Values of nitric oxide and superoxide dismutase in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis

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Abstract

Background: Neurons are highly energy-dependent and highly specialized cells, showing great sensitivity to oxidative stress (OS). This study aimed to contribute to the further elucidation of the role of OS in the pathogenesis of amyotrophic lateral sclerosis (ALS).

Matherial and methods: We assessed nitric oxide () and superoxide dismutase (SOD) levels in cerebrospinal fluid (CSF) of 24 sporadic ALS (sALS) patients (13 of them presented with spinal form while 11 patients had bulbar form) and 20 controls (CG).

Results: The obtained SOD levels in sALS patients were lower than those in CG ($p<0.001$), while NO showed higher levels compared to CG ($p < 0.001$). Observed separately, there were no significant differences in the levels of NO and SOD in CSF between patients about their clinical presentations ($p > 0.05$). There were significant negative correlations between SOD and NO levels in all sALS patients ($r = 0.31$, $p=0.025$). Significant correlation between SOD and functional rating scale as well as disease progression index were recorded in patients with sALS (r=0,618 r= 0.425 $p < 0.01$), while NO levels were significantly associated with disease progression only (r=0.348 $p < 0.01$).

Conclusion: The data presented here clearly support the role of impaired oxidant/antioxidant balance in the pathogenesis of ALS. The CSF SOD and NO level might serve as a useful biomarker for functional disorder and progression of the disease, but these facts need to be reevaluated in future research which should be performed on a large cohort of ALS patients

Introduction

Amyotrophic lateral sclerosis (ALS) has long been viewed as a progressive, fatal neurodegenerative disease mainly affecting vulnerable motor neuron populations. The vast majority of ALS cases are sporadic (sALS), while less than 10% are familial (fALS) (Turner et al 2017). In general, these two forms of the disease do not differ clinically. The discovery that 20% of fALS is caused by mutations in the gene for Cu/Zn superoxide dismutase (SOD), stimulated considerable evaluation of oxidative stress (OS) in both familial and sporadic forms of the disease (Bergeron 1995). OS is defined as a consequence of the imbalance between the production of reactive species such as free radicals and oxidants and the antioxidant capacity. The most important free radicals are reactive oxygen species (ROS), which include oxygen and its reduction products superoxide (O2-), hydrogen peroxide (H2O2) and hydroxyl radical, and the reactive nitrogen species (RNS) such as the free radical nitric oxide (NO) and its by-products, including the powerful oxidant peroxynitrite (ONOO-) (Sharma et al. 2012). In vivo, superoxide anion radicals (O2-) are mostly removed enzymatically, by SOD, which is one of the most important antioxidant enzymes and represents the first line of enzymatic cell protection against O2-. SOD is abundantly present in cell bodies, axons, and dendrites of human motor neurons (Pardo et al. 1995). Because the concentrations of SOD and O2- in a given tissue are relatively constant, the primary driving force for

peroxynitrite formation is the NO concentration (Torreilles et al. 1999). NO itself is neither highly reactive nor particularly toxic under physiological conditions (Estevez et al. 1999). However, its toxicity is caused by more potent and specific oxidant peroxynitrite formed through a diffusion-limited reaction with superoxide resulting in the nitration of tyrosine residues in neurofilaments, irreversible inhibition of the mitochondrial respiratory chain, and inhibition of the glutamate transporter (Beckman 1996, Pardo et al. 1995).

Excessive production of superoxide anion radicals, nitric oxide, and peroxynitrite may, thus, be a significant pathogenetic factor for neuronal damage. However, despite more than three decades of research, there are insufficient reports in the literature on NO levels and SOD activity in cerebrospinal fluid (CSF) of patients with sALS and the results are inconsistent. While some studies indicated a decrease in SOD activity in both fALS and sALS the results of other studies indicated an increase in SOD activity in the CSF of patients with sALS (Boll et al. 2003, Ihara et al. 1995, Ihara et al. 2005, Kokic et al. 2005). As for NO, the results of previous studies show elevated or unchanged levels. The different outcomes may depend either on the ALS type, the disease duration, or the sampling time (Süssmuth et al. 2008).

This study was performed to assess the contribution to the further elucidation of the role of OS in the pathogenesis of ALS. In this regard, the activity of SOD and NO levels, as markers of OS in CSF of patients with spinal and bulbar form sALS were measured.

Methods

The study was approved by the Ethical Committee of the Faculty of the Medicine University of Nis and informed consent was obtained from each patient before entry into the study, according to the declaration of Helsinki.

Twenty control participants (CG), non-smokers, (10 male, 10 female), aged 23–65 years, who were admitted at Clinic for Neurology, Clinical Center Nis underwent the complete diagnostic procedure due to suspected neurological disorder, which had been presented as reversible, nonspecific, neurological symptoms without any objective abnormalities found at the laboratory, MRI scan, and CSF examination. Their final diagnosis was mainly functional disorders and tension-type headaches.

Twenty-four patients (13 male and 11 female) aged 40–77 years, with clinical signs of peripheral and central motor neuron lesions, admitted at Clinic for Neurology, Clinical Center Nis underwent the complete diagnostic procedure to verify ALS diagnosis and exclusion of other diseases that can mimic ALS. The clinical, laboratory, electromyoneurography (EMNG), and MRI investigations were performed. About the main clinical presentation of ALS, all study patients were divided into those presented with spinal form as well as predominantly bulbar symptomatology. Patients with the predominantly bulbar form of the disease are further divided into two subgroups: patients with isolated bulbar involvement and patients with the bulbar onset and spinal generalization. Patients were mostly nonsmokers. They have not previously used drugs with antioxidant effects. Their life history was not burdened with other diseases. For all patients, there was no positive family history for ALS.

ALS diagnosis was made based on the revised El Escorial Criteria (Torreilles et al.1995). ALS diagnosis is defined within the evidence of signs of impairment of lower motor neuron, using clinical examination, electrophysiological or neuropathological changes, associated with clinically proven impairment of upper motor neuron, with chronic and progressive development. It is still necessary, for diagnosis, the absence of electrophysiological and pathological findings characteristic of other diseases that explain the degeneration of motor neurons. These criteria define four categories of ALS: Clinically possible ALS, Clinically Probable - Laboratory-supported ALS, Clinically probable ALS, and Clinically definite ALS. To assess the functional status of patients at the time of admission to the clinic, we used the ALS Functional Rating Score (ALSFRS) (Estevez et al. 1999). A rating scale has been developed to provide a quantitative estimate of clinical status and disease progression. This scale includes assessment of swallowing, speech, and respiratory function, and both strength and function of upper and lower extremity musculature (maximal ALSFRS score – 48 (the best finding)). In our cross-sectional study, ALSFRS was accessible only at the time of the patient's inclusion in the study. Thus, we can assume that the patient had a score of 48 just before the onset time. That is why the disease progression index (DPI) was not assed as DeltaFS or ALSFRS-R score over time, as it has been early proposed, and already DPI was calculated as 48-actual ALSFRS/time (disease duration) for each case.

CSF samples were obtained by a lumbar spinal tap at the hospital admission. The CSF samples were immediately centrifuged at 10000g for 3 min at 4°C to remove any contaminating cells and kept on ice (–80°C) until the final biochemical assays. All CSF samples showed no bleeding or other pathological findings. CSF was obtained from all the subjects by lumbar spinal tap. The CSF samples for NO measuring were immediately centrifuged at 10,000 g for 3 min at 4 C to remove any contaminating cells and kept on ice (-80 C) until the biochemical assays. All CSF samples (obtained from control and CIS and RRMS patients) showed no bleeding. Total SOD activity was determined by the spectrophotometric method of Minami and Yoshikawa (1979), based on formazan colored product formation. In the reaction with NBT (nitro blue tetrazolium), superoxide anion (O2-), produced by pyrogallol autooxidation, forms a colored product. SOD, as O2- a scavenger, inhibits this reaction. The enzyme activity was expressed as U/ml.

All statistical calculations were performed using appropriated nonparametric tests after verification of values distribution in each group, using Mann–Whitney U test and Kruskal–Wallis test. Linear regression analysis was used for the assessment of correlation between tested parameters. All data are presented as medians with range throughout the text, or when it was appropriate as means ± SD. The $p < 0.05$ was considered significant. Cohen's d as the appropriate effects size measures was calculated. All statistical calculations were done using “SPSS 21.0 for Windows” (SPSS Inc., USA)

Results

The demographic, biochemical, and basic clinical characteristics of the study subjects are shown in Tables 1–3. Although blood-brain barrier (BBB) permeability was significantly increased in all sALS patients, there was no BBB disruption (revealed by increased albumin CSF/serum ratio > 7.0 × 10 − 3).

Regarding the clinical presentation at the moment of inclusion in this study, 11 of sALS patients (45.8%) were presented with the bulbar form of ALS (5 of them had isolated bulbar involvement - failing any clinical manifestation of spinal disorder; 6 patients had bulbar onset and spinal generalization - the combination of bulbar and spinal symptomatology), while 13 of sALS patients (54.2%) had a diagnosis of a spinal form of ALS. No significant differences were comparing the study patients' ages, basic biochemical findings of CFS (cells and proteins) as well as ALSFRS, while DPI and disease duration was higher in patients with the bulbar onset and spinal generalization about those with isolated bulbar involvement (Tables 2 and 3).

Table 1
Demographic, biochemical and clinical data of sALS patients

	Control group	sALS†
Number of patients	20	24
Female/Male	10/10	11/13
Age (years)	60 (23–65)	64 (30–77)
Serum protein/albumin (g/l)	66.4 ± 17.2/39 ± 10.1	64.2 ± 3.59/41 ± 4.67
CSF‡ protein/albumin (g/l)	0.40 ± 0.1/0.16 ± 0.02	0.49 ± 0.1/0.26 ± 0.04
Index of BBB§ permeability	4 ± 1.05	6.2 ± 0.6*
Disease duration (years)	/	1 (0.25–2)
ALSFRS ||	/	42.5 (29–46)
DPI ¶	/	6 (1–26)

* $p < 0.001$ (sALS vs control group); †sporadic amyotrophic lateral sclerosis, ‡cerebrospinal fluid; § blood brain barrier; || ALS Functional Rating Score, ¶ Disease Progression Index = 48-ALSERS/disease duration.

Table 2
Demographic, biochemical and clinical data of sALS patients with different clinical presentation.

	Bulbar form	Spinal form
Number of patients (%)	11 (45.8%)	13 (54.2%)
Female/Male (%)	6 (54.5%)/5 (45.5%)	5 (38.5%)/8 (61.5%)
Age (years)	63 (30–77)	65 (42–77)
Serum protein/albumin (g/l)	64.3 ± 9/40.3 ± 3.8	68.6 ± 4.9/42.6 ± 3.5
CSF* protein/albumin (g/l)	0.47 ± 0.1/0.25 ± 0.03	0.50 ± 0.11/0.27 ± 0.03
Index of BBB† permeability	6.2 ± 0.6	6.3 ± 0.5 ns
Disease duration (years)	1 (0.5 – 2)	1 (0.5 – 2)
ALSFRS ‡	42 (32–45)	43 (29–46)
DPI §	8 (5–26)	6 (1.20)

*Cerebrospinal fluid; † blood brain barrier; ‡ALS Functional Rating Score, §Disease Progression Index = 48-ALSERS/disease duration.

Table 3
Demographic, biochemical and clinical data of sALS† patients with bulbar form.

	Isolated bulbar involvement	With spinal generalization
Number of patients (%)	5 (45.4%)	6 (54.6%)
Female/Male (%)	4 (80%)/1 (20%)	2 (33.3%)/4 (66.7%)
Age (years)	57(47–63)	67.5 (30–77)
Serum protein/albumin (g/l)	67.3 ± 8/40.2 ± 2.8	61.8 ± 6.9/31.1 ± 5.4
CSF‡ protein/albumin (g/l)	0.44 ± 0.1/0.26 ± 0.04	0.49 ± 0.15/0.24 ± 0.04
Index of BBB§ permeability	6.4 ± 0.5	6 ± 0.45
Disease duration (years)	0.5 (0.5 – 1)	1.25 (0.5 – 2)*
ALSFRS ||	42 (35–46)	37.5 (32–43)
DPI ¶	6 (4–26)	10 (8–12) *

* $p < 0.05$ (Isolated bulbar involvement vs Bulbar onset and spinal generalization), †sporadic amyotrophic lateral sclerosis, ‡cerebrospinal fluid; § blood brain barrier; || ALS Functional Rating Score, ¶ Disease Progression Index = 48-ALSERS/disease duration.

The obtained NO values in the control and the study patients are presented in Fig. 1 (sALS patients: 7.29 ± 2.44 µmol/L, CG: 12.3 ± 1.82 µmol/L). The NO values of sALS patients were significantly higher than

those obtained in the control group ($p < 0.001$). Observed separately, there were no significant differences in the values of NO in CSF between patients about their clinical presentations ($p > 0.05$) and these results are not presented. The obtained SOD values in the control group and the study patients were presented in Fig. 2 (sALS patients: 100.87 ± 29.74 U/ml, CG: 132 ± 19.57U/ml). The total CSF SOD level was significantly decreased in ALS patients compared to the control group ($p < 0.001$). Although the SOD values in the CSF of patients with purely spinal clinical manifestations were higher than in patients with bulbar manifestations, this difference was not statistically significant ($p > 0.05$). There was also no statistically significant difference in SOD values in the CSF of patients with pure bulbar form and patients with a combined bulbospinal form of the disease. These results are not presented. There was moderate negative correlation between SOD and NO values in all sALS patients ($r = 0.31$; $p = 0.025$). The SOD values correlated significantly with the study group's age, ALSFRS, disease duration, and progression, while NO values were significantly associated with disease progression only (Table 4).

Table 4
Correlation of NO and SOD in CSF of sALS† patients with age, ALSFRS ||, disease duration and disease progression

		SOD	Age	ALSFRS	Disease duration	Disease progression
NO‡	r¶	− 0.306*	0.184	0.314	0.319	0,347**
	p-value	**0.025**	0.096	0.056	0.285	**0.001**
SOD §	r	1.000	0,808**	0,618**	0,702**	0.425**
	p-value		**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**

* $p < 0.05$,** $p < 0.001$, †sporadic amyotrophic lateral sclerosis, ‡ nitric oxide, § superoxide dismutase; || amyotrophic lateral sclerosis Functional Rating Score, ¶ Correlation coefficient,

Discussion

Neurons are highly energy-dependent and highly specialized cells, showing great sensitivity to the OS, due to the high metabolic activity combined with neurotransmission (Shaw and Eggett 2000). Motor neurons receive a high level of glutamatergic excitatory input. In the case of hyperactivity of glutamate neurotransmission, the excessive intracellular calcium accumulation leads to abnormal activation of calcium-dependent enzymes, including the nitric oxide synthase (Trotti et al. 1998). Emerging evidence suggests that NO and its oxidation products play a central role in both triggering and amplifying oxidative damage in neurodegeneration (Drechsel et al. 2012). The excess NO reacts with O2- to form powerful oxidant peroxynitrite, which is responsible for the induction of cell death. Because nitric oxide reacts with O2- three-fold faster than SOD, NO is the only known biomolecule capable of effectively competing SOD for available O2- (Toreilles et al. 1998). A tenfold increase in superoxide anion radicals and nitric oxide has been found to increase peroxynitrite production one hundred times (Djordjevic 2004). Moreover, excess NO may cause a transient inhibition of the mitochondrial electron flow yielding an increase in O2-

synthesis, thus favoring the intracellular production of peroxynitrite (Cassina and Radi 1996). NO, in addition to the direct neurotoxic effect through peroxynitrite production, could underlie the glutamate-induced neurotoxicity, since NO inhibits glutamate transport (Dawson and Dawson 1996, Taskiran et al. 2000). Peroxynitrite is a substrate for SOD, which catalyzes the nitration of tyrosine on other proteins (Ischiropoulos, 2003). NO might be a causative molecule of motor neuron death in ALS (Beckman et al. 1993). They reported the nitration of tyrosine residues in neurofilaments which are supported by the following reports showing the nitration of tyrosine residues in neurofilaments and increased levels of NO metabolites in CSF of ALS patients (Beal et al. 1997, Beckman et al. 2001, Ikeda et al. 1995, Toghi et al 1999). The structural proteins that form neurofilaments are particularly susceptible to tyrosine nitration by peroxynitrite. Because of their long axons, motoneurons contain enormous quantities of neurofilament proteins (Beckman et al. 1993).

Evidence to date confirms that peroxynitrite has the potential to initiate and sustain the process of neuronal damage in neurodegenerative diseases. However, despite several lines of evidence suggesting that the production of NO is involved in motor neuron death in vivo, there are insufficient reports in the literature on NO levels and SOD activity in CSF of patients with sALS and the results are inconsistent. Ikeda et al. measured nitrite, nitrate, and cyclic GMP in CSF samples from patients with different degenerative neurologic diseases (DND: Parkinson's disease, spinocerebellar ataxia, and amyotrophic lateral sclerosis) (Ikeda et al. 1999). They found no significant change in CSF nitrite, nitrate, or cyclic GMP in patients with any DND compared with control levels. Milstein et al. investigated CSF nitrate and nitrite levels in neurologic diseases did not observe changes in patients with ALS as well (Milstien et al. 1994). On the other hand, Taskiran et al. found that the levels of stable NO metabolites levels were higher in CSF of ALS patients (2000). Kokic et al have also recorded a greater nitrite production in the CSF from sALS patients compared to controls (2005). The results of our study also show elevated levels of NO in the CSF of patients with sALS compared to controls. Regarding the antioxidant enzyme activity of SOD, we have observed a significantly lower CSF SOD activity in sALS patients as compared to control which is by the results of certain authors (Boll et al. 2003, Milstien et al. 1994). These authors reported elevated NO levels and decreased SOD activity, which coincides with our results. Decreased SOD activity together with an increased NO level strongly suggests an increased level of superoxide anion radicals in the CSF of sALS patients that reacts with NO, forming peroxynitrite. In contrast to our results, no statistically significant reduction in SOD activity in the CSF of ALS patients was found in some studies (Iwasaki et al. 1993, Jacobsson et al.2001). Jakobson et al did not record differences in the enzymatic activity of CuZn SOD in the cerebrospinal fluid of patients with sporadic, familial ALS and control group (2001) The authors explain the lack of expected leakage from compromised motor neurons with the fact that the disease has a slow course and afflicts only a limited portion of the cells in the CNS. On the other hand,

Kokic et al found increased CuZn-SOD activity and NO level in the CSF from sALS patients (when compared to control subjects) indicating conditions for the reaction of SOD with superoxide forming hydrogen peroxide, which supports the idea that oxidative stress may proceed via the hydrogen peroxide, in addition to peroxynitrite pathways (Tórsdóttir et al. 2000, Wiedau-Pazos et al. 1996). The results of our research, in addition to the elevated NO levels and decreased SOD activity, also show a correlation

between these two parameters, which confirms the involvement of these parameters in the pathogenesis of sALS.

The SOD levels correlated significantly with study groups' age, ALSFRS, disease duration, and progression, while NO levels were significantly associated with disease progression only (Table 4). These results are consistent with the results of studies in both experimental animals and patients with ALS. These changes were recognized during disease progression and in early-stage and end-stage in SOD-1 mutant mice and ALS patients (Butkovsky et al, 2012, Cunha et al 2018, Yim et al. 1996). As the disease progresses, oxidative stress might be increased by nutritional deficiency, cachexia, psychological stress, and impending respiratory failure (Di Pietro et al. 2017). In addition, a correlation between oxidative stress measured by positron emission tomography of the brain and clinical severity in ALS was recently reported (D'Amico et al.2013). Oxidative stress might be both a cause and consequence of the disease and is associated with pathogenesis as well as disease progression (Ikawa et al. 2015). Given the higher incidence of the disease in men, some studies have investigated the vulnerability of the male and female nervous systems to OS and neurotoxic effects (Takashi et al, 2021).

Taskiran and al. suggested that NO may be involved in the pathogenesis of ALS directly or indirectly and in a sexually dimorphic manner (2000). They reported that CSF nitrites and nitrates were significantly increased in both genders while serum nitrites and nitrates were increased in male patients. Boll et all. found CSF nitrates to be significantly increased in female ALS patients and slightly but not significantly increased in male ALS patients as compared to gender-matched controls (2003). The results of our study did not show significant gender differences in the levels of NO and SOD in CSF of patients with sALS.

This study is a continuation of our previous research on CSF toxicity and the role of OS in the pathogenesis of sALS (Djordjevic et al. 2018). The data presented here clearly support the role of impaired oxidant/antioxidant balance in the pathogenesis of sALS, where NO overproduction and decreased SOD defense activity seem to be particularly involved. The CSF SOD and NO level might serve as a useful biomarker for functional disorder and progression of the disease, but these facts need to be reevaluated in future research which should be performed on a large cohort of ALS patients.

Declarations

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Ethics approval and consent to participate: The study was approved by the Ethical Committee of the Faculty of the Medicine University of Nis and informed consent was obtained from each patient before entry into the study, according to the declaration of Helsinki.

References

1. Beal MF, Ferrante RJ, Browne SE, Matthews RT, Kowall NW, Brown Jr RH (1997) Increased 3-nitrotyrosine in both sporadic and familial amyotrophic lateral sclerosis. Ann Neurol 42: 644–654.
2. Beckman JS (1996) Oxidative damage and tyrosine nitration from peroxynitrite. Chem Res Toxicol. 9:836–844.
3. Beckman JS, Carson M, Smith CD, Koppenol WH (1993) ALS, SOD and peroxynitrite. Nature 364(6438):584.
4. Beckman JS, Estévez AG, Crow JP, Barbeito (2001). Superoxide dismutase and the death of motoneurons in ALS. Trends Neurosci 24(11):S15-20
5. Bergeron C (1995) Oxidative stress: its role in the pathogenesis of amyotrophic lateral sclerosis. J Neurol Sci 129: 81–84.
6. Boll M, Alcaraz-Zubeldia M, Montes S, Murillo-Bonilla L, Rios C (2003) Raised nitrate concentration and low SOD activity in the CSF of sporadic ALS patients. Neurochem Res 28:699–703
7. Butovsky O, Siddiqui S, Gabriely G, Lanser AJ, Dake B, Murugaiyan G, et al (2012) Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. J Clin Invest 122(9):3063–87.
8. Cassina A, Radi R (1996) Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. Arch Biochem Biophys 328:309–316.
9. Cunha C, Santos C, Gomes C, Fernandes A, Correia AM, Sebastião AM, et al (2018) Downregulated Glia Interplay and Increased miRNA-155 as Promising Markers to Track ALS at an Early Stage. Mol Neurobiol 55(5):4207–4224.
10. D'Amico E, Factor-Litvak P, Santella RM, Mitsumoto H (2013) Clinical perspective on oxidative stress in sporadic amyotrophic lateral sclerosis. Free Radic Biol Med 65:509–527.
11. Dawson VL, Dawson TM (1996) Nitric oxide neurotoxicity. Journal of Chemical neuroanathomy 10:179–190.
12. Di Pietro L, Baranzini M, Berardinelli MG, Lattanzi W, Monforte M, Tasca G, et al (2017) Potential therapeutic targets for ALS: MIR206, MIR208b and MIR499 are modulated during disease progression in the skeletal muscle of patients. Sci Rep 7(1):9538.

13. Djordjevic VB (2004) Free radicals in cell biology.Int Rev Cytol 237:57–89.
14. Djordjevic G, Ljubisavljevic S, Sretenovic S, Kocic G, Stojanovic I, Stojanovic S (2017) The cerebrospinal fluid values of advanced oxidation protein products and total thiol content in patients with amyotrophic lateral sclerosis. Clin Neurol Neurosurg 163:33–38.
15. Drechsel DA, Estévez AG, Barbeito L, Beckman JS (2012) Nitric oxide-mediated oxidative damage and the progressive demise of motor neurons in ALS. Neurotox Res 22(4):251–64.
16. Estévez AG, Crow JP, Sampson JB, Reiter C, Zhuang Y, Richardson GJ, et al (1999) Induction of nitric oxide-dependent apoptosis in motor neurons by zinc-deficient superoxide dismutase. Science 286(5449):2498–2500.
17. Ihara Y, Mori A, Hayabara T, Nobukuni K, Sato K, Kibata M, et al (1995) Superoxide dismutase and free radicals in sporadic amyotrophic lateral sclerosis: relationship to clinical data. J Neurol Sci 134:51–56.
18. Ihara Y, Nobukuni K, Takata H, Hayabara T (2005) Oxidative stress and metal content in blood and cerebrospinal fluid of amyotrophic lateral sclerosis patients with and without a Cu, Zn-superoxide dismutase mutation. Neurol Res 27:105–108.
19. Ikawa M, Okazawa H, Tsujikawa T, Matsunaga A, Yamamura O, Mori T, et al (2015) Increased oxidative stress is related to disease severity in the ALS motor cortex: A PET study. Neurology 84(20):2033–2039.
20. Ikeda M, Sato I, Yuasa T, Miyatake T, Murota S (1995) Nitrite, nitrate and cGMP in the cerebrospinal liquid in degenerative neurologic diseases. J Neural Transm Gen Sect 100: 263–267.
21. Ischiropoulos H. Biological selectivity and functional aspects of protein tyrosine nitration (2003) Biochem Biophys Res Commun. 305:776–783.
22. Iwasaki Y, Ikeda K, Kinoshita M (1993) Decreased cerebrospinal-fluid superoxide dismutase in amyotrophic lateral sclerosis. Lancet 342(8879):1118.
23. Jacobsson J, Jonsson PA, Andersen PM, Forsgren L, Marklund SL (2001) Superoxide dismutase in CSF from amyotrophic lateral sclerosis patients with and without CuZn-superoxide dismutase mutations. Brain. 124(7):1461–1466.
24. Kokic AN, Stevic Z, Stojanovic S, Blagojevic DP, Jones DR, Pavlovic S,et al. (2005) Biotransformation of nitric oxide in the cerebrospinal fluid of amyotrophic lateral sclerosis patients. Redox Rep 10:265–270
25. Milstien S, Sakai N, Brew BJ, Krieger C, Vickers JH, Saito K, Heyes MP (1994) Cerebrospinal liquid nitrite/nitrate levels in neurologic diseases. J Neurochem 63: 1178–1180.
26. Minami M, Yoshikawa H (1979) A simplified assay method of superoxide dismutase activity for clinical use. Clin Chim Acta 92(3):337–42
27. Pardo CA, Xu Z, Borchelt DR, Price DL, Sisodia SS, Cleveland DW (1995) Superoxide dismutase is an abundant component in cell bodies, dendrites, and axons of motor neurons and in a subset of other neurons. Neurobiology 92: 954–958.

28. Sharma P, Jha AB, Dubey RD, Pessarakli M (2012) Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. Journal of Botany 217037.
29. Shaw PJ, Eggett CJ (2000) Molecular factors underlying selective vulnerability of motor neurons to neurodegeneration in amyotrophic lateral sclerosis. J Neurol 247 (1):I17-27.
30. Süssmuth SD, Brettschneider J, Ludolph AC, Tumani H (2008) Biochemical markers in CSF of ALS patients. Curr Med Chem 15:1788–1801
31. Takashi H, Hiroshi T, Akira T (2021) Biomolecular Modifications Linked to Oxidative Stress in Amyotrophic Lateral Sclerosis: Determining Promising Biomarkers Related to Oxidative Stress. Biomolecular Processes 9:1667.
32. Taskiran D, Sagduyu A, Yuceyar N, Kutay FZ, Pogun S (2000) Increased cerebrospinal fluid and serum nitrite and nitrate levels in amyotrophic lateral sclerosis. Int J Neurosci 101: 65–72.
33. Tohgi H, Abe T, Yamazaki K, Murata T, Ishizaki E, Isobe C (1999) Increase in oxidized NO products and reduction in oxidized glutathione in cerebrospinal liquid from patients with sporadic form of amyotrophic lateral sclerosis. Neurosci Lett 260: 204–206.
34. Torreilles F, Salman-Tabcheh S, Guerin MC, Torreilles J (1999) Neurodegenerative disorders: the role of peroxynitrite. Brain Research Reviews 30:153–163.
35. Tórsdóttir G, Kristinsson J, Gudmundsson G, Snaedal J, Jóhannesson T (2000) Copper, ceruloplasmin and superoxide dismutase (SOD) in amyotrophic lateral sclerosis. Pharmacol Toxicol 87(3):126–30.
36. Trotti D, Danbolt NC, Volterra A (1998) Glutamate transporters are oxidant-vulnerable: a molecular link between oxidative and excitotoxic neurodegeneration? Trends Pharmacol Sci 19(8):328–34.
37. Turner MR, Al-Chalabi A, Chio A, Hardiman O, Kiernan MC, Rohrer JD, et al (2017)Genetic screening in sporadic ALS and FTD. *J Neurol Neurosurg Psychiatry* 88:1042–1044.
38. Wiedau-Pazos M, Goto JJ, Rabizadeh S, Gralla EB, Roe JA, Lee MK, Valentine JS, Bredesen DE (1996) Altered reactivity of superoxide dismutase in familial amyotrophic lateral sclerosis. Science 271(5248):515–518.
39. Yim MB, Kang JH, Yim HS, Kwak HS, Chock PB, Stadtman ER (1996) A gain-of-function of an amyotrophic lateral sclerosisassociated Cu, Zn-superoxide dismutase mutant: an enhancement of free radical formation due to a decrease in Km for hydrogen peroxide. Proc Natl Acad Sci USA 93: 5709–5714.

Figures

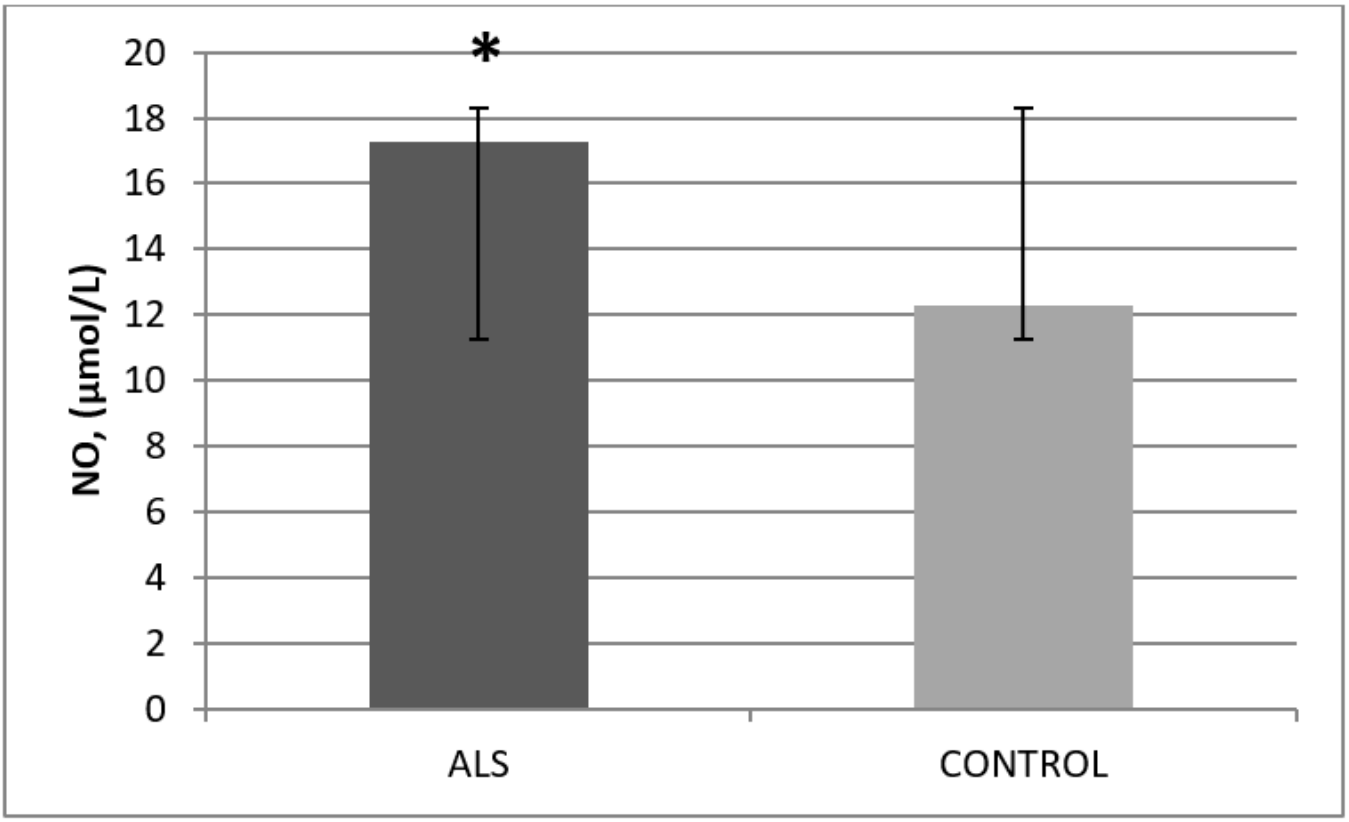

Figure 1

Values of nitric oxide in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis (ALS) and control group (*p<0.001)

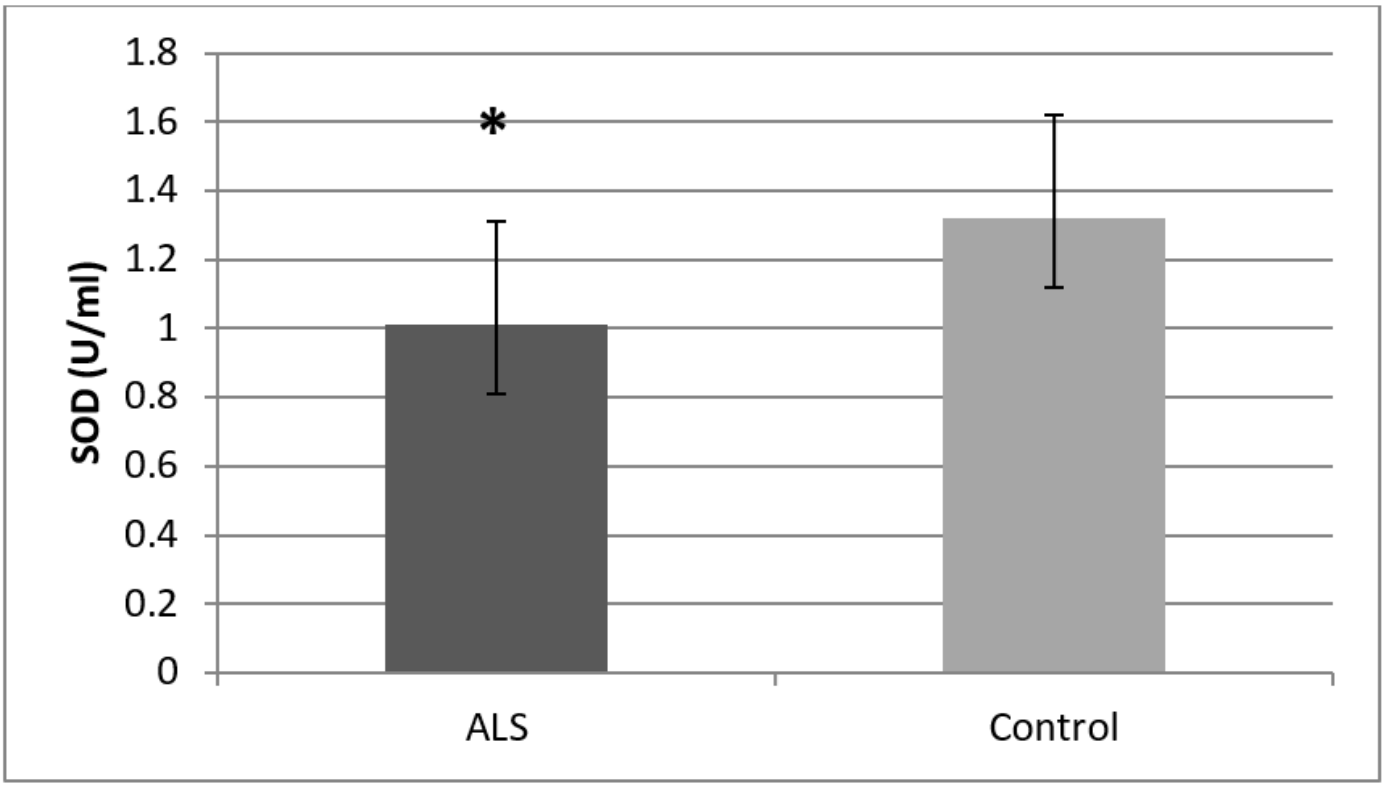

Figure 2

Values of superoxide dismutase in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis (ALS) and control group (*p<0.001)