SPECIFIC MARKER OF NEURODEGENERATION IN OPTIC NEURITIS

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ABSTRACT

Early diagnosis of optic neuritis (ON) is essential to prevent or limit the structural damage and permanent loss of visual function. The aim of this study was to estimate prognostic importance of the neuron-specific enolase (NSE) in blood serum and lachrymal fluid in optic neuritis. The clinical-diagnostic examination was performed on patients with optic neuritis, including optical coherence tomography (OCT) and Visually Evoked Potentials (VEP), MR – tractography, as well as analysis of NSE content in blood serum and in lachrymal fluid.

Decreased level of NSE in lachrymal fluid in patients with acute stage of optic neuritis and increased level of NSE in patients with optic neuritis at the stage of transition into atrophy of optic nerve disc were detected. Comparative analysis demonstrated relatively high level of NSE in lachrymal fluid at transitional stage to atrophy as compared to acute phase.

UDC CODE & KEYWORDS

■ UDC: 617.731-002:612 ■ Optic neuritis ■ Lachrymal fluid ■ Neuron-specific enolase

INTRODUCTION

Determination of specific markers of apoptosis in serum plays important role in diagnosis and prognosis of optic nerve diseases (Brambilla et al., 2012). Gradual destruction of neurons in neurodegeneration leads to efflux of neuron-specific enzymes and their isoforms. They exit into extracellular space out of cells. Increased levels of these enzymes allow us to judge the depth and intensity of structural and functional abnormalities of biomembranes in the central nervous system (Berger, Pierce, Wisniewski, Adelson & Clark, 2002).

Neuronal ionic proteins play an important role in neuron development, particularly in the formation of synaptic structures, as well as in the pathogenesis of neural diseases. Among the known neuron-specific proteins neuron-specific enolase (NSE) appeared to be the most investigated and adequately describing membranous functions of the blood-brain barrier. NSE is a glycolytic enzyme that is primarily localized to the neuronal cytoplasm and synaptic plasma membrane. NSE was reported to be associated with cytoskeleton and to play important roles in cell growth, proliferation and survival of neurons. According to literature, the qualitative and quantitative assessment of this protein in the blood serum at diseases of nervous tissue provide valuable information about the degree of manifestation of neuron damage and disorders of whole integrity of the blood-brain barrier (Chekhonin, Gurina & Rabukhin, 2000).

The detection of neuron-specific enolase in biological fluids has been investigated as an indirect marker of neuronal damage in humans. This protein was measured by a sandwich enzymeimmunoassay in serum and cerebrospinal fluid samples of patients with consciousness disorders of various etiologies. Neuron-specific enolase level was significantly increased in serum of patients with comas resulting from anoxemia, head injury, septic state, cirrhosis and fulminant hepatitis. On the other hand, there was no change of this marker level in patients with meningitis (abnormality not normally accompanied with neuronal lesion). The statistical analysis of the results suggested that in neurological disorders neuron-specific enolase levels in cerebrospinal fluid could have some prognostic value. The correlation between its level in cerebrospinal fluid and in serum was also demonstrated. Neuron-specific enolase increase in biological fluids thus represents a useful and promising marker biochemically characterizing various stroke types possibly resulting in neuronal damage (Chekhonin, Lebedev, & Rabukhin, 2004).

Gelderblom, Tristan, Benjamin, & Peter (2013) studied NSE levels in plasma. They established the direct correlation dependence between NSE increase and brain cell loss on the basis of pilot study on rats with modeling ischemic stroke and multiple sclerosis using MRT and immunological investigation. Herewith, on day 15 of the study the authors revealed NSE increase which tended to decrease from day 21 to day 30. On author's opinion informative value of the method allows its broader application in various pathologies of nervous system.

The same studies were made in glaucoma (Maruyama, Ohguro, & Ikeda, 2010), cataract and congenital retinal pathologies (Audrey et al., 2010), which confirmed the high sensitivity of this technique and the possibility to use it broader in visual-nervous apparatus diseases.

Pleines et al. (2001) proved NSE informative value in patients with severe cerebral injuries. The authors confirmed that NSE is a marker of neuroinflammation. The researchers diagnosed the onset of inflammatory process after craniocerebral injury by NSE detection in cerebrospinal fluid.

Composition of serum is similar to one of lacrimal fluid and characterizes metabolic processes occurring in the eye. This is another aspect suggesting suitability of lacrimal fluid evaluation in diagnosis and management (Brjevskiy & Somov, 1995).

Evaluation of this factor in serum and lacrimal fluid and its comparative analysis in various stages of optic neuritis (ON) is one of the promising applications. Changes of this marker along the flow of disease may indirectly indicate the transition of optic nerve inflammation from the stage of swelling to the stage of ischemia and atrophy, which will serve as a basis for correction of treatment. This is particularly important as ophthalmoscopic appearance often changes at later stages and depends on subjective estimation of a physician.

The purpose of the study was evaluation of prognostic significance of NSE in blood serum and lachrymal fluid in patients with optic neuritis.

Material and methods

Thirty-seven patients with ON and 10 controls were evaluated at the Department of Ophthalmology at the Tashkent Institute of Postgraduate Medical Education. Approval was obtained for the study from Institutional Review Board Ethics Committee. The study followed the principles of the Declaration of Helsinki and informed consent was obtained from all participants. The participants were included in the study on the basis of the following: optic neuritis in acute stage and in stage of ischemia (atrophic stage). Two groups of patients with optic neuritis between 15 and 55 years (mean age 27 ± 1.4 years) were examined. Among the examined were 14 males and 16 females. They were randomized by sex and age. According to international classification group 1 included 19 patients (19 eyes) in acute stage of optic neuritis. Group 2 consisted of 18 patients (18 eyes) with optic neuritis in the stage of transition to atrophy of optic disc (OD). Group 3 (control) included 20 absolutely healthy people (20 eyes) at the same age category. All the participants gave their informed consent to participate in the clinical trial.

Patient inclusion criteria for group 1: swelling and hyperemia of the optic disc on ophthalmoscopy, swelling of the disc and neuroretinal rim on OCT, MR tractography: swelling of neural fibers and normal parameters of fractional anisotropy. Patient inclusion criteria for group 2: mild swelling and pallor of the optic disc, thinning of RNFL on OCT, MR tractography: thinning and interruption of neural fibers, diminished levels of fractional anisotropy in the area of interrupted neural fibers of the optic tract.

All patients were performed complex examination including standard ophthalmological methods and special methods of investigation: brain and orbit MRI with tractography, optical coherence tomography (OCT) - (Zeiss, Spectral Domain Technology), examination of the visually evoked potentials (VEP) (Neuron-spectrum 4-VPM). Visual parameters were assessed by visual acuity (VA) using the standard Snellen acuity chart and Humphrey automated static perimetry for visual field (VF) defects.

OCT examination. The subjects underwent OCT scanning of the optic nerve head (ONH) region and macular area. Peripapillary RNFL thickness parameters were automatically calculated by the software including average thickness and thickness in each one of the 12 clock segments.

Neurovisualisation was performed in the radiology department of the Tashkent Institute of Postgraduate Medical Education. BRIVO-355 MRT with 1,5 Tl magnetic field induction was used for brain MRI. For MR-tractography DTI and Grad Echo MPRage protocols were used. Data analysis was performed for each patient after the procedure with the help of "Neuro 3D" software designed for "Siemens" standard workstation. It included fiber tractography in three dimensional syntheses (3D). Individual tracts as well as general overview of the tracts of white matter were composed.

Robert, Ranji & Elias (2004) studied patients with retrobulbar neuritis caused by herpes virus, meningitis and HIV infection. On brain MRI they detected lesions of chiasm, optic tracts, lateral geniculate bodies and optic radiation.

Lacrimal fluid studies were performed in every participant. It has been studied in the morning right after waking up to exclude influence of hygienic procedures. Lacrimal fluid samples (0,3 ml) were collected with canula from lacrimal lake and lower conjunctival space avoiding contact with conjunctiva and cornea. Blood samples (5 ml) were taken from ulnar vein. It was centrifuged to separate serum. Collected samples of lacrimal fluid and serum were immediately placed in test tube and sent for lab studies. The levels of NSE in lacrimal fluid and serum were detected in accordance with standard methods with the use of Cobas e 411 automated electrochemoluminescent immunoanalyzer for chemoluminescent immunoanalysis (Roche Diagnostics, Swetzerland). CRP was studied in lachrymal fluid and in plasma. The qualitative investigation of this protein was performed. The concentration of C-reactive protein was determined by the ELISA test system «IBL» ("BioKhimMak," Russia).

Statistical analysis of the data was performed by Student's t test for continuous variables and Fisher's exact test for categorical variables. As a control (normal) we used the results of NSE levels, VEP, MRI and OCT of 20 healthy individuals of the same age group. The results that we have obtained were within normal limits.

Results and discussion

We have detected significant functional abnormalities of the affected eyes in patients with optic neuritis. Visual acuity (VA) in the affected eyes in group 1 averaged to 0.4 ± 0.06 , in group 2 0.08 ± 0.02 , visual fields $354.17 \pm 12.7^\circ$ and $286.2 \pm 8.7^\circ$ respectively. Herewith, visual field constriction with central scotoma was observed. The results of VA change over time for both groups are shown in Table 1.

On slit-lamp examination anterior segment was within normal limits. In group 1 in acute stage of the disease direct ophthalmoscopy revealed round or oval pink optic disc with poorly defined margins. The number of vessels passing through the disc margins increased (18.4 ± 2.2), arteries of normal caliber, dilated veins, peripapillary edema and undetectable macular reflex.

Group 2 with transition stage of disease was characterized by pale disc with ill-defined margins. The number of vessels passing through the disc margin reduced to 13.1 ± 2.0 . The arteries were constricted with normal veins. Appearance and quantity of vessels were estimated on ophthalmoscopy. Group 3 (control) did not show any fundus abnormalities.

Table 1: Parameters CRP, NSE, OCT, VEP of patients with optic neuritis

Parameters	Study group 1 (acute stage of optic neuritis n=19)	Study group 2 optic neuritis at the stage of transition to atrophy of the optic disc (n=18)	Healthy individuals (n=20)
Visual acuity	0.4 ± 0.06	0.08 ± 0.02	0.9 ± 0.04
Visual field	354.17 ± 12.7°	286.2 ± 8.7°	550 ± 9.3°
CRP in LF, mcg/mL	182.0 ± 7.81*	157.0 ± 6.72	5.56 ± 0.23
CRP in serum, mcg/mL	8.2 ± 0.23	7.6 ± 0.31	5.6 ± 0.24*
NSE in LF, ng/ml	0.834 ± 0.175*	20.77 ± 1.24*	15.7 ± 0.2*
NSE in BS, ng/ml	13.67 ± 1.44*	14.76 ± 1.23*	16.8 ± 0.12*
RNFL thickness	185.5 ± 12.4	98.25 ± 2.2*	107.3 ± 3.4
Rim Area	2.26 ± 0.21*	1.4 ± 0.2*	1.5 ± 0.14
Disk Area	2.3 ± 0.23*	1.72 ± 0.13	1.73 ± 0.12
Cup Volume	0.01 ± 0.001*	0.02 ± 0.01*	0.03 ± 0.01
VEP Latency	115.2 ± 4.3*	133.5 ± 5.7*	102.3 ± 4.2*
VEP Amplitude	8.2 ± 0.92*	4.4 ± 0.3*	8.4 ± 0.6*
Note: * - Significant difference when compared with the control group (p<0.05)			

Source: Authors

The study of correlation between OCT and NSE levels showed that in acute phase in patients with ON OCT demonstrated optic disc and peripapillary area swelling whereas the NSE levels decreased (reverse correlation $r = -0.45$). On transition to optic atrophy NSE levels were noted to increase and thinning of RNFL thickness on OCT (reverse correlation $r = -0.56$).

At the same time, in acute phase C-reactive protein levels in lachrymal fluid and serum, as a specific inflammatory marker, were increased. This suggests development of acute inflammation. In patient of group 2 at transition stage this criterion had tendency to reduce (Table 1).

According to VEP for pattern, latency index P100 in group 1 in acute stage of the disease increased in comparison with group 2 patients at transition stage and reached 115.2 ± 4.3 ms. The normal readings in patients is 102 ms. VEP amplitude reduced significantly in group 2 patients at transition stage of the disease as opposed to group 1 patients in acute stage of the disease (4.4 ± 0.3 mcV; normal 8.4 mcV). Decrease in amplitude suggested reduction of the number of functional axons.

MR tractography in 7 patients of group 2 in transition stage of the disease revealed thinning and partial tear. Four patients of the same group showed interruption of several fibers of occipital forceps at the site of attachment to bundle of optic radiation, whereas these abnormalities were not observed in group 1 patients in acute stage of the disease.

The analysis of NSE levels showed significant changes depending on the stage and rate of progression of the disease. The level of NSE in health is 15.7-17.0 ng/ml. In 19 eyes of group 1 patients in acute stage with disc hyperemia the level of NSE in lachrymal fluid was noted to reach 0.834 ± 0.175 ng/ml ($p < 0.05$) on average. NSE in serum was detected at the level 13.67 ± 1.44 ng/ml. The NSE levels in lachrymal fluid in the experimental group were compared with the levels in lachrymal fluid in the control group. Herewith, early stages of ON were characterized by 19 fold ($p < 0.05$) reduction of NSE levels in lachrymal fluid. Consequently, at the stage of hyperemia and swelling of optic disc NSE level in lachrymal fluid was below normal limit, whereas in serum it was within normal limits.

In group 2 patients in transition stage of the disease NSE level in lachrymal fluid reached 20.77 ± 1.24 ng/ml. Thereby, at the stage of ischemia and atrophy this index increases significantly in lachrymal fluid. Analysis revealed tendency of NSE in lachrymal fluid to increase in relation to progression of the disease. On transition from acute phase to atrophy NSE level in lachrymal fluid rose 1.22 times of normal ($p < 0.05$).

In control group (healthy individuals) the NSE levels in lachrymal fluid reached 15.7 ± 0.2 ng/ml and in plasma 16.8 ± 0.12 ng/ml. The study of serum levels of NSE revealed no difference in control and experimental groups. The readings were within normal limits.

In group one with acute disease the level of NSE in lachrymal fluid was 19 times lower than normal ($p < 0.05$). Presumably, this is determined by increased hypoxia and activation of anaerobic glycolysis in neural cells. In group 2 at the stage of transition to atrophy of optic disc, the NSE levels increased 1.22 times ($p < 0.05$). We assume, this is related to onset of destructive processes in neural tissue with gradual death of neurons in neurodegeneration. This leads to exit of neuron-specific enolase (NSE) from injured cells into extracellular space. Study of NSE level allows evaluation of degree and intensity of structural and functional abnormalities of biomembranes in optic nerve.

A pattern of NSE change in relation to progression of the disease should be noted. In acute phase levels of NSE showed reduction to 0.834 ± 0.175 ng/ml ($p < 0.05$), whereas chronic stage is characterized by gradual increase of NSE up to 20.77 ± 1.24 ng/ml ($p < 0.05$). Finally, the atrophic stage is featured by 22% increase of NSE.

Our study did not detect any correlation of serum NSE with stages of disease. Consequently, evaluation of serum NSE does not provide reliable information on severity of pathological process. Also, we noted reverse correlation of NSE in lachrymal fluid and visual functions. The higher the level of visual acuity and visual fields, the lower the level of NSE and the lower the level of visual acuity the higher the level of NSE.

Optic disc hyperemia and swelling were characterized by decreased NSE in lachrymal fluid in group 1. Pale disc and constricted vessels were associated with gradual increase of NSE in lachrymal fluid in group 2. In patients with pathological tractography in the form of thinning of fibers of greater occipital forceps NSE levels in lachrymal fluid were increased by 22%.

Conclusion

Neuron-specific enolase increase in biological fluids thus represents a useful and promising marker biochemically characterizing various stroke types possibly resulting in neuronal damage (Chekhonin et al., 2000). Changes of this marker along the flow of disease may indirectly indicate the transition of optic nerve inflammation from the stage of swelling to the stage of ischemia and atrophy which will serve as a basis for correction of treatment. This is particularly important as ophthalmoscopic appearance often changes at later stages and depends on subjective estimation of a physician.

In acute stage of ON NSE level decreases to 0.834 ± 0.175 ng/ml. In atrophic stage it increases to 20.77 ± 1.24 ng/ml, which we assume, is related to increased destruction of neural fibers. For evaluation of prognosis of ON, along with clinical signs, detection of NSE in lachrymal fluid as a subclinical marker of neurodegeneration is required. Comparative analysis demonstrated relatively high level of NSE in lachrymal fluid at transitional stage to atrophy as compared to acute phase. Study of NSE in lachrymal fluid in patients with ON is informative diagnostic criterion for evaluation of optic nerve damage. Detection of NSE in lachrymal fluid should be included in examination of patients with ON as an additional marker of inflammation which is present at early stages of disease.

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