to poor nutrient intake, malabsorption problems or, in fact, to a higher expenditure of anti-oxidant compounds in these particular patients is still not completely understood and warrants further investigation.

ADHD research is still scarce regarding antioxidant pathways markers. Only two articles evaluated lipid peroxidation in these population (PON and arylesterase), and only one found them to be decreased in a population of adults with ADHD (Bulut et al., 2013). TBARS is the end product of lipid peroxidation in the body. It is abundantly produced from PUFA's in oxidative stress environments. Pro-oxidative status measured by lipid peroxidation markers, specifically by MDA levels, is the most prominent area of research regarding oxidative stress in these pathologies. Several articles found increased levels of MDA in ASD, with levels in ADHD being more controversial, since two authors report increased levels (Ceylan et al., 2010; Bulut et al., 2013) while one study reported decreased levels of MDA in ADHD patients (Oztop et al., 2012). However, the inclusion of adults in the study population may not be the most accurate way to establish a link between oxidative stress and ADHD. Being a neurodevelopmental disorder, the core symptoms emerge in infancy and may attenuate with age. Possible adaptive changes may occur through childhood and adolescence, like neuroplasticity adaptations to consistent cognitive behavioural training and learning, so that is why pathological links should try to be established before or during these periods of development.

Clinically, ASD and ADHD are quite distinctive disorders. Nonetheless, symptoms of ADHD are commonly seen in clinical practice in children with ASD diagnosis and the opposite is also true. Moreover, both disorders seem to co-occur in families and both show heritability links. All of those factors have a huge impact on the way

both disorders are diagnosed – and possibly may account for some inflated rates of both diagnosis – and ultimately, will have impact on the therapeutic approach on these children. Realizing that oxidative stress could be behind the pathophysiology of both ASD and ADHD could in fact help explain why these two disorders can co-occur in the same patient at such high rates.

CONCLUSIONS

The negative imbalance between antioxidants and pro-oxidants levels in children with autism spectrum disorder and attention deficit hyperactivity disorder suggest that these disorders are accompanied by increased oxidative damage. Whether this fact is due to increased metabolic stress induced by the disorder or can indeed account as a cause for the disorder is still not understood. Longitudinal studies may contribute to disentangle this circular relationship. Nevertheless, the role for oxidative stress in both disorders can very well explain the difficulty in finding a single candidate gene responsible for ASD and ADHD, so as the rise in the co-occurrence of the disorders. Chronic exposure to oxidative stress can result in changes in gene expression and protein synthesis, resulting in alteration in the synaptic patterns and epigenetic modification of gene functions.

The methods used to measure oxidative status vary among the studies included making comparisons of study findings difficult to achieve. To be used as diagnostic tools, it is critical that the biomarkers used can be stable, able to accumulate to different detectable concentrations and correlate to symptoms severity. In order to clarify the incongruities found, more studies are needed in the study of neurodevelopmental disorders, with more reliable study designs and particular attention to the accuracy of the diagnostic tools used for the cases included. The etiology of neurodevelopmental disorders such as ASD and ADHD cannot be fully understood unless using a bio-psycho-social conceptualization, integrating a careful and robust clinical approach. At the same time, adjusting the accuracy of biological markers for these disorders will allow for early identification and therapeutic intervention, contributing to ameliorate prognosis.

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