Measurement of Gelatinase B (MMP-9) in the Cerebrospinal Fluid of Patients With Vascular Dementia and Alzheimer Disease

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Background and Purpose—Vascular causes of dementia are increasing in importance because of the aging of the population. Biological markers to distinguish patients with vascular dementia (VaD) from Alzheimer disease (AD) would be very useful. Because cerebrovascular disease increases expression of brain matrix metalloproteinases (MMPs) and tissue inhibitors to metalloproteinases (TIMPs), we hypothesized that MMPs would be elevated in the cerebrospinal fluid (CSF) of patients with VaD, but not in patients with AD.

Methods—Fifteen patients with VaD were identified, including dementia caused by multiple infarcts and progressive dementia caused by disease of the small cerebral blood vessels. Patients were followed-up for 4 to 10 years to confirm the diagnosis. Thirty patients with AD were also studied. Patients had CSF collected at their initial evaluation. Gelatinase A (MMP-2) and gelatinase B (MMP-9) were quantified by gelatin-substrate zymography, and TIMPs were measured by reverse zymography. Control CSF was obtained from neurologically normal subjects.

Results—MMP-9 levels were significantly elevated in the CSF of VaD patients compared either to those with AD ($P<0.0001$) or to controls. MMP-2, TIMP-1, and TIMP-2 were similar in patient groups and controls.

Conclusions—Patients with multiinfarct and small vessel VaD have elevated levels of MMP-9 in the CSF compared with AD and controls. Although CSF MMP-9 increases in other neurological conditions and is not specific for VaD, it could provide an additional biological marker for the separation of patients with VaD and AD. (Stroke. 2004;35:e159-e162.)

Key Words: matrix metalloproteinase ■ dementia

Vascular dementia (VaD) is defined as a loss of multiple cognitive functions caused by cerebrovascular disease. VaD may involve multiple ischemic lesions, a single strategic lesion, or a progressive small vessel disease with demyelination. Separating a progressive dementia caused by small vessel disease from Alzheimer disease (AD) can be difficult. Postmortem studies that document the concurrence of vascular and degenerative pathologies further complicate their clinical distinction. Although clinical features alone often suffice to render a "probable" diagnosis, biological markers can help increase diagnostic specificity. Patients with VaD have elevated cerebrospinal fluid (CSF) albumin, reduced N-acetylaspartate on proton magnetic resonance spectroscopy, and extensive abnormal white matter signal on magnetic resonance imaging (MRI).

At autopsy, patients with VaD show fibrosis of the small blood vessels with adjacent demyelination, suggesting that extracellular matrix (ECM) remodeling may be occurring. Matrix-degrading metalloproteinases (MMPs) are increased in neuroinflammation, including that accompanying cerebrovascular disease. Because brain tissues from patients with VaD contain several MMPs, including gelatinase A (MMP-2), gelatinase B (MMP-9), and stromelysin-1 (MMP-3), we hypothesized that patients with dementia related to vascular disease would have elevated levels of MMPs in the CSF. Furthermore, we postulated that MMPs would not be increased in AD, making it a potential biological marker. To test these hypotheses, levels of MMPs and tissue inhibitors of metalloproteinases (TIMPs) were measured in patients with clinically diagnosed VaD and compared with those of AD patients and a group of control subjects without neurological disease.

Patients and Methods

CSF Collection

The study was approved by the Human Research Review Committees at the University of New Mexico Health Sciences Center.

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RESULTS

Clinical Data

Of the 15 patients with VaD, 8 patients had a slowly progressive course associated with hypertension, focal findings, gait difficulties, and an elevated Hachinski score, and the diagnosis for these patients was consistent withBinswanger disease. These patients’ scans all showed extensive areas of periventricular white matter changes without prominent cortical atrophy. The other 7 patients with VaD manifested syndromes more consistent with multiple infarcts, including multiple episodes of symptomatic cerebral ischemia associated with a discontinuous course of cognitive decline. Their MRI scans demonstrated multiple focal lesions cortical and subcortical signal abnormalities. All patients with VaD underwent neuropsychological testing, which showed deficits characteristic of frontal/subcortical-type cognitive impairments.

The groups did not differ significantly in age, gender distribution, or duration of dementia. The VaD patients had elevated CSF protein concentrations with a mean of 87.3±39 mg% (mean±SEM) and with a normal mean leukocyte count (1.8±2.4). The AD patients did not show an elevation in either CSF measure.

None of the clinical diagnoses of probable AD changed during follow-up after the CSF examination. During this period, 6 of 30 AD patients died and the diagnosis was confirmed at autopsy. Two of the VaD patients died, but permission for autopsy was not given.

CSF Gelatinases and TIMPs

Quantification of the zymograms showed a significant increase in the bands at 92-kDa from the MMP-9, but there was not a significant increase in the levels of MMP-2 compared with the controls (P<0.003) (Figure 1). There were no differences in MMP-9 levels between the patients with presumed small vessel disease and those with multiple infarcts.

Reverse zymograms for the VaD patients and controls showed bands at 21-kDa and 28-kDa, which are the molecular weights of TIMP-2 and TIMP-1, respectively, as confirmed by Western immunoblots. Neither of the TIMP activities differed significantly from the control values. Three VaD patients with repeated CSF measurements over 5 years showed a gradual decrease in MMP-9 levels without a loss inTIMP-1 or TIMP-2, and the MMP-2 levels were also unaffected by time (data not shown).

CSF samples from patients with AD yielded values of MMP-2 and MMP-9 that were similar to those of the controls.
Likewise, there was no group difference with regard to TIMP activity. Comparison of the VaD data from UNMH with the AD data from OHSU was accomplished through normalizing each sample by the controls’ mean values, because the same control samples were used separately with each patient set. Normalized data showed that MMP-9 levels from the VaD group were significantly higher than values from AD patients ($P < 0.0047$) (Figure 3).

**Discussion**

VaD caused an increase in the levels of MMP-9 in the CSF. Patients with AD did not show a similar increase and had values in the control range. Diagnostic criteria for VaD are controversial because of the group of patients with a slowly progressive course and damage to the myelin secondary to the vascular disease. Because VaD is a heterogeneous disorder and can follow a variable course, diagnostic criteria for large vessel, multi-infarct, and small vessel forms of the illness were used.\(^7\)\(^8\) Recently, a combination of several previous diagnostic criteria has been proposed, and these new criteria fit our patient groups.\(^11\) Because autopsies were not performed, the diagnosis was confirmed by long-term follow up. In this series, approximately half of VaD patients had a progressive course consistent with Binswanger disease,\(^8\) whereas the others had a course more consistent with multiple infarcts.\(^12\) This breakdown of patients is similar to that found in an autopsy series.\(^6\)

Elevation of MMP-9 in the CSF is a nonspecific finding reported in a number of neuroinflammatory conditions, including multiple sclerosis, AIDS dementia, and viral infections.\(^13\) The present study is the first description, to our knowledge, of elevated MMP-9 levels in patients with VaD. There are several sources for MMP-9 in the CSF: extravasation from the blood, release by infiltrating leukocytes, and endogenous production by brain cells. A recent report described elevated levels of MMP-9 in the plasma in patients with AD.\(^14\) However, in our series and in 1 other published report, levels of MMP-9 were not found to be elevated in the CSF in AD patients.\(^15\) In patients with VaD, the CSF cell count was normal, but protein was elevated, suggesting an abnormality of the blood–brain barrier. Accordingly, some of the MMP-9 in the CSF may come from the blood. The lack of a change in MMP-2 levels argues against this account, but confirmation must await further studies that index CSF levels to those in the blood.

In an autopsy study of patients with VaD, stromelysin-1 (MMP-3) and MMP-2 were the major MMPs detected.\(^6\) One possible explanation for finding an acute marker of inflammation (ie, MMP-9) in the CSF of patients with a chronic disease is that they were studied during a clinically active phase. Longitudinal analysis in several patients showed a gradual decrease in MMP-9, consistent with the autopsy findings.

Although the number of patients in the present study was small, the differences between the VaD and AD patients were robust. The results suggest that CSF MMP measurements, although not diagnostic, might be combined with factors such as clinical course, psychometric profile, and imaging results to improve the early distinction between VaD and AD, potentially improving patient selection in future clinical trials.

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**References**


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