Abnormalities of tau-protein and beta-amyloid in brain ventricle cerebrospinal fluid

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Abstract

OBJECTIVE: Determination of various biomarkers, such as beta-amyloid, tau-protein, phosphorylated tau-protein in CSF and their sensitivity and specificity in neurodegenerative brain processes, in particular Alzheimer Dementia (AD), has been recently investigated to monitor their abnormalities in the CSF at early stages of diseases before the clinical manifestation.

DESIGN AND SETTING: In the pilot group of our patients (10 men / 5 women) who underwent a drainage neurosurgical procedure for diagnosis of hydrocephalus, CSF was obtained from the brain ventricles and the influence of a different compartment of the CSF on the level of biomarkers, tau-protein and beta-amyloid, was investigated.

RESULTS: The mean tau-protein level for all 15 patients was 812.0 pg/ml, with median value 363.7 pg/ml; while mean beta-amyloid level for all 15 patients was 526.7 pg/ml, with median value 239.5 pg/ml, respectivelly. The abnormal tau-protein and beta-amyloid levels were found in the subgroup of patients in whom hydrocephalus was caused by a severe pathological process, such as brain tumor. The beta-amyloid values were significantly lower also in comparison with our previously published results in patients with AD in the CSF obtained by lumbar puncture in the spinal canal.

CONCLUSIONS: CSF in the brain ventricles is theoretically more stable and the values in this CSF probably provide more reliable informations for clinical diagnostic procedure than those for the CSF obtained by lumbar puncture in the spinal canal.

ORIGINAL ARTICLE

Abbreviations :

CSF	- Cerebrospinal Fluid
AD	- Alzheimer Dementia
MS	- Multiple Sclerosis
CJD	- Creutzfeldt-Jacob Disease
Stroke	- Ischemic vascular cerebral insult
SAH	- Subarachnoid hemorrhage
HCF	- Hydrocephalus
Normal-HCF	- Normotensive hydrocephalus
CNS	- Central Nervous System
β-amyloid	- Beta-Amyloid
Aβ-42	- Beta-Amyloid-42 Isoform
ROC	- Receiver Operating Characteristic
UNK	- Unknown

INTRODUCTION

Determination of levels of the biomarkers beta–amyloid, tau–protein, and phosphorylated tau–protein and their sensitivity and specificity in neurodegenerative brain processes, in particular Alzheimer Dementia (AD) has become a subject of investigation due to a chance to determine their abnormalities in the CSF early before the onset of clinical manifestations of the neurodegenerative processes, most frequently dementia.

Formation of the beta–amyloid seems to be the primary degenerative event, while the generation of tau–protein has been found to be the secondary event (Hashimoto *et al.* 2003). Tau–protein has physiologically an intracellular localization, wherein it strengthens the fibers of microtubuli. In Alzheimer Dementia (AD), the tau-protein is abnormally phosphorylated and formes shorter abnormal filaments. Filaments constitute a part of tangles. Neurons containing tangles are subject to apoptosis (Goedert *et al.* 2006). Although accumulation of beta–amyloid and tau–proteins has been linked to AD for many years, no direct evidence is available, explain fully (based on experimental data) the mechanisms by which these peptides contribute to the development of a cognitive deficit and neurodegeneration. It is not clear whether their accumulation is a cause or consequence of another mechanism for neuronal loss.

Above menched three biomarkers are the best-studied. The first biomarker is beta–amyloid which forms from the amyloid precursor protein participating in the formation of the cellular membrane. Beta–amyloid in patients with AD constitutes a part of perineuronal neuritic plaques. This explains reduced beta-amyloid level in the cerebrospinal fluid (CSF) of patient with AD (Wolfe, 2002). The most widely used approach is determination of the Aβ-42 isoform (Alonso *et al.* 2000), which displayes the highest level of adherence in the plaques and the highest decrease in its levels in patients with AD (Small *et al.* 1999). Tau-protein is another marker under investigation. It represents one of the principal components of the cytoskeleton network and takes part in the axonal transport system. It is shed to the CSF during the neuronal loss, particularly in atrophic-degenerative disorders which are characteristic for AD. It can be concluded that the amount of tau–protein in the CSF is reflecting the rate of progression of the disease and damage to the nerve cells. The highest level of tau-protein has been reported in patients with CJD (Kapaki *et al.* 2001). Finally, the phosphorylated tau–protein has also been identified as a specific and sensitive marker of AD. Hyperphosphorylation of the tau–protein occurs during the development of the nervous system and is found to be elevated in CSF in a broad spectrum of neurodegenerative disorders. Tau-protein glycosylation and ubiquitinization probably also contribute to the formation of typical neuronal tangles (Ledesma *et al.* 1996).

For the purpose of differential diagnosis, a high level of both specificity and sensitivity of a proposed biomarker is needed. In addition to overall tau–protein and phosphorylated tau–protein levels, other markers are also monitored in Creutzfeldt Jacob Disease (CJD) such as protein 14-3-3, metallothionein and laminin receptor protein (Baloui *et al.* 2004). Previous research in this area suggested that differences in the levels of tau–protein and tau/phospho-tau ratio in the CSF of patients are enough sensitive and specific to differentiate CJD and AD (Kapaki *et al.* 2001).

Multiple cerebrospinal sclerosis (MS) is characterized by the formation of multiple inflammatory lesions in the white matter of brain and spinal cord with the maximum in the periventricular regions, brain stem and corpus callosum. Although the mechanisms leading to damage of the cerebral tissue in patients with this disease are well defined, the cause of development of MS is still unknown. This disease is characterized by demyelination and is a combined inflammatory disorder and axonal loss in the CNS. The histopathologic pattern seems to be probably identical during the entire course of the disease. It includes plaque with the same cellularity and destruction pattern, presence of immunoglobulins and complement components in CSF in various stages of development.

Examination of the CSF provides valuable information regarding the course of the chronic inflammatory reaction in the CNS. CSF is examined preferentially for the presence of oligoclonal immunoglobulins, which are present in 75 to 85% of patients with definitive diagnosis of MS (Rudick *et al.* 1987). Axonal destruction was found to play an important role during initial phase of demyelination (Trapp *et al.* 1998). The research has been focused on the identification of specific biomarkers of this process (Lunque *et al.* 2007). Tau protein seems to be the only promising marker, although results of studies are still unequivocal (Brettschneider *et al.* 2005; Guimarães *et al.* 2006; Kapaki *et al.* 2000).

OBJECTIVES OF THE STUDY

To determine levels of the biomarkers, beta–amyloid and tau–protein in a unique biological material, CSF, obtained from the brain ventricles during the drainage neurosurgery procedure for hydrocephalus.

To evaluate the significance of the CSF collection site as well as the etiology of hydrocephalus on the level of biomarkers.

MATERIAL AND METHODS

A total of 15 patients diagnosed with hydrocephalus (Table 1) were enrolled into the study group (10 men / 5 women). All patients were of Caucasian origin, Czech nationality, and they were examined and treated at the Department of Neurology and Department of Neurosurgery of the University Hospital in Hradec Kralove. The patients or their legal representatives/guardians signed the informed consent form for hospitalization and invasive diagnostic and therapeutic procedures. This study was approved by Ethic Commitee of University Hospital in Hradec Kralove. All patients enrolled into the study were examined by MRI or CT scan of the brain. Cerebrospinal fluid samples from the brain ventricles were collected for further analysis. During the course of the neurosurgical drainage procedure, CSF was routinely drained and collected into sterile tubes under sterile conditions. The results of examinations were compared with the control group of 38 patients, in whom the original clinical suspection for an organic neuropathological process in the nervous system was not confirmed by clinical, laboratory and imaging methods and techniques. Patients suffering from suspected subarachnoidal bleeding or neuroinfection were enrolled as control group. Cerebrospinal fluid in the control group was collected by a standard lumbar puncture technique using an atraumatic needle for single use. The CSF samples were frozen at –70 °C and stored for subsequent analysis.

Concentrations of the tau–protein and beta–amyloid42 were examined using enzyme immunoassays (ELISA), INNOTEST β–AMYLOID$_{(1–42)}$ and INNOTEST hTAU Ag kits purchased from INNOGENETICS N.V. (Belgium). MedCalc software (Belgium) was used for statistical evaluations.

RESULTS

Concentrations of tau-protein and beta-amyloid in CSF samples obtained from the exceptional compartment of brain ventricles are listed in Table 3. More detailed analysis for the respective subgroups divided according to the cause of hydrocephalus (Table 2) is provided in the discussion.

The levels of these markers in the cerebrospinal fluid obtained by lumbar puncture in our control patients were for tau–protein up to 169 pg/ml (specificity/sensitivity = 77.8/78.2%) and for the beta–amyloid42 up to 1180 pg/ml (specificity/sensitivity = 72.2/80.0%), respectively. These limits were detected using the receiver operating characteristic (ROC) and the borderline/limit values of biomarkers with the highest specificity and sensitivity for the study group of patients (Talab *et al.* 2003, Talab *et al.* 2004, Valis *et al.* 2008).

Compared to the values in the control group collected by a standard lumbar puncture technique, which were defined as reference values, the mean abnormal value from the brain ventricles for the tau-protein increased to 812.0 pg/ml (median 363.7 pg/ml), while the mean beta-amyloid value decreased to 526.7 pg/ml (median 239.5 pg/ml) (Table 4).

Tab. 1. Demographic data for patients and controls.

Group	Age	Age (median)	Number	Men	Women
1.	2–20	4.5	4	2	2
2.	20–60	52.5	6	5	1
3.	60–80	72	5	3	2
Total	2–80	53	15	10	5
Control group	18–77	45	38	11	27

Tab. 2. Causes of hydrocephalus.

Cause of hydrocephalus	Men	Women
Tumors	4	1
Developmental anomalies	2	2
Trauma	1	0
SAH	0	1
Normotensic HCF	1	0
Others	2	1

Tab. 3. Results of the CSF analysis.

	ID	Age (years)	Gender	Diagnosis	Tau-protein (pg/l)	β-amyloid (pg/l)
1.	J-P	23	M	Tumor	534.4	189.5
2.	L-R	53	M	Tumor	16.8	175.1
3.	O-S	57	M	Tumor	705.1	1465.1
4.	A-N	68	F	Tumor	404.1	137.7
5.	V-S	72	M	Tumor	2050.4	185.6
6.	G-P	15	M	Anomaly	572.3	239.5
7.	V-S	2	M	Anomaly	270. 7	1253.4
8.	L-O	6	F	Anomaly	164.3	164.3
9.	K-V	3	F	Anomaly	105.6	416.5
10.	V-C	23	M	Trauma	3430.3	1628.1
11.	P-J	63	M	Normal-HCF	80.5	354.7
12.	M-J	75	F	SAH	3175.4	687.6
13.	F-K	72	M	UNK	201.5	713.5
14.	M-K	58	F	UNK	104.5	172.4
15.	M-H	56	M	Stroke	363.7	126.4

Tab. 4. Comparison of mean (∅) values measured for the tau-protein and beta-amyloid in the brain ventricle CSF with mean reference values of the spinal-CSF in the control group.

Protein/concentration	Spinal-CSF ∅ normal (pg/ml)	Brain ventricle CSF ∅ concentration (pg/ml)
Tau-protein	<169 pg/ml	812.0 pg/ml
Beta-amyloid42	>1180 pg/ml	526.7 pg/ml

DISCUSSION

Cerebrospinal fluid (CSF) is in a direct contact with the extracellular space of the brain and therefore it is well reflecting biochemical changes in the CNS. The source of total tau–protein in the CSF remains unclear, but it is probably related to degeneration of neurons filled with neurofibrillar tangles. Assumption that the presence of tau–protein in the CSF is indicating neuronal damage and degeneration is based on the observations of transiently elevated total tau-protein levels in the CSF in the course of ischemic stroke. This elevation is correlated with the detection of a focus in the brain using the CT scan. In agreement with these findings, our patient No. 15 diagnosed with ischemic stroke complicated with hydrocephalus also showed elevation of tau-protein levels in CSF.

Any insult irrespective endogenous or exogenous, which results in a neuronal damage will lead to elevated CSF levels of these biomarkers. Predictions of severity and progression should also include the level and extent of damage. This may also be observed in the respective subgroups of the study population, which had a common diagnosis of hydrocephalus. In the group with developmental anomalies (patients No. 6, 7, 8, 9), in whom the disorder was not caused by an axonal destruction, no abnormal tau protein levels were recorded in majority of patients. In contrast, abnormal tau-protein and beta-amyloid levels were found in the subgroup of patients in whom hydrocephalus was caused by a severe pathological process, such as brain tumor. In this context, extremely high concentrations of these biomarkers in patients with craniocerebral trauma (patient No. 10) or subarachnoid bleeding (patient No. 12) seems to be relevant. Brain tumors, which caused hydrocephalus in this group, showed large variability as to the values measured, and based on these values no clear conclusion from our results can be drawn. Abnormal tau-protein concentration probably relies more on the particular mechanism leading to the formation of hydrocephalus rather than the effects of elevation of the intracranial pressure.

Determination of markers of myelin disintegration, axonal destruction or immunological dysfunction in the CSF seems to be sensitive and specific approach in the differential diagnosis of many neurological disorders, exspecially during the early stages of the disease. Markers of myelin disintegration are detected in any disease, in which primary or secondary destruction of myelin is present (MS, stroke, trauma or neurodegenerative disorders). Markers of axonal damage, such as tau-protein, beta-amyloid, 14-3-3 protein or neurofilament light chains may serve as predictors for disease progression, because axonal loss is reflected by the irreversible disability in neurologically diseased patients (Martinez-Yelamos *et al.* 2001, 2004; Petzold *et al.* 2006).

Markers of axonal damage reflect the process of axonal loss and can serve as a predictor of disease severity and progression in early stages. Delineation of reference (physiological) values and intervals is the main problem in the study of biomarkers, such as tau-protein and beta-amyloid, in newly introduced analytical procedures. In this work, borderline (cut-off) physiological values of these markers were determined as an optimal ratio between the sensitivity and specificity using ROC analysis in the control group. In general, when increasing the limit (threshold for the determination of pathological findings) the sensitivity of test is increased while the specificity is decreased. The value of tested parameter when the ratio between the sensitivity and specificity is optimal, is called the cut-off value. All values above this cut-off represent pathological results. The same approach is applied to the reduced values (if their detection makes sense).

Determination of tau–protein levels in CSF is preferably performed by ELISA techniques. This test is available in many modifications depending on the type of antibodies used and target structures in the tau-protein chain.

Low availability of the analyzed samples of brain ventricle CSF is associated with the invasiveness of the procedure used to obtain this valuable biological material from the brain ventricles. In our study CSF was obtained exclusively as part of the therapeutic neurosurgical procedure related to diagnosed hydrocephalus. It was performed for a wide spectrum of brain pathologies, which ultimated in a high variability of the levels of both tau-protein and beta-amyloid. The mean tau-protein level for all 15 patients was 812.0 pg/ml, with median value 363.7 pg/ml; while mean beta-amyloid level for all 15 patients was 526.7 pg/ml, with median value 239.5 pg/ml, respectivelly. Unfortunatelly, heterogeneity in our studied group does not allow statistical analysis. However, beta-amyloid level in this group was remarcable lower. The beta-amyloid values were significantly lower also in comparison with our previously published results in patients with AD (Talab *et al.* 2003, Talab *et al.* 2004, Valis *et al.* 2008).

Reduced Aβ–42 levels in the CSF of patients with AD are explained by its increased adherence in the perineural neuritic plaques. Nevertheless, some studies reported reduced CSF Aβ–42 levels also in neurological diseases characterized by an absence of beta–amyloid plaques. Such disorders comprise CJD (Otto *et al.* 2000),

amyotrophic lateral sclerosis (Sjögren *et al.* 2002) and multisystemic atrophy (Holmberg *et al.* 2003). However, recent studies based on autopsy findings suggest a significant correlation between low Aβ–42 levels in the ventricular cerebrospinal fluid and high number of plaques in the neocortex and hippocampus. Low Aβ–42 levels in the CSF are probably caused by an accumulation of the beta–amyloid in the plaques (Strozyk *et al.* 2003).

Other plausible explanations could comprise the compartment, from which the CSF was taken. Formation, circulation and resorption of the CSF in the brain ventricles, unlike the spinal CSF, are influenced only by the pathogenic mechanisms, caused by hydrocephalus and subsequently by increased intracranial pressure. CSF in the brain ventricles is theoretically more stable and leves of biomarkers in this biological matherial should be more reliable and informative than those for the spinal CSF obtained by lumbar puncture.

The compartment of CNS from which CSF sample is collected and method of analysis of the CSF sample, seems to be a hypothetic way for an increase in sensitivity and specificity of methods for the determination of study biomarkers of axonal loss. However, the invasiveness of the procedure used to obtain the ventricular CSF is limiting this approach compared to the level of sensitivity and in particular specificity of the results, that it could be applied only in similar indications and situations like that in the presented group, i.e. during an indicated surgical procedure.

CONCLUSION

Tau-protein and beta-amyloid concentrations were examined in the cerebrospinal fluid obtained from a unique compartment, brain ventricles, using a neurosurgical drainage procedure in patients with hydrocephalus. Given the abnormal values of these biomarkers, it can be admitted that CSF in the brain ventricles is theoretically more stable and the values in this CSF probably provide more reliable informations for clinical diagnostic procedure than those for the CSF obtained by lumbar puncture in the spinal canal.

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