Clinical Neurology and Neurosurgery 163 (2017) 33–38

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

Full length article

The cerebrospinal fluid values of advanced oxidation protein products and total thiol content in patients with amyotrophic lateral sclerosis

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ARTICLE INFO

Keywords:
Oxidative stress
Amyotrophic lateral sclerosis

ABSTRACT

Objectives: Amyotrophic lateral sclerosis (ALS) is recognized as a progressive neurodegenerative disorder of unknown origin. Oxidative stress (OS) is considered as one of the most challenging hypothesis in the disease pathogenesis. The aim of this study was to contribute to the understanding of what extent there is involvement of OS in ALS.

Patients and methods: We assessed Advanced Oxidation Protein Products (AOPP) and total thiol (-SH) groups in cerebrospinal fluid (CSF) of 24 ALS patients (13 of them presented with spinal form while 11 patients had bulbar form) and 20 controls (CG).

Results: The obtained AOPP levels in ALS patients were higher than those in CG ($p < 0.001$), while −SH groups showed lower values compared to CG ($p < 0.001$). The AOPP values were higher in ALS patients with bulbar compared with ALS patients with common spinal manifestation ($p < 0.001$). There were no differences in −SH group's levels among these different clinical forms ($p > 0.05$). The negative correlation between AOPP and the levels of −SH groups was confirmed ($p < 0.01$). Significant mild correlations between tested parameters and functional rating scale as well as disease progression index were recorded for both of tested parameters in spinal form of ALS ($p < 0.01$).

Conclusion: The data presented here clearly support the fact that OS is involved in patophysiology of ALS, where oxidation of −SH groups represents an important aspect of protein oxidation. The CSF AOPP level and −SH groups may serve as potential useful biomarker for functional disorder and progression of the disease in the spinal form of ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is recognized as a progressive neurodegenerative disorder of unknown origin which characterizes by signs of peripheral and central motor neurons damages. It is possible to distinguish sporadic and familial ALS, although its clinical manifestation is very similar. Amyotrophic lateral sclerosis is characterized by heterogeneity of its clinical manifestations, depending on the proportion of damaged peripheral and central motor neurons. However, it is believed that these differences are meaningful only in the beginning of the disease until later in the majority of patients manifest the characteristics of involvement of damages of both groups of motor neurons [1].

The main histopathological characteristics of ALS represent degeneration and loss of neurons in the anterior horn of the spinal cord, brain stem, and cerebral cortex. With the losing of cortical cells, there are degenerations of the pyramidal tract with the losing of large myelinated fibers. Despite the well-documented degenerative processes in ALS, there is still no answer to the question of what causes or runs this degeneration. It is assumed that there is no a unified mechanism, but this is a complex multifactorial etiopathogenetic disorders. In this complex milieu, it is believed that oxidative stress (OS) plays a very important role and attracted special research attention in recent years [1,2].

Despite numerous evidences that OS can determine the appearance of motor neuron degeneration [2–4], there is still a debatable question whether the increased OS appears as a cause or also contributing factor in ALS pathogenesis as a result of previous damage, and does OS influence the course as well as the clinical phenotype of the disease. This dilemma, however, does not diminish the pathogenetic importance of OS in neurodegenerative diseases in general, considering that OS by

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http://dx.doi.org/10.1016/j.clineuro.2017.10.015
Received 3 July 2017; Received in revised form 8 October 2017; Accepted 17 October 2017
Available online 18 October 2017
0303-8467/

itself has the capacity to cause serious damage in cellular systems [1–3]. It seems that for a better understanding of this phenomenon, the further researches are needed.

Authors performed this study in order to assess the contribution to the further elucidation of the role of OS in the pathogenesis of ALS. In this regard, were measured levels of total advanced oxidation protein products (AOPP) and total thiols content (-SH groups), as markers of OS and its intensity, in the cerebrospinal fluid (CSF) of patients with spinal and bulbar form of ALS.

2. Patient and methods

2.1. Ethical permission

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

2.2. Control patients

Twenty control patients, no smokers, (10 male, 10 female), aged 23–65 years, admitted at Clinic for Neurology, Clinical Center Nis who 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 laboratory, MRI scan and CSF examination. Their final diagnosis was mainly functional disorders and tension type headache.

2.3. ALS patients

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 investigation were performed. In reference the main clinical presentation of ALS all study patients were divided into those presented with spinal form as well as predominantly bulbar simptomatology. Patients with predominantly bulbar form of the disease are further divided into two subgroups: patients with isolated bulbar involvement and patients with 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.

2.4. Clinical assessment

ALS diagnosis was made on the basis of the revised El Escorial Criteria [5]. ALS diagnosis is defined within the evidence of signs of impairment of lower motor neuron, by means of 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) [6]. 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 patients inclusion in the study. Thus, we can assume that the patient had score 48 just before the onset time. That is why 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 individual case.

2.5. Biochemical assessment

2.5.1. CSF sampling

CSF samples were obtained by lumbar spinal tap at the hospital admission. The CSF samples were immediately centrifuged at 10000*g* 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.

2.5.2. Advanced oxidation protein products

Advanced oxidation protein products were determined in CSF mixed with H_2O, acetic acid and potassium iodide. The absorbance was read spectrophotometrically at 340 nm and compared with a solution of chloramine T dissolved in the same buffer. The data were expressed as μML^{-1} of chloramine equivalents and related plasma or CSF total protein [7].

2.5.3. Sulfhydryl (-SH) groups

The amount of total (protein and non protein) −SH groups, was estimated in CSF, by the spectrophotometric assay using 2, 2-dithiobisnitrobenzoic acid (DTNB), and the results were expressed as μML^{-1} [8].

2.5.4. Total protein and albumin determination

The total protein and albumin concentrations were measured in serum and CSF samples to assess CSF/serum albumin ratio as an index of blood brain barrier (BBB) disruption. It was defined as disrupted when this ratio was increased more than 7.0×10^{-3} (Brochure Sysmex XT-2000i and XT-1800i, Switzerland).

2.5.5. Chemicals

Chemicals were purchased from Sigma (St. Louis, MO, USA). All used chemicals were of analytical grade. All drug solutions were prepared on the day of the analyses.

2.6. Statistics

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 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 as a significant. Cohen's *d* as the appropriate effects size measures was calculated. All statistical calculations were done using "SPSS 13.0 for Windows" (SPSS Inc., USA).

3. Results

The demographic, biochemical and basic clinical characteristics of the study subjects are shown in Tables 1–3. Although BBB permeability was significantly increase in all ALS patients, there were 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 ALS patients (45.8%) were presented with bulbar form of ALS (5 of them had isolated bulbar involvement − failing any clinically manifestation of spinal disorder; 6 patients had bulbar onset and spinal generalization − combination of bulbar and spinal symptomatology), while 13 of ALS patients (54.2%) had diagnosis of spinal