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Short Communication

Cerebrospinal fluid proteomics and human immunodeficiency virus dementia: Preliminary observations

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Protein profiling using mass spectrometry may be useful in identifying previously unknown protein markers in human immunodeficiency virus (HIV) dementia and provide insight into disease pathogenesis. Six samples of matched cerebrospinal (CSF) and blood serum from patients with no, mild, and moderate dementia were prepped for biomarker screening by the Ciphergen system. Chips were analyzed in the matrix-assisted laser desorption/ionization (MALDI) mass spectrometer at low mass (700 to 20,000 Da) and at higher mass (5000 to 100,000 Da). In both serum and CSF samples, differences in protein intensity appeared to correlate with degree of dementia. This preliminary study suggests that protein markers of HIV dementia may be detected by MALDI mass spectrometry. *Journal of NeuroVirology* (2005) **11,** 557–562.

Recent advances in molecular biological technology, particularly proteomic analysis, combine high-performance mass spectrometry instrumentation with highly efficient chromatographic and electrophoretic separations. The technique of surface-enhanced laser desorption and ionization protein chip analysis now permits a rapid means of obtaining a protein fingerprint of minute quantities of bodily fluids, such as blood and cerebrospinal fluid (CSF) (Ferguson and Smith, 2003). Proteomics simplifies the identification of potential diagnostically and therapeutically important biomarkers identified by comparing the proteomic profiles of control states and disease (Aldred *et al*, 2004). Proteomic analysis of the serum and cerebrospinal fluid has been applied to a variety of central nervous system (CNS) disorders.

To date, there has been limited application of this technology to human immunodeficiency virus (HIV) dementia (HIVD) (Luo *et al*, 2003; Persidsky and Gendelman, 2003; Ciborowski *et al*, 2004; Wojna *et al*, 2004; Pocernich *et al*, 2005a, 2005b).Wojna and colleagues, using proteomic fingerprinting to study incubated circulating monocytes from HIV-infected persons with cognitive impairment, identified seven unique protein peaks between 3.0 and 20.0 kDa in the samples of this patient population (Wojna *et al*, 2004). Other studies have used proteomics to investigate the potential neuropathogenesis of HIVD, including protein expression changes in human astrocytes that expressed HIV-1 Tat intracellularly (Pocernich *et al*, 2005a, 2005b) and neurotoxic secretory products of HIV-infected macrophages (Persidsky and Gendelman, 2003). This preliminary analysis was undertaken to determine whether there were unique protein patterns in HIVD CSF and serum.

Six study subjects were selected from among 56 subjects enrolled at the time in studies of the pathogenesis of HIVD (Table 1). Samples of matched CSF and blood serum from patients with no dementia, mild dementia, and moderate dementia (two from each group) were prepped for biomarker screening by the Ciphergen system. The two samples of like types were pooled and the pooled samples were diluted for

Table 1 Study subjects

Group	Age	Gender and race	CD4 Count	Viral load	MSK Score
Nondemented	38	M AA	231	<400	0
	44	M W	374	<400	0
Mild dementia	35	M W	880	5978	0.5
	42	M W	820	<400	1.0
Moderate	48	M W	248	842	2.0
dementia	30	M W	372	7042	2.0

M = male; AA = African American; MSK = Memorial Sloan Kettering Scale for AIDS dementia complex.

application onto the chemically derivatized surfaces of the Ciphergen protein chips. The survey screening used protein chips with hydrophobic (H4), immobilized metal affinity (IMAC-3), weak cation exchange (WCX-2), and strong anion exchange (Q-10) surfaces. Each sample was applied in duplicate for each analysis condition. The serum and CSF samples were bound to the anion and cation exchange chips at pH = 10, 8, 6, and 4. All the chips were analyzed

in the matrix-assisted laser desorption/ionization (MALDI) mass spectrometer at low mass (700 to 20,000 Da) and a matched set was analyzed at higher mass (5000 to 100,000 Da). The resulting mass spectra were compiled by fluid type (serum or CSF), target chemistry (protein chip type and wash conditions), and mass range analyzed.

In the serum samples, the presence of dementia was associated with lack of binding of a 15-kDa and a 15.7-kDa protein to the target, as seen in weak cation exchange at pH = 4 (Figure 1) and anion exchange at pH = 10 (Figure 2). Also missing in the dementia serum but present in the HIV-infected normal samples were 7.6-, 7.9-, and 8.6-kDa species that bound to the anion exchange chip (Figure 3). In the CSF samples, the presence of dementia was associated with decreased amounts of a 6.7-kDa protein binding to copper-loaded IMAC (Figure 4) and on the zinc-loaded IMAC (Ferguson and Smith, 2003) chip (Figure 5). A protein at 8.9-kDa was seen in the moderately demented sample, but not in the mildly demented or normal sample. Weak cation exchange
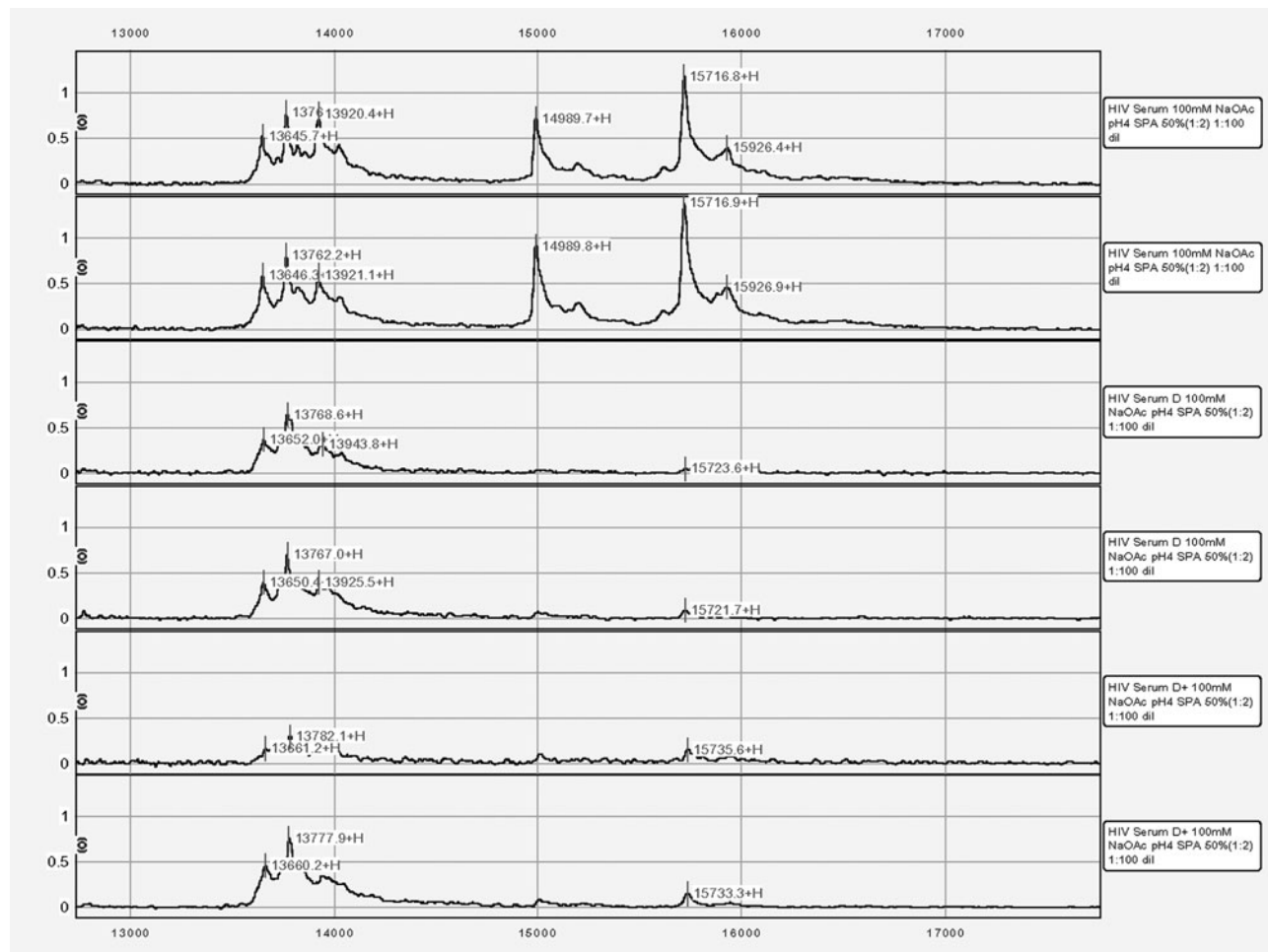

Figure 1 Mass spectra of serum samples in weak cation exchange (pH = 4) showing lack of binding of 15 dDa and 15.7 kDa protein in HIVD sera.
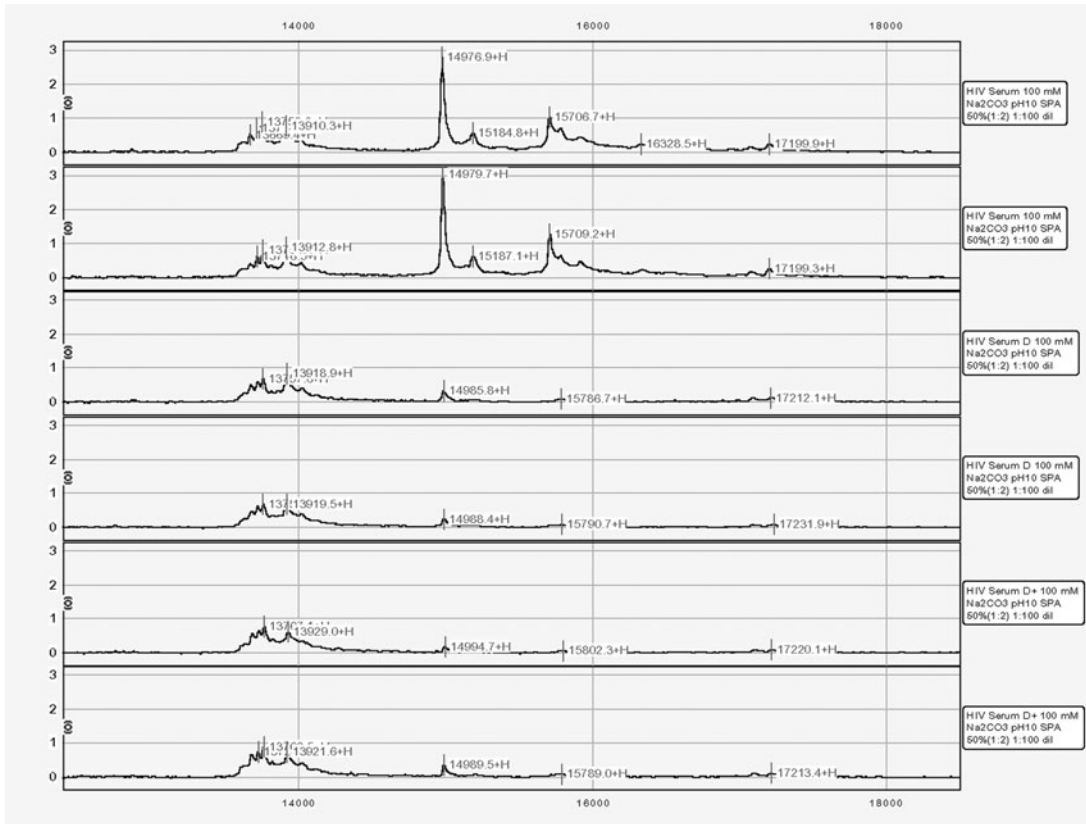
Figure 2 Mass spectra of serum samples in anion exchange (pH = 10) showing lack of binding of 15 dDa and 15.7 kDa protein in HIVD sera.
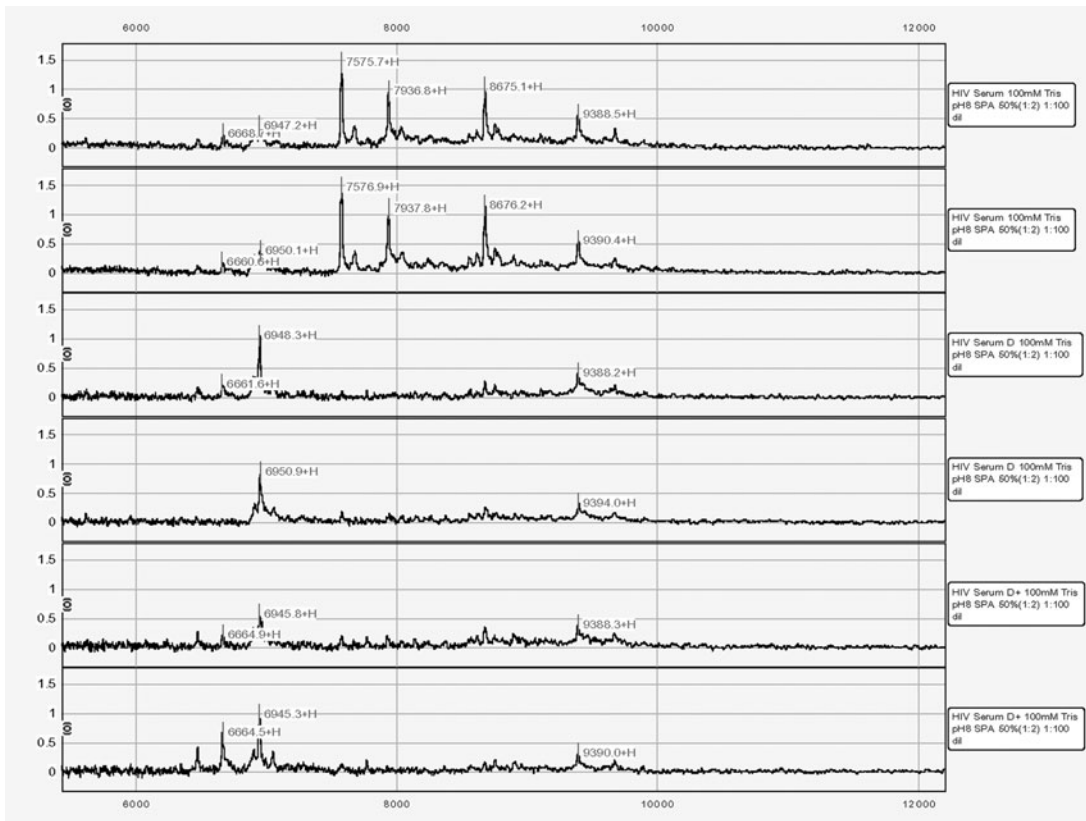

Figure 3 Mass spectra of serum samples in anion exchange (pH = 10) showing missing 7.6, 7.9 and 8.6 kDa proteins in HIVD sera.
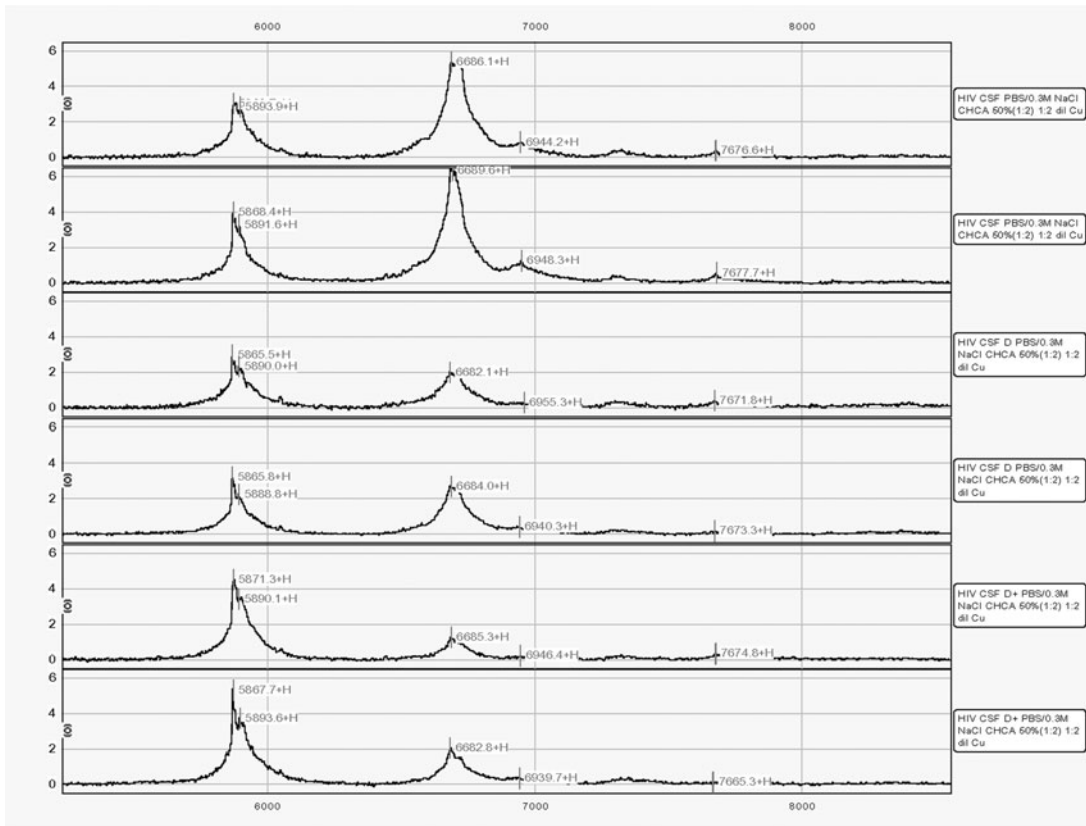
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Figure 4 Mass spectra of CSF samples showing decreased binding of 6.7 kDa protein in.
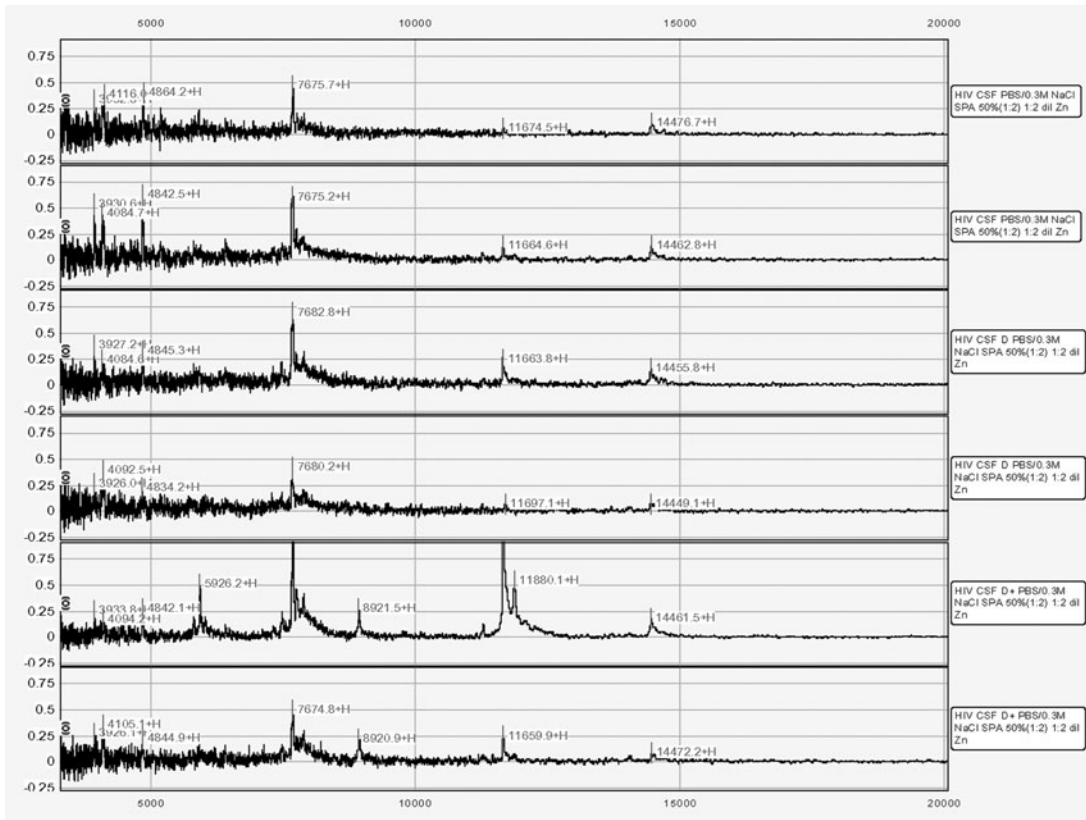

Figure 5 Mass spectra of CSF samples showing decreased binding of 6.7 kDa protein in zinc-loaded IMAC chip.
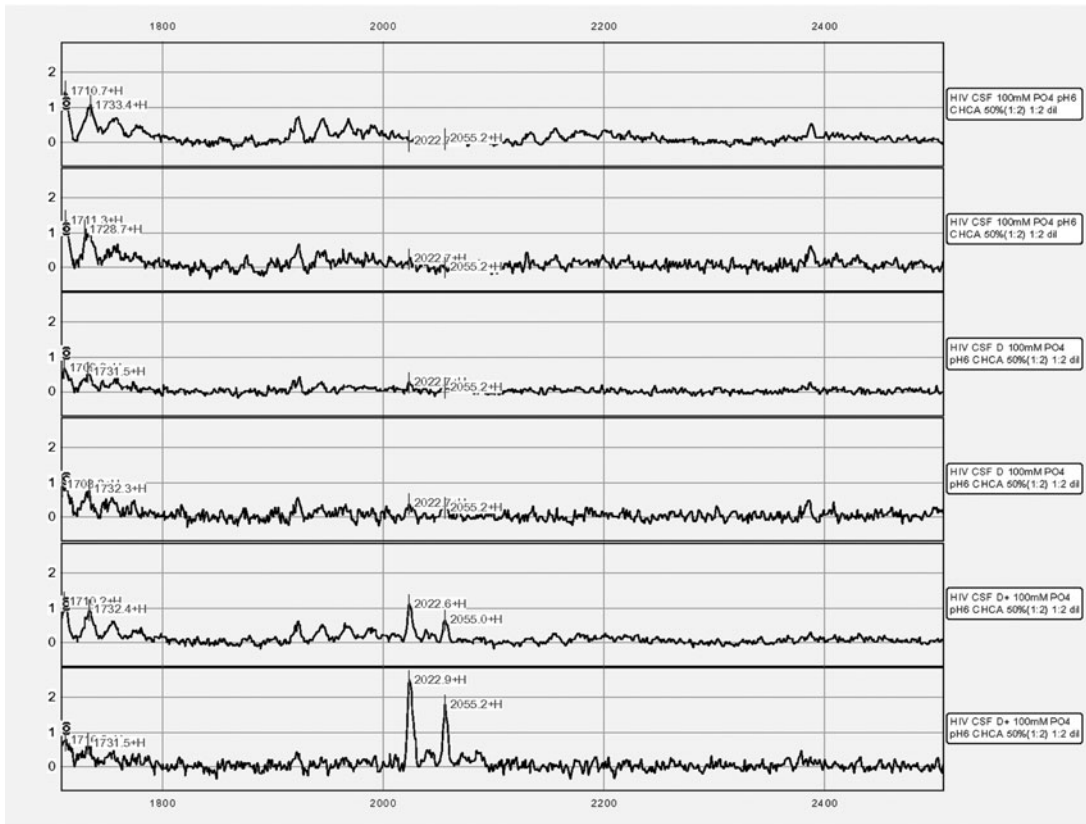
Figure 6 Mass spectra of CSF samples showing an increased of 2 proteins at approximately 2 kDa in the HIVD samples.
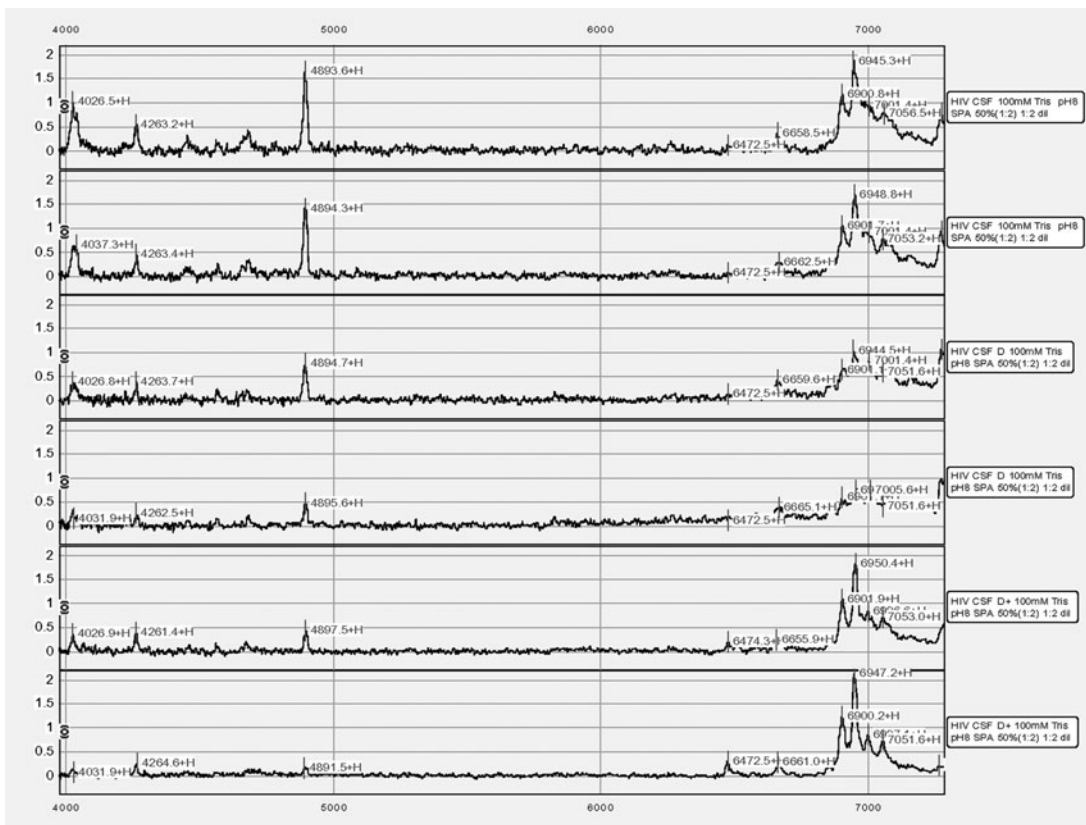

Figure 7 Mass spectra of CSF samples showing a decreased in 4.9 kDa protein in HIVD samples.

(pH = 6) saw an increase in two proteins at about 2 kDa with increase in dementia level (Figure 6), and there was a decrease in a 4.9-kDa protein with an increase in degree of dementia seen in CSF on anion exchange (Figure 7).

The application of proteomics to the study of HIVD may prove valuable in identifying biomarkers for the disorder. A number of biomarkers have been proposed for HIVD, including CSF neopterin (Brew *et al*, 1990), quinolinic acid (Brouwers *et al*, 1993). β_2-microglobulin (McArthur *et al*, 1992), tumor necrosis factor-α (Benveniste and Benos, 1995), interferon-γ (Benveniste and Benos, 1995), and soluble Fas (Towfighi *et al*, 2004). Protein fingerprints obtained from these fluids may provide an excellent strategy for identifying the presence

of the disorder and, perhaps quantifying its severity. Their precise identification may provide a very important insight into disease pathogenesis.

Our IMAC data exhibit similarities to that recently reported by J. LeBlanc *et al* (LeBlanc *et al*, 2001) in CSF of patients with HIVD. Although the numbers of patients studied remains small and the results must be regarded as preliminary, the unique pattern of protein expression in moderate HIVD and the lesser degree of these changes in the mild HIVD group when compared to the cognitively unimpaired HIV-infected controls suggest that these changes are likely significant. If reconfirmed in repeated analyses and larger studies, these protein signatures may prove a useful biomarker for HIVD.

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