ORIGINAL ARTICLE

Serum biomarkers in normal pressure hydrocephalus

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ABSTRACT

Aim To investigate the serum value of brain derived neurotrophic factor (BDNF), proteins S-100, NSE, IL-6 in normal pressure patients (NPH) compared to control (healthy) group and also a possible correlation with radiological findings in NPH patients.

Methods Study patients were included during the period of 2020-2022. All NPH patients met the diagnostic criteria for probability of NPH. Control patients group included patients without known brain disorder, without clinical symptoms of NPH. Blood samples were taken before planned surgery for NPH. BDNF serum concentrations were assessed by a sensitive ELISA kit, and serum concentrations of S-100, NSE and IL-6 were assessed by using ECLIA technology for immunoassay detection.

Results Among 15 patients who were included, seven NPH patients were compared to eight control patients. Non-significant decrease in BDNF serum concentrations, an increase of protein S-100 serum concentrations, a decrease of NSE serum concentrations, as well as an increase of IL-6 serum concentrations in NPH patients compared to healthy controls was found. Strong positive correlation between BNDF and Evans index was observed (p=0.0295).

Conclusion We did not find a significant difference of BDNF, protein S-100, IL-6 and NSE between serum concentration in NPH and healthy patients. More future research is needed to find the role of BDNF in NPH patients.

Keywords: biomarkers, brain-derived neurotrophic factor, serum

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INTRODUCTION

Normal pressure hydrocephalus (NPH) is a clinical state characterized with triad of symptoms (Adam-Hakim triad of symptoms) urinary incontinence, dementia, and gait impairment without an increase of intracranial pressure. However, only 51% of patients only have the complete clinical triad (1). The prevalence of NPH is 3.7% among individuals aged over 65 years of age, with higher prevalence in elderly groups, 80 years or older (2). Normal pressure hydrocephalus is considered to be one of the potentially curable clinical states, where after shunting, as the most common treatment option, comes to resolution or improvement of symptoms (1).

The presence of NPH as an individual clinical entity has been questioned. It has been proposed as a sub-entity of Alzheimer's disease, sharing the similar clinical picture and even a temporary clinical response upon a spinal tap (3,4). The molecular pathology of NPH is not well understood. One of the proposed molecular mechanisms that are involved in many brain processes are related to neurotrophins, which are the group of brain proteins which can regulate a neuron survival (5). Brain-derived neurotrophic factor is one of the most investigated neurotrophins, which is involved in synaptic plasticity, neuronal survival, and differentiation (5,6). Furthermore, it has been proposed that some biomarkers associated with Alzheimer's disease, among them BDNF, IL-6, protein S-100 and NSE might as well be associated with developing and outcome of NPH (7-11).

The guidelines for diagnosing NPH are changing and bring a new knowledge about NPH (12).

The aim of this study was to investigate serum value of BDNF in NPH patients and healthy individuals, serum value of proteins S-100, NSE, IL-6 in these two patient groups, and a possible correlation with radiological findings in NPH patients.

PATIENTS AND METHODS

Patients and study design

In this study patients from the Department of Neurosurgery and Neurology, Clinical Centre of the University of Sarajevo were included during the period January-November 2022. All NPH patients met the diagnostic criteria for probable NPH according to the Guidelines for the Management of Idiopathic Normal Pressure Hydrocephalus (Third Edition): Endorsed by the Japanese Society of Normal Pressure Hydrocephalus (12). A control patients group included patients without known brain disorder, without clinical symptoms of NPH.

All patients underwent clinical, neurological, psychological examination. NPH patients had a magnetic resonance imaging (MRI) of the brain, with measured Evans index. All blood samples were collected during the hospital stay at the Department of Neurosurgery or Neurology. All patients underwent ventriculoperitoneal shunting; only one patient was not shunted due the bad general condition, and serum samples were collected for microbiological and biochemical analysis.

An ethical approval was obtained from the Ethical Committee of the Clinical Centre of the University of Sarajevo.

A written informed consent was obtained from all patients; for patients without possibility to take and informed consent, their legal representatives provided an approval.

Methods

Measurement of BDNF concentrations in serum. Venous blood (5 mL) was collected from each patient in the morning (between 7 and 8 a.m.) after overnight fasting. Blood samples were collected into tubes without anticoagulant and allowed to clot at room temperature for 1/2 h. Serum was separated by centrifuge at approximately 1000 x g for 15 minutes and then stored at −-20 °C to -80 °C until assay.

BDNF levels were measured by the enzyme-linked immunosorbent assay (ELISA) method using the human BDNF reagents kit (DRG Instruments GmbH, Germany), according to the manufacturer's instructions. The BDNF ELISA Kit allows the preferential quantification of mature BDNF in less than 3 hours. This kit consists of a pre-coated mouse monoclonal anti-BDNF capture antibody, a biotinylated anti-BDNF detection antibody and horseradish peroxidase (HRP)-conjugated streptavidin. The addition of a substrate (3,3',5,5'- tetramethylbenzidine, TMB) yields a coloured reaction product, which is directly proportional to the concentration of BDNF present in samples and protein standards. This BDNF ELISA kit employs a recombinant human

antibodies bind epitopes within the mature domain of the protein and therefore recognize the mature, as well as the proform of BDNF. However, cross-reactivity to the full-length proBDNF protein is low (13).

All samples and standards were measured in duplicate. Samples of patients with different diagnoses and control participants were analysed together in the ELISA templates, and possible variability between different assays was controlled using two samples of known BDNF concentration.

The inter-assay precision was expressed as the coefficient of variation (%); it was from 4.98% to 8.26%. Two different patient samples were diluted with the sample diluent to 1:200, 1:400 and 1:800. The percentage of recovery was 98.3-102.9%. Typical normal human blood values are 2 -20 ng/mL.

Measurement of S-100, NSE and IL-6. S-100, NSE and IL-6 samples were collected, separated from the clot and stored at 2-8 °C for up to 24 hours. If the serum was not tested within 24 hours, the samples were stored at -20°C.

Electrochemiluminescence- ECLIA –COBAS 601 is used for immunoassay detection of S-100, NSE or IL-6 in human serum. The development of immunoassays is based on the use of a ruthenium-complex and tripropylamine (TPA) (14).

Statistical analysis

The data were presented as the mean±SD. The statistical analysis of differences between two groups was performed using the Mann-Whitney U test two-tailed t-test. The correlation between two variables was determined by using Pearson correlation coefficient. Significance for the results was set at $p<0.05$.

RESULTS

Our study included seven patients diagnosed with NPH, average age 70.42±4.92 years compared to eight control patients, average age 50.87±15.53 years.

A decrease of BDNF serum concentrations in NPH patients (7.4286 ng/mL) compared to healthy controls (10.62 ng/mL) was found without statistical significance (p=0.28) (Figure 1).

An increase of protein S 100 serum concentrations in NPH patients (8.35 ug/L) compared to healthy controls (7.68 ug/L) was found (p=0.77) (Figure 2).

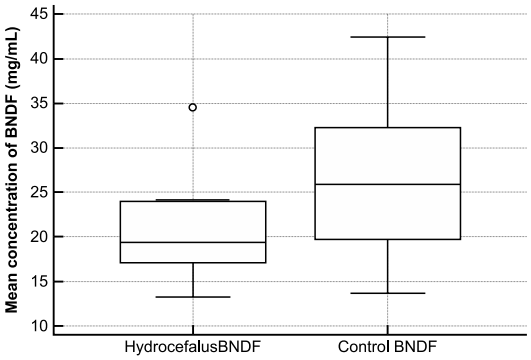

Figure 1. Average value of the brain derived neurotrophic factor (BDNF) in the group with normal pressure hydrocephalus (NPH) and the controls

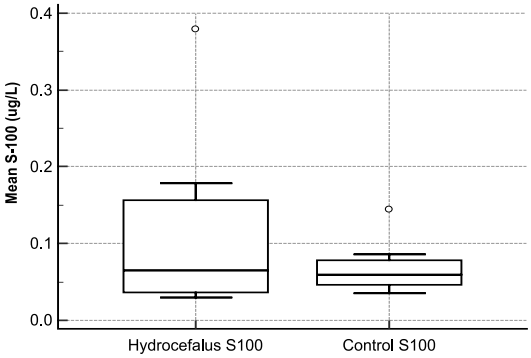

Figure 2. Average value of S100 protein in the group with normal pressure hydrocephalus (NPH) and the controls

A decreased NSE serum concentrations in NPH patients (6.14 ng/mL) compared to healthy controls (9.25 ng/mL) was found (p=0.15) (Figure 3).

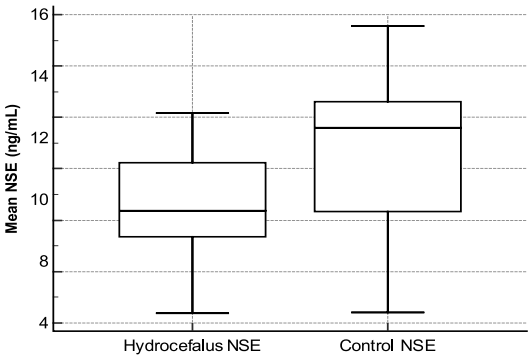

Figure 3. Average value of the neuron specific enolase (NSE) in the group with normal pressure hydrocephalus (NPH) and the controls

An increase of IL-6 serum concentrations in NPH patients (8.35pg/mL) compared to healthy controls (7.68 pg/mL) was noticed (p=0.77) (Figure 4).

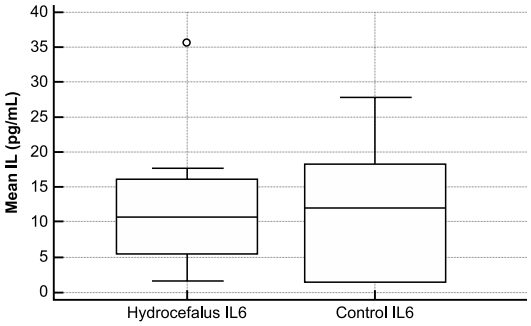

Figure 4. Average value of IL 6 in the group with normal pressure hydrocephalus (NPH) and the controls

The correlation coefficient (Pearson's coefficient) depending on the two variables BNDF and age (Hydrocephalus group) indicated that the regression coefficient was low (r=0.18) or that the correlation was weak, with low statistical significance (p=0.707) (Figure 5).

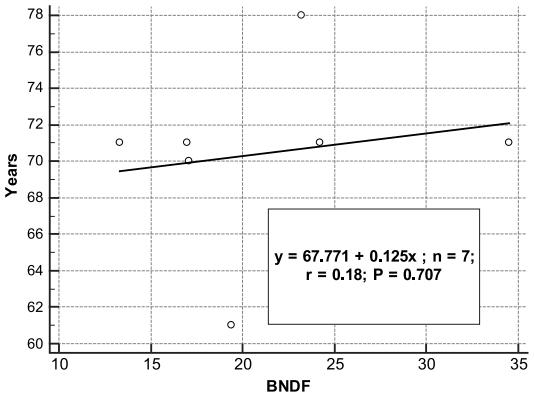

Figure 5. Correlation between brain derived neurotrophic factor (BDNF) (ng/mL) and age in patients with normal pressure hydrocephalus (NPH)

The correlation coefficient depending on the two variables BNDF and Evans index indicated that the regression coefficient was high (r=0.8036), or that the correlation was strong with high statistical significance (p=0.0295).

The correlation coefficient depending on the two variables IL 6 and Evans index indicated that the negative regression coefficient was significant (r= - 0.5661), or that the correlation was of medium strength with low statistical significance (p=0.1853) (p<0.05).

The correlation coefficient depending on the two variables NSE and Evans index indicated that the negative regression coefficient was high (r= -0.8148) i.e. that the correlation was strong with high statistical significance (p=0.0256) (p<0.05).

The correlation coefficient depending on the two variables S 100 and Evans index indicated that the negative regression coefficient was low (r= - 0.1257), that is, the correlation was of weak with low level of statistical significance (p=0.7883) (p<0.05).

DISCUSSION

In this study we did not find a significant correlation of BDNF, S-100, NSE, IL-6 serum concentrations in NPH patients compared to healthy controls. A strong statistically significant positive correlation between Evans index and BDNF serum concentration was found in NPH patients.

Brain-derived neurotrophic factor is neurotrophin present in human blood, also found in the brain, and has been widely associated with neurodegenerative diseases, such as Huntington's disease (15,16) and Alzheimer's disease (17). Studies have found that the decreased level of BDNF in serum correlates with the progression of the neurodegeneration (5,17). In animal studies it is shown that lower BDNF is related with neuronal death in the striatum, and post mortem analyses in Alzheimer disease patients found a decreased level of BDNF mRNA in the hippocampus, cortex and two areas critical for learning and memory (6,17). On the other hand, some recent research have presented conflicting results given that some authors observed higher concentrations (18,19) and others observed lower concentrations of serum BDNF in patients with Alzheimer disease (AD) (20- 23). In Huntington disease, there are studies which reported a decrease of BDNF level in striatal neurons, but the peripheral blood concentration of BDNF did not reflect progression and is not associated with the examined clinical and imaging measures in Huntington patients (5,6).

When it comes to NPH, previous studies found that there was no difference between CSF concentration between NPH and other non-NPH groups (17). One animal study with infant congenitally hydrocephalic rats found that BDNF expression was aberrant in the ventricular zone of the cerebral cortex that can be associated with etiology of infant congenital hydrocephalus (24). Laske et al. (17) found that BDNF is decreased in serum of NPH patients without known exact mechanism underlining.

The influence of age on lowering the serum value of BDNF has been clearly recognized (25,26). According to our results, BDNF serum concentrations in NPH patients were lower compared to healthy controls, but the difference was not statistically significant. Moreover, we observed only a weak correlation between the age of our respondents and the value of serum concentrations of BDNF.

Most of the conducted studies examined the levels of inflammatory cytokines in cerebrospinal fluid. However, Rota et al. (27) examined the serum levels of interleukin (IL)-12 and a panel of related cytokine levels in NPH patients, where no statistically significant differences were found compared to healthy controls. Moreover, Sosvorova et al. (28) determined an increase in various cytokine levels, predominantly in CSF of NPH patients, which is consistent with studies reported by other authors (29).

In our study an increase of IL-6 serum concentrations in NPH patients was found compared to healthy controls. Sosvorova et al. (28) observed a lowering level of IL-6 in CSF following the first day of lumbar drainage.

Recent research investigated the role of neuron specific enolase (NSE) and protein S-100 as specific markers for developing NPH (30). A meta-analysis including the total of 3,204 respondents reported significantly increased protein S-100 serum levels in AD patients (31). Levada and Trailin (32) observed an elevation of serum level of protein S 100 in patients diagnosed with vascular dementia. On the other hand, research evaluating NPH patients did not observe any aberration of serum levels of protein S-100 (33). In our study, we observed elevated protein S-100 serum concentrations and lower NSE concentrations, as well as insignificant increase of protein S-100 serum concentrations, and insignificantly decreased NSE serum concentrations in NPH patients compared to healthy controls.

Neuron specific enolase, a glycolytic enzyme has been proposed as a significant marker of neurodegeneration in AD (36). However, most of the conducted research measured the CSF NSE levels, rather than serum levels but the results remain conflicting (34). Meta-analysis in 2016 reported increased CSF but equivalent NSE serum levels in AD patients compared to controls (35). Nooijen et al. (36) observed lower NSE CSF levels in 57 patients with NPH compared to the patients with AD and controls, but the difference was not statistically significant. The significance of NSE as a biological marker in NPH still remains to be confirmed.

Evans index has been considered as a predictor of NPH outcome (37). In our research, we found a strong positive correlation between BNDF and EVANS index, medium strength negative correlation between IL -6 and Evans index, strong negative correlation between NSE and Evans index and weak negative correlation between protein S-100 and Evans index. There is a possible clinically significant relation between Evans index and BDNF, but more clinical studies are needed to confirm this.

We performed an extensive literature search; however, we did not find studies exploring potential influence of BDNF, IL-6, protein S 100 and NSE as serum biomarkers of NPH on Evans index.

In conclusion, our results, although derived from the small-size population, indicate a need for more research in this area.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

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