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The longitudinal profile of CSF markers during external lumbar drainage

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The longitudinal profile of CSF markers during external lumbar drainage

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Abstract

External lumbar drainage (ELD) is known as a good predictor of favourable outcome in shunting patients suffering from idiopathic normal pressure hydrocephalus (iNPH).

Methods: Eleven patients suffering from iNPH had a lumbar drain (LD) inserted for 72 hours and participated in a research study to quantify any improvement in their clinical symptoms. The lumbar CSF levels of lactate, 8-isoprostane, vascular endothelial growth factor (VEGF), glial fibrillary acidic protein (GFAP), neurofilament (heavy chain) protein (NF (h)), A β_{1-42} (β - amyloid) and total tau were assayed samples from all three time points.

Results: The concentrations of lactate, VEGF, GFAP and tau increased significantly during the 72 hours of drainage. There were also increases in 8-isoprostane and A β_{1-42} (non significant). The concentration of NF (h) was reduced significantly following 72 hours of drainage. There was a significant positive correlation between A β_{1-42} and total tau in the first sample. GFAP was negatively correlated in a significant fashion with both A β_{1-42} and total tau. NF (h) was negatively correlated with VEGF.

Conclusion: We provided evidence that ELD is producing measurable changes in the CSF composition of patients with iNPH. We discuss how such changes may be implicated in the pathophysiology of the condition.

Introduction

The pathophysiology of idiopathic normal pressure hydrocephalus (iNPH) has remained more or less an enigma since its original inception in 1965 [1]. We now know that there is an element of ischaemia characterising these patients [2, 3], as well as a degree of neurodegeneration not dissimilar to that found in patients with Alzheimer's type of dementia (AD) [4]. Drainage of cerebrospinal fluid (CSF) is known to improve the symptoms and assist in selecting patients for CSF diversion [5].

The aim of this study was to examine the changes that occur in the composition of the CSF of patients with iNPH by measuring markers that characterise the pathological changes postulated so far in this group of patients. For this purpose we have chosen to measure lactate, 8-isoprostane, vascular endothelial growth factor (VEGF) to investigate the element of cerebral ischemia. Glial fibrillary acidic protein (GFAP) was chosen as a marker of gliosis and irreversible damage. The heavy chain of neurofilament protein (NF (h)), total tau and $A\beta_{1-42}$ were chosen to investigate the degree of neurodegeneration. Our hypothesis is that drainage of CSF will produce changes in the concentrations of these markers that might provide a further insight into the pathophysiology of this condition.

Material and methods

Eleven patients were recruited from the hydrocephalus research programme carried out in our institution between February 2005 and February 2007. Inclusion criteria for patients were those of “probable” iNPH as suggested by recently published guidelines [6]. All 11 patients of the above group suffered from the idiopathic form of normal pressure hydrocephalus. **The patients have been screened for the diagnoses of Vascular Cognitive impairment and Alzheimer’s dementia and they did not fit the diagnostic criteria. None of the patients presented with symptoms or signs of cervical stenosis.** The patients had continuous external lumbar drainage for 72 hours after insertion of a lumbar drain in the operating theatre. All participants had signed a consent form with regards to the aims of the study and the study has been approved by the local research ethics committee.

Mean patient age was 71.2 (range: 62-82) years and the male: female ratio was 9:2.

Mean pre-operative duration of symptoms was 37.2 (range: 12-60) months.

The CSF samples used in our study were obtained during and after the insertion of the external lumbar drain at three different time points (during the insertion, 24 and 72 hours after and prior to the removal of the catheter). The drainage was set at 15 mL/hour (approximately 360 mL in total for every 24 hours of drainage). There was no statistically significant difference between the total amount of CSF drained between all 11 patients.

Red cell and white cell counts were measured on all “day 0” samples. CSF albumin was measured and CSF/serum albumin ratio calculated to look for disruption of the

blood brain barrier and aid in interpretation of results. All samples were centrifuged within 20 minutes following collection and the supernatant aliquoted, frozen and stored at -80°C until analysis.

CSF albumin was measured by an in-house immunoturbidimetric assay. CSF lactate was analyzed enzymatically on an YSI 2700 STAT PLUS analyzer (YSI, USA).

VEGF, 8-isoprostane, $\text{A}\beta_{1-42}$, and total tau were assayed using commercial assays (Amersham BiotrakTM human VEGF ELISA, GE Healthcare, U.K.; 8-Isoprostane EIA kit, Cayman Chemical Company, MI, US; Innotest[®] β -amyloid₍₁₋₄₂₎, Innogenetics, Belgium; Innotest[®] hTAU Ag, Innogenetics, Belgium). Heavy chain of the neurofilament protein and GFAP were assayed by techniques described previously [7, 8].

All results were calculated and compared for each group using the non-parametric Wilcoxon signed ranks test. The Spearman rank order test was used to assess bivariate correlations. All statistical tests were calculated using SPSS version 13.0 for Windows.

Results

There were no differences in red cell count, white cell count or CSF/serum albumin ratio between the samples. The results of the assays for the markers can be seen in Table 1. Lactate levels increase as drainage progresses, and this increase reaches statistical significance within 72 hours of drainage. Furthermore, even though lactate levels are within normal limits at time 0 (upper normal limit: 2.40 mmol/L) they become pathological at 48 and 72 hours post drainage.

The levels of the 8-isoprostane increased over 72 hours with the difference reaching statistical significance only between 48 and 72 hours. Concentration of VEGF also increased in a statistically significant manner, particularly between day 0 and 2, where the change is most prominent. Similar results occur for GFAP, with the most prominent change happening within the first 48 hours and the significance reaching $p \leq 0.001$ after 72 hours of drainage. The levels of NF (h) decrease significantly between day 0 and 3. The levels of $A\beta_{1-42}$ increase overall but in a non-significant fashion. A consistent increase is noted in the concentration of total tau as drainage continues.

Correlations between markers

Table 2 presents the correlations between the markers for day 0. Lactate has virtually no correlation with either 8-isoprostane or VEGF in day 0, 2 and 3. Equally 8-isoprostane has no correlation with any marker in any of the 3 day samples. VEGF has a negative correlation with NF (h) ($r = -0.758$, $p = 0.007$) in the first sample (Figure 1).

GFAP has also a significant negative correlation with total tau level on day 0 ($r = -0.785$, $p = 0.004$) (figure 2). $A\beta_{1-42}$ also has a significant negative correlation with GFAP level on day 0 ($r = -0.676$, $p = 0.022$).

$A\beta_{1-42}$ and tau have a moderately positive correlation ($r = 0.636$, $p = 0.035$) which is statistically significant during day 0.

Discussion

No study in the literature to date has recorded the changes in the CSF composition during external drainage. Our study therefore is the first, to our knowledge, which attempts to record such changes. Tullberg et al. on a recent study have recorded increased postoperative levels of several markers; their findings of increased tau levels agrees with our current findings [9].

The significant increase in lactate levels was against our expectations that lactate level would decrease since the element of ischaemia is alleviated following drainage. We postulate that the hydrostatic pressure gradient from the ventricles to the interstitial space is reversed during drainage and therefore the resorption of lactate by the subependyma occurring in normal circumstances [10] might cease. **Another explanation would be that this increase is the product of extended CSF drainage producing mild inflammatory changes.**

The temporal profile of 8-isoprostane remains mostly unaltered during the 72 hours of drainage. The most likely cause is that the ischaemic process cannot be reversed within such a short time and therefore any changes will not be reflected in the CSF profile obtained during this study. Similar suggestions have been made in a recent study by Lenfeldt et al., [11].

The level of VEGF increased during the drainage. Our study supports a degree of angiogenesis in patients with iNPH, even though this angiogenesis does not seem to be induced by ischaemia since VEGF is not correlated with the other 2 markers we chose to characterise hypoxia.

The heavy subunit of the neurofilament protein (NF (h)) has not been investigated so far in iNPH. NF (h) levels were reduced significantly within 72 hours. This result might explain the reversal of neuronal degeneration that one would expect to occur with shunting. However, the levels on all three days were within normal limits (upper limit of normal = 0.73 ng/ml [12]). In the day 0 sample the levels of NF (h) are negatively correlated with the levels of VEGF which might signify a reverse established association between neurodegeneration and angiogenesis/ neurogenesis.

Increased GFAP levels in iNPH patients compared to controls is well known [13]. Our results might signify a release of GFAP from a ventricular pool as result of the underlying astrocytic damage; similar findings have been published [14]. GFAP had a statistically significant negative correlation with both $A\beta_{1-42}$ and total tau in the first sample.

Tau has been suggested as a potential biomarker for NPH [15]. Our results from the initial lumbar sample for these two markers agree with those of Agren-Wilsson and colleagues [16] pointing out that there does not appear to be a co-existence of AD pathology in our study group. However, the increase in total tau levels over the 3 days of drainage was significant signifying possibly a release from a ventricular pool. The levels of $A\beta_{1-42}$ and tau are positively correlated in the first 24 hours

It is possible that the changes observed in day 2 and day 3 represent the levels of ventricular rather than lumbar CSF. Simultaneous sampling of lumbar and ventricular CSF might provide some of these answers in future studies.

One of the limitations of our study was that the markers were measured after 72 hours of external lumbar drainage which might prove insufficient in order to provide any significant alterations in the neuronal environment and the effect this will have on the composition of the CSF. Nevertheless the correlations between markers might have been strengthened further if we were able to obtain CSF samples at a later time point.

Conclusion

In our study we recorded the pathophysiological changes occurring in the CSF of patients with idiopathic NPH during three days of external lumbar drainage. In future studies it would be necessary to correlate the findings of this study with imaging findings reflecting the underlying pathophysiological processes to further elucidate on the significance of these changes.

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Tables and figures

CSF markers	Normal values Median (range)	Day 0		Day 2		Day 3	Day 0-3
CSF/serum albumin (X 10 ³)	<10.2 §	9 (6.8)	↑, ns	11.6 (10.7)	↓, ns	9.3 (5.8)	↑, ns
Lactate (mmol/L)	Upper reference range: 2.4 mmol/L ‡	2.27 (.26)	↑, ns	2.68 (.23)	↑, ns	2.74 (.26)	↑, * [0.027-0.033]
8-Isoprostane (pg/ml)	‡	38.13 (.29)	↓, ns	37.8953 (.33)	↑, * [0.027-0.034]	38.66 (.32)	↑, ns
VEGF (ng/ml)	‡	4.08 (1.14)	↑, * [0.010-0.014]	13.61 (2.81)	↑, ns	16.8 (4.09)	↑, * [0.007-0.011]
GFAP (pg/ml)	Upper reference range: 9 pg/mL	2.01 (1.41)	↑, * [0.022-0.028]	6.9750 (.99)	↑, ns	16.3 (6.46)	↑, ** [0.001-0.003]
NF (h) (ng/ml)	Upper reference range: 0.73 ng/ml	0.1618 (.052)	↓, * [0.07-0.010]	.0156 (.015)	↑, ns	.0345 (.016)	↓, * [0.013-0.018]
Aβ 1-42 (pg/ml)	849 (682-1063) f	366.05 (74.71)	↑, ns	464.04 (43.62)	↓, ns	449.02 (74.50)	↑, ns
Tau (pg/ml)	195 (121-294) f	151.88 (27.28)	↑, * [0.006-0.010]	310.57 (51.68)	↑, * [0.038-0.046]	415.55 (69.78)	↑, * [0.002-0.004]

Table 1 Concentrations of markers in all 3 days of collection and the relationship between days 0 and 3. Values represent mean and standard error of mean in brackets. The one-tailed Wilcoxon signed ranks test was used to identify significant differences. Levels of significance: * p<0.05, ** p<0.001, ns: non significant. In brackets the 95% Confidence Intervals of the levels of significance. §: *Principles of albumin and IgG analyses in neurological disorders. I. Establishment of reference*

values. Tibbling G et al. *Scand J Clin Lab Invest.* 1977 Sep;37(5):385-90. f: median values as suggested by ELISA manufacturer taken from *Improved discrimination of AD patients using beta-amyloid(1-42) and tau levels in CSF.* Hulstaert F, et al. *Neurology.* 1999 May 12;52(8):1555-62. ‡: No data available for ELISA used.

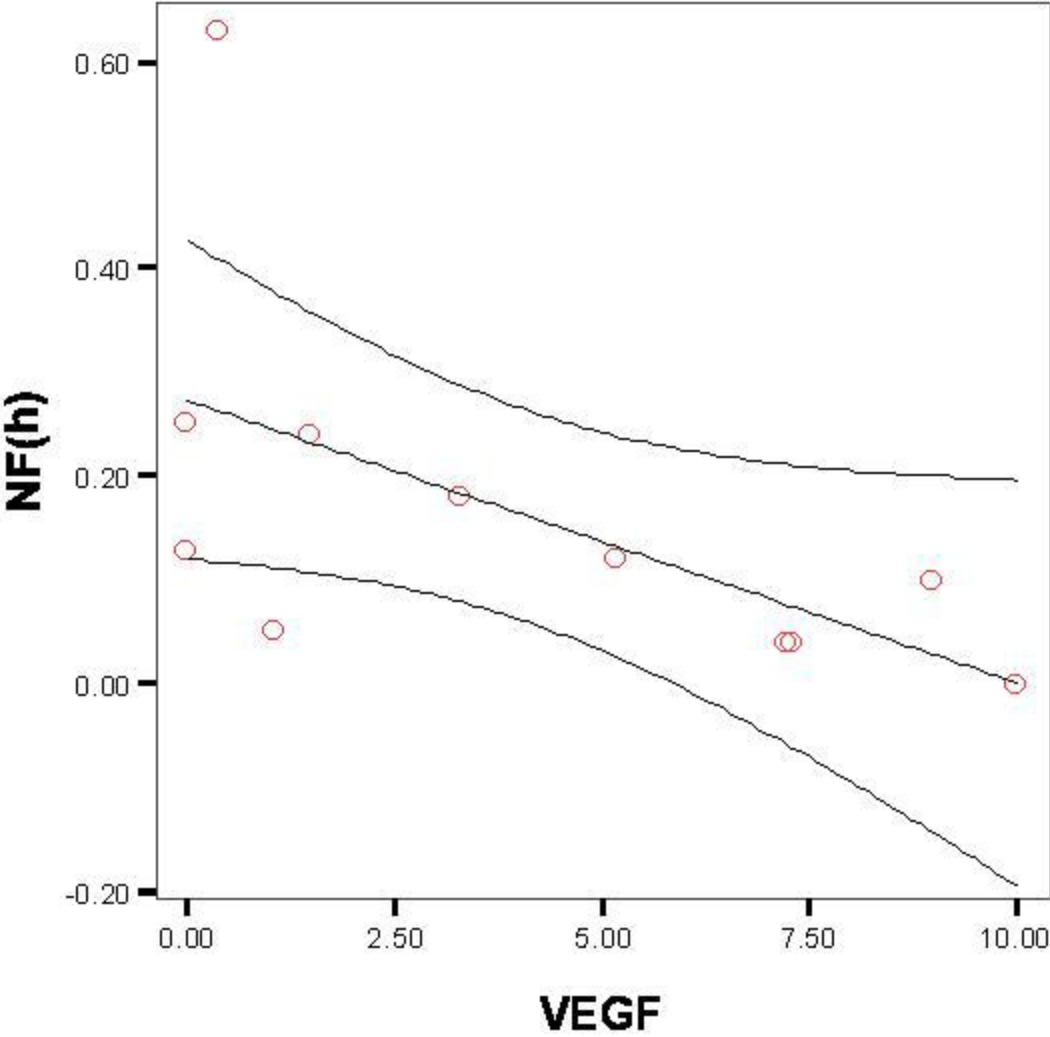
		Lactate	IP	VEGF	GFAP	NF (h)	A β ₁₋₄₂	tau
Lactate	Correlation Coefficient							
	Sig. (2-tailed)		NS	NS	NS	NS	NS	NS
8-isoprostane	Correlation Coefficient							
	Sig. (2-tailed)	NS		NS	NS	NS	NS	NS
VEGF	Correlation Coefficient					-0.758		
	Sig. (2-tailed)	NS	NS		NS	0.007	NS	NS
GFAP	Correlation Coefficient						-0.676	-0.785
	Sig. (2-tailed)	NS	NS	NS		NS	0.022	0.004
NF (h)	Correlation Coefficient							
	Sig. (2-tailed)	NS	NS	0.007	NS		NS	NS
Aβ₁₋₄₂	Correlation Coefficient							
	Sig. (2-tailed)	NS	NS	NS	0.022	NS		0.035
tau	Correlation Coefficient						0.636	
	Sig. (2-tailed)	NS	NS	NS	0.004	NS	0.035	

Table 2. Correlations of CSF markers. Table demonstrates only results for the samples collected at day 0. The two-tailed Spearman's test for bivariate correlations was used. Levels of significance: * p<0.05, ** p<0.001, NS: non significant

Figure 1. Scatterplot and linear regression analysis of lumbar NF(h) and VEGF levels. Solid line is best-fit regression line with 95% CIs (curved lines).

Figure 2. Scatterplot and linear regression analysis of lumbar GFAP and total tau levels. Solid line is best-fit regression line with 95% CIs (curved lines).

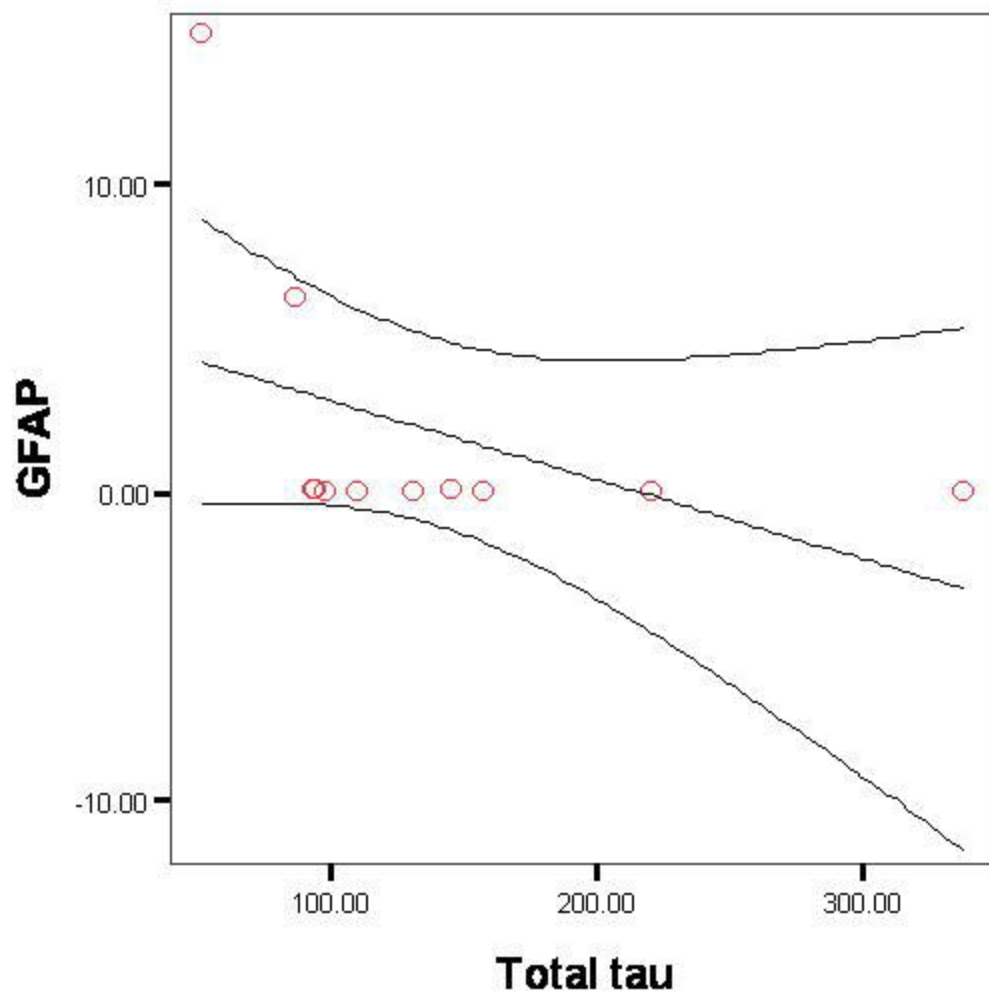
Correlation of NF(h) levels with VEGF levels at day 0 sample



Linear Regression with
95.00% Mean Prediction Interval

$NF(h) = 0.27 + -0.03 * VEGF$
R-Square = 0.35

Correlation of GFAP levels and total tau levels at day 0 sample



Linear Regression with
95.00% Mean Prediction Interval

$$\text{GFAP}_0 = 5.56 + -0.03 * \text{TAU}_0$$

R-Square = 0.19