Tau protein, phosphorylated tau protein and beta-amyloid42 in the cerebrospinal fluid of multiple sclerosis patients

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Abstract

OBJECTIVES: The presented study focuses on the importance of measurement of beta-amyloid42 (Aβ-42) levels, total tau protein, and phosphorylated tau (p-tau) protein in the cerebrospinal fluid (CSF) of cerebrospinal multiple sclerosis (MS) and clinically isolated syndrome (CIS) which represents an early phase of multiple sclerosis. **METHODS**: A total of 23 patients with clinically isolated syndrome and suspected MS were enrolled into the study. Of this number, 14 patients met the criteria for definitive MS according to McDonald. The control group consisted of 40 patients examined for the possibility of organic damage to the brain, which was not confirmed. We used method of enzyme immunoanalysis to examine concentrations of tau protein, p-tau protein, and beta amyloid42. Differences between the respective groups were examined by test statistics. In addition, dependence of the total tau protein, p-tau protein, and beta-amyloid42 levels on demographic variables, diagnosis and duration of disease was examined by correlation analysis. Correlation of the concentrations obtained in the measurements was evaluated based on the calculated correlation coefficient (r) and level of significance (p).
RESULTS: Compared to the control group, no statistically significant difference was found in the levels of tau protein and p-tau protein between the CIS group and definitive MS group. A significant increase was found only for beta-amyloid42 levels in patients with diagnosed MS vs. control group. We demonstrated no correlation between the beta-amyloid42 and tau protein levels, p-tau protein and age of patients and duration of disease in patients with MS, CIS and the control group.
CONCLUSION: Results of our study show that use of tau protein, p-tau protein and beta-amyloid42 in the diagnosis of multiple sclerosis seems to be non-beneficial.

ORIGINAL ARTICLE

We confirmed no importance for the differential diagnosis of an early stage MS.

Abbreviations:
AD – Alzheimer's disease
Aβ42 – Beta-Amyloid42
CIS – Clinically Isolated Syndrome
CNS – Central Nervous System
CSF – Cerebrospinal Fluid
CT – Computed Tomography
MS – Multiple Sclerosis
MRI – Magnetic Resonance Imaging
NS – Non significant
NSE – Neuron Specific Enolase
p – Level of significance
p-tau – Phosphorylated Tau Protein
r – Correlation Coefficient
tau – Tau Protein

INTRODUCTION

Cerebrospinal multiple sclerosis is a chronic handicapping disease, characterized by demyelination and combined inflammatory disorder and axonal loss in the CNS. Since the year 2001, McDonald's criteria have been valid for diagnosis. These criteria were revised in the year 2005 (Mc Donald *et al*., 2001, Polman *et al*., 2005). Apart from the clinical picture, presence of specific findings in the MRI of the brain, and its dissemination in time and space, detection of positive synthesis of oligoclonal immunoglobulins in the CSF, which are absent in the serum, and abnormal visual evoked potentials is used for diagnosis. The understanding of MS has increased rapidly in recent years. Since axonal destruction plays a significant role at the beginning of demyelination in MS, researchers have focused on identification of specific biomarkers for this process in the cerebrospinal fluid (Luque *et al*., 2007). Being in direct contact with the extracellular space of the brain, the cerebrospinal fluid (CSF) is able, in the majority of cases, to reflect well any biochemical changes in the CNS (Raedler *et al*., 2006). Promising biomarkers of neurodegeneration in the CNS include NSE, neurofilaments, 14-3-3 protein, and tau protein. While NSE levels in the CSF are usually normal in MS patients, increased levels of neurofilaments should correlate with degree of disability and relapse rate, revealing axonal damage in the initial phase of MS (Lycke *et al*., 1998). Increased tau protein concentrations in the CSF were found in MS patients compared with patients suffering from non-inflammatory neurological disease (Bartosik-Psujek *et al*., 2004; Kapaki *et al*., 2000). Results of other studies of tau protein are not so convincing (Brettschneider *et al*., 2005; Guimarães *et al*., 2006; Vališ *et al*, 2008). On the other hand, the importance of determination of tau protein, p-tau protein, and beta amyloid42 levels in the differential diagnosis of AD and other dementias, in particular Creutzfeld-Jacob's dementia (CJD) has been clearly demonstrated (Munroe *et al*., 1995; Nagga. *et al*., 2002; Otto *et al*., 1997; Hulstaert *et al*., 1999).

Tau–protein is a neuronal microtubule-associated protein (MAP). It is primarily located in the neuronal axons. It was discovered in the year 1975 at Mark Kirchner's laboratories in Princeton University (Weingarten *et al*., 1975). Microtubules are the main component of neuronal cellular processes. Tau protein plays a significant role in the arrangement of tubulin monomers into microtubules. It maintains their structure and contributes to the connection of microtubules to other proteins and elements of the cytoskeleton (Buée *et al*., 2000).

Tau proteins are present in particular in the neuronal axons of the central and peripheral nervous systems, but they are also detectable in minor quantities in astrocytes and oligodendroglia (Trojanowski and Lee, 2002).

Tau-protein is a phosphoprotein and its biological activity is regulated by phosphorylation. Increased phosphorylation was described during the development of neurons (which means that the embryonic brain has much more phosphorylated tau protein than the adult brain) and in some neurodegenerative disorders. Phosphorylated sites in the tau–protein are grouped around the area of connection to microtubules. Hyperphosphorylation disconnects tau–protein from the surface of microtubules, thus causing damage to axonal integrity and accumulation of toxic tau–peptides (Drewes, 2004). However, hyperphosphorylation can also result in the accumulation of tau-protein (tau inclusions) with the formation of tangles, pair helical filaments, and straight filaments, which have a role in the pathogenesis of neurodegeneration (Alonso *et al*., 2001). Apart from phosphorylation, tau–protein can undergo ubiquitination, nitration, prolyl isomerization, conjugation with heparan sulfate, glycosylation, glycation, and modification by the end products of glycation (Ledesma *et al*., 1996; Trojanowski and Lee, 2002).

Source of the total tau–protein in the cerebrospinal fluid remains unclear, but it is probably associated with the degeneration of neurons filled with neurofibrillary tangles. The hypothesis that the presence of tau–protein in the cerebrospinal fluid indicates neuronal damage and degeneration is based on the observation of transient elevation of total tau CSF levels during cerebral infarction. The findings were in agreement with the detection of a lesion in the brain using CT scan. Another reason is that the highest levels of total tau protein are found in the cerebrospinal fluid of CJD patients, a disease with a very strong neuronal degeneration (Hort *et al*., 2008). In contrast to the total tau–protein levels, the phosphorylated tau CSF levels likely indicate the degree of its phosphorylation in the brain. This anticipation is based on indirect evidence as the acute cerebrovascular accident causes no changes in the phosphorylated tau CSF levels (Hesse *et al*., 2000). Phosphorylated tau CSF levels are most often determined by ELISA techniques. Many variations are available for this test which depend on the type of antibodies used and target structures in the tau–protein chain.

Beta-amyloid constitutes the main component of Alzheimer's plaques. It is produced from a substance natural to the body, known as amyloid precursor protein. This protein is present in neurons in several fractions, such as transmembrane, lysosomal and perhaps other fractions. The transmembrane fraction is cleaved by enzymes secretases to short fragments known as beta–peptides. Cleavage of the transmembrane fraction of the amyloid precursor protein by gamma-secretase results in the formation of C–terminal isoforms of Aβ having 39 to 43 amino acids (Small *et al*., 1999).

Two main isoforms of the beta-amyloid are distinguished. The Aβ–40 isoform is predominant under normal circumstances, is soluble and excreted from the body. On the other hand, Aβ–42 aggregates more rapidly than Aβ–40 and represents an initial and dominant form of Aβ deposited in plaques (Iwatsubo *et al*., 1994; Tamaoka *et al*., 1994; Miller *et al*. 1993). Patients with AD have reduced CSF Aβ–42 levels (Vanderstichele *et al*., 1998; Mehta *et al*., 2000; Sunderland *et al*., 2003; Baranowska *et al*., 2008). Increased Aβ–42 levels were found only in one study (Jensen *et al*., 1999). Patients developing a depressive syndrome had also elevated CSF Aβ–42 levels. In other studies, normal CSF Aβ–42 levels were found (Andreansen *et al*., 1999; Sjögren *et al*., 2000).

Reduction of Aβ–42 levels in the CSF of patients with AD is commonly explained by increased adherence of Aβ–42 in the perineural neuritic plaques. Nevertheless some of the studies reported reduced CSF Aβ–42 levels also in diseases without any presence of beta–amyloid plaques. These disease include CJD (Otto *et al*., 2000), amyotrophic lateral sclerosis (Sjögren *et al*., 2002) and multisystem atrophy (Holmberg *et al*., 2003). However, recent studies based on autopsy findings report a considerable relation between the low Aβ-42 levels in the ventricular cerebrospinal fluid and high number of plaques in the neocortex and hippocampus. Low CSF Aβ–42 levels are caused by the adherence of beta–amyloid in the plaques (Strozyk *et al*., 2003). Inflammatory reactions were also found to contribute to the pathogenesis of AD which is supported by proofs of positive influence of the administration of non-steroidal anti-inflammatory drugs on the development of this disease (Rich *et al*., 1995).

Beta-amyloid also likely contributes to an increased migration of T lymphocytes into the brain in patients with a dysfunction of the blood-brain barrier, which plays a significant role in the etiopathogenesis of MS (Perry *et al*., 1997, Farkas *et al*., 2003, Zlokovic B, 2008). The heterogeneity of the pathogenetic mechanisms of MS is manifested by a variable rate of axonal loss, which probably correlates with the rate of inflammation in the acute lesions.

The presented study focuses on the importance of measurement of beta-amyloid42, total tau protein, and phosphorylated tau protein levels for the diagnosis of MS. Identification of an axonal damage marker in the MS patients would contribute to a more precise differential diagnosis and help identify individuals with clinically isolated syndrome (CIS), which represents an early stage of multiple sclerosis (Brettschneider *et al*., 2006).

MATERIAL AND METHODS

A total of 23 patients with clinically isolated syndrome and suspected MS were enrolled into the study. Of this number, 14 patients met the criteria for definitive MS according to McDonald. The control group consisted of 40 patients examined for the possibility of organic damage to the brain, which was not confirmed (Table 1). All patients enrolled signed an informed consent to the collection of cerebrospinal fluid sample and were examined by MRI or CT scan of the brain. The cerebrospinal fluid sample was collected by a standard lumbar puncture technique using a single-use atraumatic needle. We examined concentrations of tau protein, p-tau protein, and beta amyloid42 by the enzyme immunoanalysis (ELISA) method, using commercial kits of the Belgium-based firm INNOGENETICS N.V. MedCalc (Belgium) software was used for statistical calculations. The patient and control groups were compared using unpaired t-test after verifying the normal distribution of data. Mutual relationships between the values measured were analyzed using Pearson's correlation coefficient (r). Dependence of the total tau protein, p-tau protein, and beta-amyloid42 levels on demographic variables, diagnosis and duration of disease was examined. The value of $p<0.05$ was used as a level of statistical significance in all cases.

RESULTS

Protein concentrations (mean ± standard deviation) in the groups of patients with MS and CIS and in the control groups are summarized in Table 2.

Table 1. Demographic variables of the MS and CIS patients and the control group

Group	Age	Age (median)	Number	Men	Women
CIS	18–55	29	9	4	5
MS	17–51	37	14	7	7
Control group	18–72	45	40	18	22

Table 2. Summary of tau protein, p-tau protein and beta amyloid42 levels (all pg/ml) in all patient groups and in the control group. Data in the table are shown as mean ± SD.

Protein	MS	CIS	Control
tau protein	167.2 ± 82.73	223.3 ± 120.1	198.1 ± 140.3
p-tau protein	32.6 ± 8.9	32.2 ± 8.2	32.3 ± 14.4
Beta-amyloid42	1477.2 ± 389.9	1429.7 ± 451.3	1204.2 ± 225.1

Table 3. A statistical comparison of the p-tau protein, tau protein and beta-amyloid42 levels between the CIS, MS and control groups.

Protein	MS vs. controls		CIS vs. controls		MS vs. CIS	
tau protein	$p = 0.442$	NS	$p = 0.619$	NS	$p = 0.197$	NS
p-tau protein	$p = 0.933$	NS	$p = 0.989$	NS	$p = 924$	NS
Beta-amyloid42	$p = 0.0076$	**	$p = 0.057$	NS	$p = 791$	NS

Table 4. Correlation between the tau protein, p-tau protein and beta-amyloid42 levels in the MS group and age of patients or duration of clinical symptoms.

	Tau x age	tau x duration	p-tau x age	p-tau x duration	Aβ42 x age	Aβ42 x duration
r	0.1854	–0.0607	–0.1387	0.1224	–0.3863	–0.3264
p	0.526	0.844	0.636	0.690	0.173	0.274
Significance	NS	NS	NS	NS	NS	NS

No statistically significant difference in tau protein and p-tau protein levels was found for both groups of patients as compared to the control group. The MS group had significantly increased beta-amyloid42 level (p=0.0076) vs. control group. No difference was found in the beta-amyloid42 levels between the CIS group and the control group.

No differences in the tau protein, beta-amyloid42 and p-tau-protein concentrations were found between the group of patients with the clinically isolated syndrome (CIS) and the group with a definitive diagnosis of MS. Statistical comparison between the patient and control groups is summarized in Table 3.

We demonstrated no statistically significant correlation between the beta-amyloid42, tau protein and p-tau protein levels and age of patients or duration of disease, both in the MS, and CIS patient and in the control group (Table 4.).

DISCUSSION

In our work we examined the possibility of using the following three biomarkers in the cerebrospinal fluid: tau protein, p-tau protein and beta-amyloid42, in multiple sclerosis patients.

Analysis of cerebrospinal fluid is one of the constituents of the diagnostic procedure in multiple sclerosis patients. However, no MS-specific test has been available to date (Mc Donald *et al*., 2001, Luque *et al*., 2007). Certain analogies in the pathogenesis of MS and AD (a variable combination of degenerative and inflammatory involvement) and clinical use in the diagnosis of dementia brought us to the idea of determining tau protein, p-tau protein and beta-amyloid42 levels in MS patients. The idea of testing tau protein levels in this disease is based on the findings of early axonal damage in the MS patients (Trapp *et al*, 1998).

However, the anticipated elevated CSF levels in patients compared to the healthy controls were confirmed by several studies only (Brettschneider *et al*., 2005, 2006, Kapaki *et al*., 2003). In contrast, Guimaraes *et al*. demonstrated no difference in the tau protein and p-tau protein levels between the MS patients and healthy controls and no dependence of this marker on the course and duration of disease or age in a study involving the group of 50 MS patients (Guimarães *et al*., 2006). In our study, we reached corresponding results and the same conclusions as in the latter study. Tau protein seems to be no suitable biomarker for the diagnosis of MS.

The only statistically significant findings were those of elevated beta-amyloid42 levels in patients with definitive MS compared to the control group and CIS. The difference in the values between the CIS group and control group was not statistically significant. No correlation was demonstrated with the age of patients and duration of disease.

Elevation of beta amyloid levels in our group of patients could be perhaps explained by the fact that our study population contained the relapse remittent form of MS only. In this form, the inflammatory component of the disease is predominant with a significant damage to the blood-brain barrier. A clinically non-significant difference between the control group and CIS is in accordance with the expectations, since it is an early phase of the diseases with suspected MS.

Based on our results, the possibility of using tau protein, p-tau protein and beta-amyloid42 levels in the routine diagnosis of MS seems to be non-beneficial. To date no single marker is available that would m*eet al*l the required criteria to justify its routine implementation. Research for MS-specific surrogate markers is eased by the availability of new technologies (proteomics, metabolomics, pharmacogenomics) (Dumont et.al, 2004).

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