unstable atoms, molecules, or ions with unpaired electrons. They are harmful because the unpaired electron oxidatively reacts with other ions and molecules, or, by "stealing" an electron from other molecules, to pair that electron. This produces disruption to other molecules and damage to cells [49]. One of the main problems is that ROS "steal" electrons from lipid membranes (the cell membrane of most living organisms is made of a lipid bilayer). The oxidative degradation of the lipid membrane is referred to as lipid peroxidation. Lipid peroxidation results in loss of membrane integrity and fluidity, which ultimately leads to cell death [50,51]. ROS also react with proteins and nucleic acids which can lead to cell death via apoptosis or necrosis [52].

Under normal conditions, a dynamic equilibrium exists between the production of ROS and the antioxidant capacity of the cell [53]. Oxidative stress occurs when there is an imbalance between free radicals and the ability to neutralize them (i.e. an excess of pro-oxidants, a decrease in antioxidant levels, or both). The brain is highly vulnerable to oxidative stress due to its limited antioxidant capacity, higher energy requirement, and higher amounts of lipids and iron [54].

In ASD, several post-mortem studies reveal that affected areas of the brain in individuals diagnosed with an ASD showed accelerated cell death under conditions of oxidative stress [55-61]. For example, Lopez-Hurtado and Prieto [55] found that the density of lipofuscin, a matrix of oxidized lipid and cross-linked protein that forms as a result of oxidative injury in the tissues, was observed to be greater in cortical brain areas concerned with communication in individuals diagnosed with an ASD than in controls. As mentioned earlier, individuals diagnosed with an ASD typically lose speech and language abilities at the time of regression.

Other studies have found elevated oxidative stress markers in areas of the brain associated with ASD symptoms. Sajdel-Sulkowska et al [59]. reported that the brain regions with the highest levels of the oxidative stress marker, 3-nitrotyrosine (3-NT), were in the orbitofrontal cortex, Wernicke's area, cerebellar vermis, cerebellar hemisphere, and pons (brain areas associated with the speech processing, sensory and motor coordination, emotional and social behavior, and memory) in individuals diagnosed with an ASD.

In two other studies by Sajdel-Sulkowska et al [57,58], they found elevated 3-NT and neurotrophin-3 (NT-3), markers of oxidative stress, in the cerebellum of individuals diagnosed with an ASD in comparison with controls. Evans et al [56] found elevated oxidative stress markers in the brains of individuals diagnosed with an ASD by evaluating the oxidative stress metabolites of carboxyethyl it was not seen in any neurotypical brains, young or aged, used as controls for the oxidative assays. Chauhan et al [60] compared DNA oxidation and glutathione redox status in postmortem brain samples from the cerebellum and frontal, temporal, parietal and occipital cortex from individuals diagnosed with an ASD and age-matched neurotypical controls. These investigators reported that DNA oxidation was significantly increased by two-fold in the frontal cortex, temporal cortex, and cerebellum in individuals diagnosed with an ASD compared to controls. Moreover, the levels of reduced glutathione were significantly reduced, and the levels of oxidized glutathione were significantly increased, in samples of the cerebellum and temporal cortex from individuals diagnosed with an ASD as compared to the corresponding levels in the control brain samples. Earlier, Chauhan et al [61] found a significant increase in the levels of lipid hydroperoxides, an oxidative stress marker, in the cerebellum and temporal cortex in individuals diagnosed with an ASD as compared to controls.

8-oxo-guanosine (8oHdG) and neurodegeneration

8oHdG is an RNA oxidative damage marker that can be used to assess oxidative stress that is found in the brain in neurodegenerative disease [62]. Urinary 8OHdG has been used successfully to measure brain damage and degeneration, showing a significant correlation ($r = 0.87$, $p < 0.01$) with serum S100beta values, which are already used to measure brain damage [63]. Sajdel-Sulkowska et al [58] conducted a study where they examined oxidative damage in the cerebellum of those individuals diagnosed with an ASD by measuring 8OHdG. The authors found that cerebellar 8OHdG showed an upward trend toward higher levels with an increase of 63.4% observed in those individuals diagnosed with an ASD in comparison to controls.

Conclusion

To date, the etiology of ASD remains under debate. There are, however, many studies that suggest toxicity in children with ASD. A recent study conducted by the Harvard School of Public Health [64], for example, found that perinatal exposures to the highest versus lowest quintile of diesel, lead, manganese, mercury, methylene chloride, and an overall measure of metals were significantly associated with ASD, with odds ratios ranging from 1.5 (for overall metals measure) to 2.0 (for diesel and mercury). Similarly, Windham et al [65] examined possible associations between ASD and environmental exposures in 284 children with ASD and 657 controls, born in 1994 in the San Francisco Bay area. They found that the

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Servizi Diagnostici

Sig.ra [redacted]

Richiesta [redacted]

[redacted]

P.P. VIA ORZINUOVI 111

Esame	Risultati	U.M.	Valori di riferimento	
TNF (Tumor Necrosis Factor)				
Metodo : EIA				
TNF (Tumor Necrosis Factor)	9,20	pg/ml	[< 8,10]	*

Esame eseguito presso il laboratorio synlab - Leinfelden

INTERFERONE GAMMA

Metodo : Citofluorimetria

Interferone gamma	4,40	pg/ml	[< 10,00]

Esame eseguito presso il laboratorio synlab - Leinfelden

ESAME COLTURALE DELL'URINA (URINOCOLTURA)

Metodo :

COLTURALE	**Nessuno sviluppo**

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Servizi Diagnostici

[redacted]

Esame	Risultati	U.M.	Valori di riferimento	
POLIOVIRUS ABS				
Metodo : NT				
Poliovirus 1 ABS	**1/128**	Titolo	[< 1/4]	
Poliovirus 2 ABS	**1/64**	Titolo	[< 1/4]	
Poliovirus 3 ABS	**1/64**	Titolo	[< 1/4]	
Esame eseguito presso il laboratorio synlab - Leinfelden				
HLA TIPIZZAZIONE COMPLETA (LOCI A-B-C, DR-DQ)				
Metodo: PCR + Ibridazione				
locus A	**A*03, A*11**			
locus B	**B*44, B*57**			
locus C	**C*06, C*07 C*07, C*12**			
locus DQ	**DQB1*03, DQB1*03**			
locus DR	**DRB1*07, DRB1*11**			
FIBRILLARINA Abs IgM	**< 1/80**		[< 1/80 Negativo]	
Metodo: Immunofluorescenza indiretta				
Substrato: cellule Hep-2				
GUANOSINA DEOSSIDATA	**24,3**	ng/mg creatinina	[< 7,0]	*
Metodo : Immunoenzimatico				
INTERLEUCHINA 2 RECETTORI				
Metodo : LIA				
SIL 2 R Recettore (Interleukin 2) U/ml	**518**	U/ml	[158 - 623]	
Esame eseguito presso il laboratorio synlab - Leinfelden				

brain tissue can result [35]. It is interesting to note that Nam et al [36] found (using a model of mitochondrial membrane potential loss) there was only 25% cell loss in SH-SY5Y (SH) neuronal mono-cultures, but that 85% neuronal loss occurred when neurons were co-cultured with BV2 microglia. These investigators reported that SH neurons overexpressing uncoupling protein 2 exhibited an increase in neuron-microglia interactions, which represented an early step in microglial phagocytosis of neurons.

Sometimes neurodegeneration involves degeneration of presynaptic terminals prior to the loss of the cell body. There is some debate as to the involvement of microglia in synaptic stripping and synapse degeneration [37].

Numerous recent studies provide evidence that individuals diagnosed with an ASD suffer from an ongoing neuroinflammatory process in different regions of the brain involving microglial activation [22,38-43]. Evidence from post-mortem brain tissue documents activated microglia and astrocytes [22,38,39].

Vargas et al [22] observed that among the brain regions studied, the cerebellum showed the most neuroglial responses in individuals diagnosed with an ASD. They stated that the selective process of neuronal degeneration and neuroglial activation appears to occur predominantly in the PC layer and the granular cell layer of cerebellum and that these findings are consistent with an active and ongoing postnatal process of neurodegeneration and neuroinflammation. They also stated that the proinflammatory chemokine, monocyte chemotactic protein-1 (MCP-1), was consistently elevated in the brain regions studied in individuals diagnosed with an ASD, and that increased expression of MCP-1 may have relevance to the pathogenesis of ASD because its elevation in the brain is linked to microglial activation, and perhaps, to the recruitment of monocytes/macrophages to areas of neurodegeneration in the cerebellum. Importantly, these diagnosed with an ASD as compared to controls. They determined that increased microglial activation was present in the: cerebellum, midbrain, pons, fusiform gyri, and the anterior cingulate and orbitofrontal cortices, in individuals diagnosed with an ASD in comparison to controls. The investigators also observed, in accord with the observations made by Vargas et al [22], that the area with the most prominent increase was the cerebellum in individuals diagnosed with an ASD in comparison to controls.

Proinflammatory cytokines and neurodegeneration

Activated microglia can release a number of potentially neurotoxic substances, such as reactive oxygen species, nitric oxide, and various proinflammatory cytokines, and evidence implicates neuroinflammation and overproduction of proinflammatory cytokines as a contributor to pathophysiology of chronic neurodegenerative disorders [44]. Proinflammatory cytokines are found in chronic neurodegenerative disorders such as AD, PD, and MS.

Studies show elevated proinflammatory cytokines in the brains and spinal cords of individuals diagnosed with an ASD. Li et al [45], for example, showed that proinflammatory cytokines (tumor necrosis factor (TNF)-α, interleukin (IL)-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF), Th1 cytokine (interferon (IFN)-γ) and chemokine (IL-8) were significantly increased in the brains of individuals diagnosed with an ASD compared with controls. A study by Vargas et al [22] demonstrated tumor growth factor-β1, derived from neuroglia, was significantly increased in the middle frontal gurus (MFG) of individuals diagnosed with an ASD, while MCP-1, IL-6 and IL-10 were increased in the anterior cingulated gurus (ACG), in comparison to controls. In addition, using a protein array approach, Vargas et al [22] also found that MCP-1, IL-6, IL-8 and IFN-γ were significantly increased in the cerebrospinal