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Original Paper

Increased Levels of Kynurenic Acid in the Cerebrospinal Fluid in Patients with Hydrocephalus

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Key Words
Hydrocephalus • Dementia • Cerebrospinal fluid • Serum • Kynurenic acid • L-Kynurenine • L-Tryptophan • CRP • Aging process

Abstract
Background/Aims: Normal pressure hydrocephalus (NPH) is a potentially reversible neurological syndrome commonly characterized by gait disturbance, urinary incontinence, and dementia. Hydrocephalus e-vacuo (He-v) is also characterized by the occurrence of dementia but does not show gait disturbance or urinary incontinence and has no evident cerebrospinal fluid (CSF) pressure elevation. Kynurenic acid (KYNA), an endogenous metabolite of the L-kynurenine (L-KYN) pathway of L-tryptophan (L-TRP) degradation, is an antagonist of glutamate N-methyl-D-aspartic acid and alpha-7 nicotinic cholinergic receptors that have been linked to dementia. We investigated KYNA, L-KYN, and L-TRP levels in human CSF and serum during the aging process in 30 healthy control individuals. In addition, clinical parameters and L-TRP metabolites in CSF and serum were evaluated in four patients with NPH and five with He-v. ***Methods:*** KYNA, L-KYN, and L-TRP levels in CSF and serum were determined using high-performance liquid chromatography. ***Results:*** Healthy controls showed a significant decrease in serum albumin with age. Compared with their corresponding controls and unlike patients with He-v, patients with NPH (age ≤ 50 years) had significant increases in CSF protein (241%, $p < 0.001$), CSF albumin (246%, $p < 0.001$), CSF IgG (328%, $p < 0.001$), and CSF:serum IgG (321%, $p < 0.001$) and CSF:serum albumin (257%, $p < 0.001$) ratios. Controls had significant increases in KYNA, L-KYN, and L-TRP levels in the CSF with advancing age but not in the serum. Compared with the corresponding controls, KYNA levels were significantly increased in the CSF of patients with NPH (141%, $p < 0.05$) and He-v (225%; $p < 0.01$). Additionally, the serum levels of KYNA were increased in patients with NPH and He-v to 161% and 156% of controls, respectively (both $p < 0.01$). The serum levels of L-KYN and L-TRP were significantly reduced in patients with He-v but not in patients with NPH. C-reactive protein, as a marker of inflammation, was significantly increased in the serum of patients with

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He-v but not in patients with NPH, compared with the corresponding controls. ***Conclusion:*** The aging process is related to elevated CSF levels of KYNA, L-KYN, and L-TRP levels. There are significant differences in clinical parameters between the two forms of hydrocephalus and these differences might have diagnostic utility. The occurrence of dementia in patients with either form of hydrocephalus might be at least partly related to elevated KYNA levels in the CNS and/or periphery.

Introduction

Kynurenic acid (KYNA), an antagonist of glutamatergic and nicotinic cholinergic neurotransmissions [1, 2], has been suggested to be involved in cognition impairment [3]. Within the last decade, a significant number of studies have found higher levels of KYNA in the CNS in various neuropsychiatric disorders with dementia and during the aging process [4-7]. Normal pressure hydrocephalus (NPH) is characterized by three typical symptoms, namely, gait disturbance, urinary incontinence, and cognitive decline, and a high protein level in CSF is a characteristic parameter in NPH [8-11]. Recently, we described increased KYNA levels in the CSF and serum of one patient with NPH [10]. Interestingly, after successful therapy via implantation of a permanent CSF shunting system, the KYNA levels in the CSF and serum also declined in this patient, suggesting a possible link between the cognitive decline and the elevated KYNA levels in the CSF [10]. Another form of hydrocephalus, called hydrocephalus e-vacuo (He-v), is characterized by ventricle enlargement without CSF pressure elevation, gait disturbance, or urinary incontinence, but with occurrence of distinct dementia [12].

L-kynurenine (L-KYN) is the primary metabolite of the enzymatic degradation of L-tryptophan (L-TRY) by tryptophan 2, 3-dioxygenase in the liver or by indoleamine-2,3-dioxygenase (IDO) in the CNS [13, 14]. Degradation of L-KYN along the so-called kynurenine-niacin pathway leads to the formation of KYNA and other metabolites [1, 7]. In the CNS, KYNA may be derived from L-KYN that is synthesized either by IDO in the CNS or from L-KYN that crossed the blood–brain barrier [14, 15, 16]. KYNA levels are regulated by kynurenine aminotransferases, which are pyridoxal-5-phosphate-dependent enzymes that catalyze the formation of KYNA from L-KYN [1, 17, 18]. In human brain tissues, KYNA is synthesized by several kynurenine aminotransferases (KAT I–IV) with different catalytic characteristics [1, 7, 17, 18].

The levels of KYNA in CSF and/or serum depend on differently regulated events involved in KYNA metabolism, such as substrate availability, substrate uptake, and KYNA formation and release into the CSF and diffusion into the blood [1, 7, 15-18]. Because the choroid plexus is involved in the secretion of various soluble factors as well as in CSF dynamics, it is likely that altered KYNA levels in the CNS might also contribute to the pathogenesis of the aging process [19]. Indeed, the aged rat shows increased L-KYN levels in the CSF [20]. Glia depressing factor, which blocks KYNA formation, might influence KYNA levels, depending on health status [21]. We questioned whether the occurrence of dementia in patients with either form of hydrocephalus might be related to elevated KYNA levels. Because patients with He-v belong to a group of older patients with increased occurrence of inflammation, we were interested in determining whether C-reactive protein (CRP) plays a role as well. This is important because there are indications that CRP is not only an inflammatory biomarker, but also an important risk factor associated with aging-related diseases [22]. Furthermore, Hsu et al. [23] suggested an association between the baseline serum CRP level and dementia development.

Thus, the aim of this study was to evaluate clinical parameters and levels of KYNA, L-KYN, and L-TRP levels in the CSF and serum and serum CRP content in patients with NPH and He-v compared with corresponding control subjects. Correlations between age and the CSF levels of KYNA, L-KYN, and L-TRP in control subjects were also performed.

Materials and Methods

Out of a larger series of patients who underwent lumbar puncture to exclude subarachnoid hemorrhage, 30 individuals were selected as normal subjects because their CSF samples did not contain erythrocytes and there were no abnormalities in neuroimaging studies and further clinical investigations, which included electroencephalography and transcranial Doppler-sonography. Their ages ranged from 18 to 80 years. In addition, nine patients with hydrocephalus, comprising four with normal pressure hydrocephalus (NPH) and five with hydrocephalus e-vacuo (He-v), were selected.

Lumbar puncture was performed to obtain CSF for routine parameter determinations, namely, erythrocyte count, cell count, protein content, detection of IgG oligo-clonal bands, the autochthonic immunoglobulin production IgG index, and albumin content. Blood samples were taken for routine investigation of leukocyte counts, IgG and IgM, and albumin content. For neurochemical analyses, samples of CSF and serum were collected in 1 ml aliquots and stored immediately at –40°C until analysis. CSF and serum samples were coded to conceal patient identity and the study was carried out according to Lower Austrian Ethical Regulations.

All chemicals used were of the highest purity commercially available.

Biological parameter investigations

Measurements of erythrocytes, protein, albumin, IgG, IgM, and white blood cells were carried out using previously reported methods [24, 25]. Serum CRP levels were determined using a routine laboratory particle enhancer immunological clouding test. CSF:serum IgG and CSF:serum albumin ratios and the IgG index were calculated as previously reported [24]. For determination of oligo-clonal IgG bands, agarose isoelectric focusing was performed, followed by transfer to cellulose nitrate membranes and double-antibody avidin-biotin-peroxidase labeling [25].

Neuroradiological investigations

Routine clinical investigations involving cranial computed tomography (CT) and magnetic resonance tomography with CT or magnetic resonance angiography of cerebral vessels and electroencephalography and transcranial Doppler-sonography were performed.

Measurement of L-TRP and its metabolites

For sample preparation, 400 µl of CSF or serum samples were mixed with 14 µl of 50% trichloroacetic acid and 0.2 M HCl (vol/vol) and centrifuged for 20 min at 14,000 rpm. The supernatant obtained was used for the measurement of KYNA, L-KYN, and L-TRP.

Measurement of KYNA

KYNA was measured according to Shibata et al. [26] and Swartz et al. [27] with the modifications described by Baran et al. [4]. The supernatant was applied to a DOWEX 50W cation exchange column pre-washed with 0.1 M HCl. Subsequently, the column was washed with 1 ml 0.1M HCL and 1 ml distilled water, and KYNA was eluted with 2 ml distilled water according to Turski et al. [15] and quantitated by a high-performance liquid chromatography (HPLC) system coupled with fluorescence detection.

Measurement of L-KYN and L-TRP

L-KYN and L-TRP levels were quantitated by isocratic HPLC with UV and fluorescence detection, respectively, as described previously [28]. The HPLC method used a mobile phase of 42 mM ammonium acetate, 7 mM sodium hydrogen phosphate, 7 mM sodium acetate, 11 mM ammonium hydroxide, 59 mM acetic acid, 1.380 mM 1-octanesulfonic acid, and 74 µM sodium disulfide (pH = 4.8) pumped through a Chromolith™ Performance RP-18e, 100-4, 6 mm column at a flow rate of 0.7 ml/min. The injection volume was 50 µl. The fluorescence detector was set at an excitation wavelength of 299 nm and emission wavelength of 420 nm. The retention time of L-TRP was approximately 16 min with a sensitivity of 800 fmol per injection (signal:noise ratio = 5). Using the same HPLC conditions and the UV detector set at 366 nm wavelength, L-KYN was eluted with a retention time of approximately 3.3 min and a sensitivity of 500 fmol per injection (signal:noise ratio = 5).

Statistical analysis

All mean values are given ± the standard error of the mean (SEM). Analyses were performed in duplicate or triplicate. One-way analysis of variance (ANOVA) and the Student's t-test were applied to determine statistical significance. The level for statistical significance was set at $p < 0.05$.

Results

Clinical data in control subjects

The clinical parameters of control subjects were evaluated with respect to age ≤ 50 years vs. age ≥ 51 years and were comparable with previously published results [6]. The mean ages of the investigated groups were 37.9 ± 2.7 (range 18 to 50) years and 65.9 ± 1.9 (range 51 to 80) years ($p < 0.001$; Table 1). Regarding the clinical parameters of the serum in the two control subject groups, no significant differences could be seen, with the exception of a significant reduction in serum albumin in controls ≥ 51 years (85%, $p < 0.001$). There was a nonsignificant increase in CRP levels in controls ≥ 51 years (171%, $p = 0.12405$). Linear regression analysis revealed no significant increase in serum CRP with age ($r = 0.21035$, $p = 0.3238$; Fig. 1). CSF:serum IgG and CSF:serum albumin ratios were non-significantly higher in the group of control subjects ≥ 51 years (131%, $p = 0.14165$ and 127% $p = 0.17208$, respectively; Table 1) vs. those ≤ 50 years. Moderate but nonsignificant increases in CSF protein (124%, $p = 0.1046$) and CSF IgG (122%, $p = 0.3032$) values were observed in the controls ≥ 51 years.

KYNA concentrations in the CSF and serum of control subjects

The level of KYNA in the CSF was significantly higher in the group ≥ 51 years than in controls ≤ 50 years (5.005 ± 0.404 nM vs. 2.769 ± 0.225 nM, respectively; 181%, p = 4.35507E-5; Fig. 2). In the serum of subjects ≥ 51 years, we observed a moderate but significant increase in KYNA vs. subjects ≤ 50 years (129%, $p = 0.01765$; Table 2). Linear regression analysis revealed a significant increase in KYNA levels with age in the CSF ($r = 0.7131$, $p < 0.0001$;

Table 1. Biological parameters in patients with normal pressure hydrocephalus (NPH) and hydrocephalus e-vacuo (He-v) and in the corresponding control subjects (CO). Data represent the mean ± SEM. % of control is given in parentheses. Significance: *p < 0.05, **p < 0.01, ***p < 0.001 vs. group age ≤ 50 years or ≥ 51 years using a Student's t-test. W/M, women/men; CRP, C-reactive protein; CSF, cerebrospinal fluid

Biological parameters	CO Control subjects Age:18-50 years	NPH Patients with normal pressure hydrocephalus (% of control)	CO Control subjects Age:51-80 years	He-v Patients with hydrocephalus e-vacuo (% of control)
Age (years) Number	37.9 ± 2.7 (15)	44.7 ± 4.6 (118) (4)	65.9 ± 1.9*** (15)	75.2 ± 2.51 (114) (5)
W/M	10/5	3/1	11/4	1/4
Protein in serum, mg/dl	7.09 ± 0.12	7.05 ± 0.26 (99)	6.74 ± 0.19	7.36 ±0.10 (109)
Albumin in serum, mg/dl	4490 ± 10	4282 ± 137 (95)	3828 ± 120***	3876 ± 167 (101)
IgG serum, mg/dl	912.9 ± 48.5	957.7 ± 82.9 (105)	874.6 ± 68.9 (12)	1132.4± 91.9 (129)
IgA serum, mg/dl	182.9 ± 21.7	149.5 ± 8.02 (82)	201.2 ± 40.4	333.4 ± 76.5 (163)
IgM serum, mg/dl	101.51 ± 16.2	106.5 ± 10.14 (104)	73.77 ± 10.6	73.42 ± 21.04 (99)
Numbers of leucocytes in serum, 10^6/l	6.54 ± 0.49	8.02 ± 0.47 (123)	7.85 ± 0.61	9.14 ± 0.86 (116)
CRP in serum	0.200 ± 0.0365	0.2250± 0.094 (124)	0.3417 ± 0.0941	4.98 ± 2.5166** (1457)
Protein in CSF, mg/dl	34.25 ± 3.84	82.62 ± 11.84*** (241)	42.41 ± 2.49	35.48 ± 1.02 (84)
Albumin in CSF, mg/dl	25.93 ± 3.69	63.85 ± 8.08*** (246)	27.88 ± 0.2.26	25.82 ± 1.17 (93)
IgG CSF, mg/dl	2.817± 0.427	9.24 ± 0.99*** (328)	3.45 ± 0.408	3.26 ± 0.333 (94)
Ratio CSF:serum IgG	3.099 ± 0.464	9.96 ± 1.589*** (321)	4.074 ± 0.422	2.904 ± 0.291 (72)
Ratio CSF:serum albumin	5.803 ± 0.837	14.917 ± 1.847*** (257)	7.374 ± 0.679	6.738 ± 0.506 (91)
IgG index	0.5333 ± 0.0171	0.6600 ± 0.0434** (124)	0.5458 ± 0.0172	0.436 ± 0.032** (80)
Oligo clonal bands	negative	negative	negative	negative
Cell count	5.64 ± 1.11	1.75 ± 0.85 (31)	3.58 ± 0.98	4.8 ± 1.49 (134)

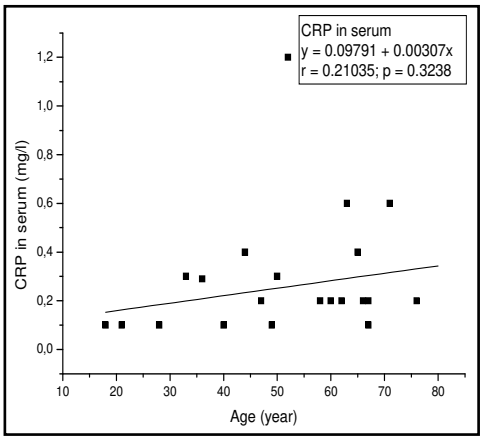

Fig. 1. Correlation between aging and the levels of C-reactive protein (CRP) in the serum of control subjects.

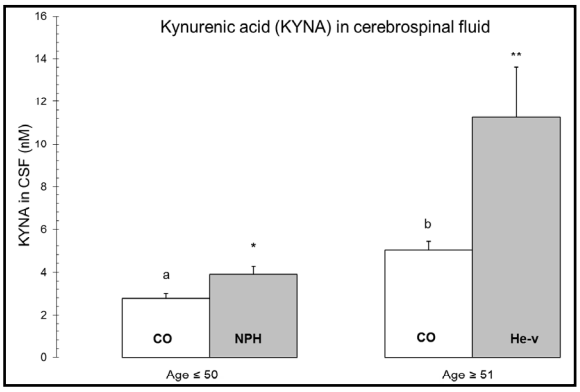

Fig. 2. Kynurenic acid (KYNA) levels in the cerebrospinal fluid (CSF) of patients with normal pressure hydrocephalus (NPH) and hydrocephalus e-vacuo (He-v) and in the corresponding control subjects (CO). Data represent mean ± SEM. Number (N) of subjects: CO group (each), N = 15; NPH group, N = 4; HE-v group, N = 5. Significance: *p<0.05, **p<0.01, vs. the group age ≤ 50 years or ≥ 51 years using a Student's t-test.

Fig. 3) but not in the serum (r = 0.3356, p = 0.06492; Fig. 4).

L-KYN concentrations in the CSF and serum of control subjects

The level of L-KYN in the CSF was significantly higher in the group ≥ 51 years than in controls ≤ 50 years (505.0 ± 94.3 nM vs. 240.1 ± 33.9 nM, respectively; 210%, p = 0.01334; Table 3). The serum of subjects ≥ 51 years showed a moderate but nonsignificant increase in L-KYN vs those ≤ 50 years (128%, p = 0.05537; Table 3). Linear regression analysis revealed a significant increase in L-KYN levels with age in the CSF (r = 0.3905, p < 0.03287; Fig. 5) but not in the serum (r = 0.2817, p = 0.13152).

Table 2. Kynurenic acid (KYNA) levels in the serum of patients with normal pressure hydrocephalus (NPH) and hydrocephalus e-vacuo (He-v), and in the corresponding control subjects (controls). Data represent mean ± SEM. Number (N) of subjects is given in parentheses. Significance: **p < 0.01 vs. the respective control group age ≤ 50 years or ≥ 51 years using a Student's t-test

Group	KYNA in serum nM (% of controls)
Control subjects (15) Age ≤ 50 years	53.37 ± 3.99 (100)
NPH patients (4) Age ≤ 50 years	85.92 ± 5.02** (161)
Control of subjects (15) Age ≥ 51 years	69.01 ± 4.76** (100)
He-v patients (5) Age ≥ 51 years	107.79 ± 12.14** (156)

L-TRP concentrations in the CSF and serum of control subjects

The level of L-TRP in the CSF showed a nonsignificant tendency to be higher in the group ≥ 51 years than in controls ≤ 50 years (4.109 ± 0.547 µM vs. 2.825 ± 0.378 µM, respectively; 145%, p = 0.06378; Table 3). Serum L-TRP levels were not significantly different between the two age groups (p = 0.88459). Linear regression analysis revealed a significant increase in L-TRP levels with age in the CSF (r = 0.4285, p < 0.01814; Fig. 6) but not in the serum (p = 0.72062).

Clinical parameters in patients with hydrocephalus

The clinical parameters of serum and CSF in patients with NPH and He-v and in the corresponding control subjects are presented in Table 1. We found a nonsignificant increase in IgA (163%, p = 0.11636) and IgG (129%, p = 0.0523) in patients with He-v and no alterations in these immunoglobulins in patients with NPH. Compared with the respective controls, serum CRP content was significantly increased in patients with He-v (1457%, p = 0.00925) but not in patients with NPH (124%, p = 0.76418). The CSF of patients with NPH showed increased levels of protein (241%, p = 8.36818E-5), albumin (246%, p = 2.56721E-4), and IgG

(328%, p = 4.19794E-6) and higher CSF:serum IgG (321%, p = 2.19994E-5) and CSF:serum albumin (257%, p = 1.443819E-4) ratios, whereas patients with He-v showed no alterations in these clinical parameters, compared with the respective controls (Table 1). The IgG index was moderately and significantly increased in patients with NPH (124%, p = 0.00517) but was moderately and significantly reduced in patients with He-v (80%, p = 0.00519; Table 1). No significant alterations in cell counts were observed in NPH patients (31%, p = 0.091479) and He-v patients (134%, p = 0.5096) (Table 1). Oneway ANOVA between the four groups revealed the following significant differences: serum CRP (F = 6.54835, p = 0.0018), CSF protein (F = 14.63323, p = 3.57433E-6), CSF albumin (F = 12.12738, p = 1.82649E-5), CSF IgG (F = 19.45617, p = 2.3043E7), CSF:serum IgG ratio (F = 16.87265, p = 9.44449E-7), CSF:serum albumin ratio (F= 11.17799, p = 3.54537E-5), and IgG index (F = 8.36858, p = 2.99938E-4). A tendency for a significant difference was observed for IgA serum (F = 2.5232, p = 0.07524) using one-way ANOVA. No significant difference was observed in the serum for protein (F = 1.91917, p = 0.14632) or IgG (F = 1.93237, p = 0.14419).

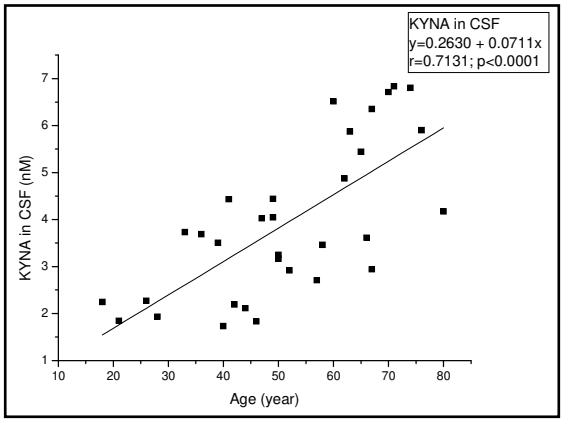

Fig. 3. Correlation between kynurenic acid (KYNA) levels in cerebrospinal fluid (CSF) and the age of control subjects.

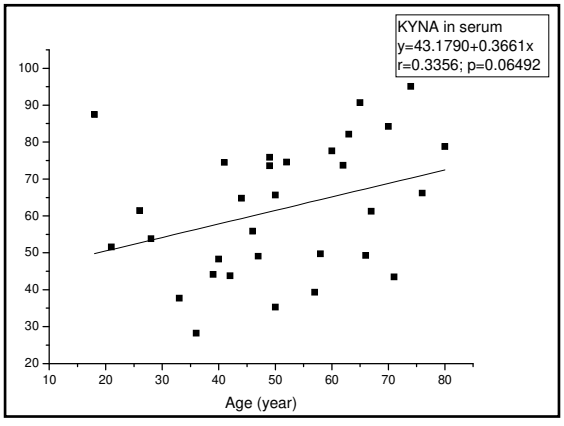

Fig. 4. Correlation between kynurenic acid (KYNA) levels in the serum and the age of control subjects.

KYNA in patients with hydrocephalus

Compared with the corresponding controls, CSF levels of KYNA were significantly increased in patients with NPH and He-v (141%, p = 0.02649 and 225%, p = 4.51705E-4, respectively; Fig. 2). One-way ANOVA between the four groups revealed significant differences (F = 20.50706, p = 7.62319E-8). KYNA levels were also significantly increased in the serum of patients with NPH and He-v (161%, p = 0.0010 and 156%, p = 0.002, respectively; Table 2) compared with the corresponding controls. One-way ANOVA between groups revealed significant differences (F = 12.99745, p = 8.13815E-9). No significant difference was seen in serum KYNA levels between patients with NPH and with He-v (p = 0.17414).

Table 3. L-Kynurenine (L-KYN) and L-tryptophan (L-TRP) levels in the cerebrospinal fluid (CSF) and serum of the corresponding control subjects and patients with normal pressure hydrocephalus (NPH) and hydrocephalus e-vacuo (He-v). Data represent mean ± SEM. Number (N) of subjects is given in parentheses. % of corresponding control subjects is given in parentheses. Significance: *p < 0.05, **p<0.01 vs. control group age ≤ 50 years or ≥ 51 years, respectively, using a Student's t-test

Groups	L-KYN in CSF (pmol/ml)	L-KYN in serum (pmol/ml)	L-TRP in CSF (pmol/ml)	L-TRP in serum (pmol/ml)
Control subjects (15) Age ≤ 50 years	240.1± 33.9	3878.3 ± 411.3	2824.9 ± 378.2	106560.9 ± 10455.3
NPH patients Age ≤ 50 years (N) (% of control)	477.4 ± 245.5 (3) (198)	4490.5 ± 616.1 (4) (116)	2864.3 ± 501.1 (5) (101)	86083.3 ± 7255.5 (4) (81)
Control subjects (15) Age ≥ 51 years	505.0 ± 94.3	4958.6 ± 350.5	4108.7 ± 547.1	108511.3 ± 8243.2
He-v patients Age ≥ 51 years (N) (% of control)	83.1 (1) (16)	2248.7 ± 375.3** (4) (45)	2250.4 ± 659.8 (4) (55)	66705.1 ± 14087.0* (4) (61)

The CSF/serum KYNA ratio was not significantly different between control groups (p = 0.08703). We observed a moderate but nonsignificant reduction in the CSF/serum KYNA ratio in patients with NPH (79%, p = 0.4595), whereas patients with He-v showed a moderate but nonsignificant increase (134%, p = 0.1225) compared with the respective controls (Fig. 7). Although no significant differences were seen between the ratios in both control subject groups (p = 0.08703), significant differences in the CSF/serum KYNA ratio were observed between the NPH and He-v patients (226%, p = 0.02635). ANOVA between the four groups revealed significant differences (F = 4.1203, p = 0.01351).

L-KYN in patients with hydrocephalus

Compared with the corresponding controls, patients with NPH tended to show a significant increase in L-KYN levels in the CSF (198%, p = 0.07146) but not in the serum (116%, p = 0.48825; Table 3). Patients with He-v had significantly lower L-KYN levels in the serum (45%, p = 0.00147), and one He-v patient had a low L-KYN level in the CSF (16%) compared with the corresponding controls. One-way ANOVA between groups revealed a tendency for significant differences for L-KYN in the CSF (F = 2.59865, p = 0.07058) and serum (F = 3.99704, p = 0.05537).

L-TRP in patients with hydrocephalus

L-TRP levels in the CSF and serum were comparable between patients with NPH and the corresponding controls (Table 3). In patients with He-v, the L-TRP level in CSF was not significantly lower (55%, p = 0.11727), whereas it was significantly reduced in serum (61%, p = 0.02966) compared with the corresponding controls (Table 3). One-way ANOVA between groups revealed no significant differences for L-TRP in CSF (F = 2.09279, p = 0.11888) and serum (F = 1.94475, p = 0.14095).

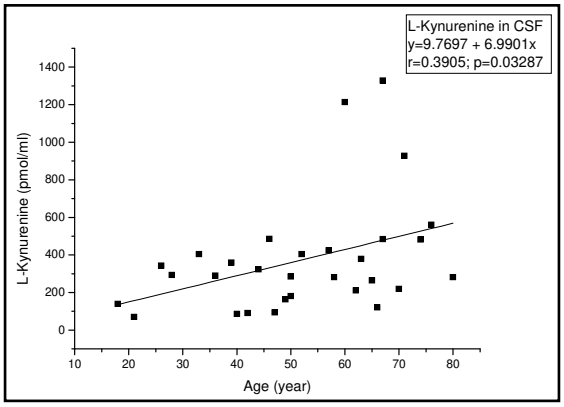

Fig. 5. Correlation between L-kynurenine levels in cerebrospinal fluid (CSF) and the age of control subjects.

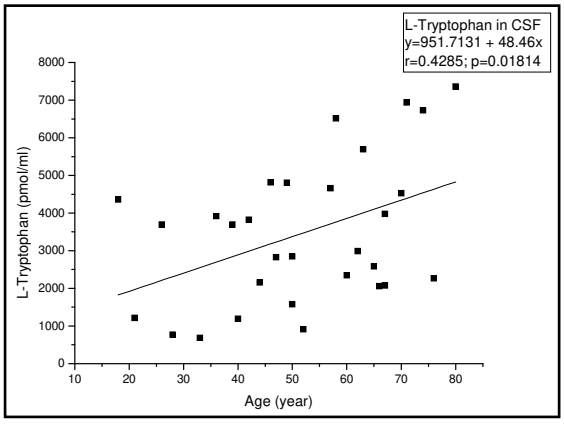

Fig. 6. Correlation between L-tryptophan levels in cerebrospinal fluid (CSF) and the age of control subjects.

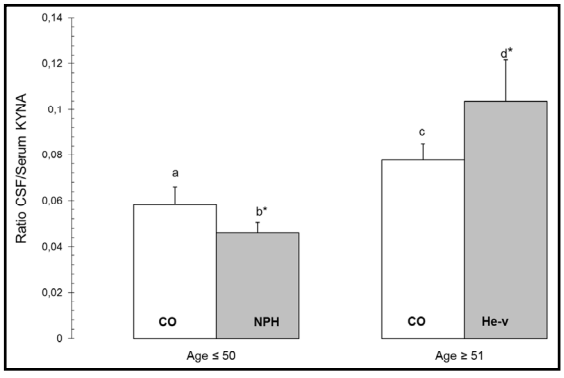

Fig. 7. CSF/serum ratios of kynurenic acid (KYNA) in patients with normal pressure hydrocephalus (NPH) and hydrocephalus e-vacuo (He-v) and in the corresponding control subjects (CO). Data represent mean ± SEM. Number (N) of subjects: CO group (each), N = 15; NPH group, N = 4; He-v group, N = 5. Significance: *p<0.05 vs. the respective control group age ≤ 50 years or ≥ 51 years using a Student's t-test.

Discussion

We recently reported elevated KYNA levels in the CSF of a NPH patient with dementia [10]. Although He-v hydrocephalus is also characterized by dementia, the two forms of hydrocephalus show significant differences in clinical parameters [8-12]. In the CSF of NPH patients, we found significant increases in protein, albumin, IgG and the IgG index, and CSF:serum IgG and CSF:serum albumin ratios, whereas these CSF parameters were mostly in the control range in He-v patients. Our present data are in line with those of a previous study [6].

In addition, this study revealed for the first time increased KYNA levels in the CSF and serum of NPH and He-v patients. The occurrence of dementia in patients with either form of hydrocephalus and the elevated KYNA levels in CSF represent an interesting and important finding because many studies of neurological disorders have found a positive correlation between elevated KYNA content in CNS and the occurrence of dementia and/or cognition impairment, including Alzheimer's disease, HIV-1 infection/AIDS, schizophrenia, and even aging [4-7]. A high risk of mortality has also been described in patients with NPH [29], a phenomenon that was also observed in demented HIV-1-infected patients with significantly high KYNA metabolism [5].

We found an increase in KYNA levels in the CSF of older control subjects and our data confirmed previously published findings on the positive correlation between an increase in KYNA levels in the CSF with advancing age [6]. This correlation was not seen in the serum in either the previous study [6] or the present one, with only a moderate increase in KYNA levels evident in the serum. In addition, we found a significant elevation of L-KYN and L-TRP levels in CSF with advancing age, and these findings correlate with the increased KYNA in CSF found here and previously [6]. It is questionable whether this observed increase of L-TRP and L-KYN levels in CSF with advancing age is linked to a shift of L-TRP in the direction toward greater KYNA formation and reduced serotonin synthesis, which is in part responsible for late-life depression [30]. Importantly, it is not clear which mechanism(s) are responsible for the increase of L-TRP in CSF with advancing age. Interestingly, L-KYN was also significantly higher in the CSF of healthy aged rats [20].

In both forms of hydrocephalus, patients showed increased levels of KYNA in the serum, but its role is not yet known. Interestingly, a lumbar puncture-mediated reduction in not only the ventricular volume, CSF protein levels, and CSF:serum IgG ratio, but also the CSF and serum levels of KYNA significantly improves cognition in patients with NPH [10]. Reversibility of dementia after shunt insertion has also been evaluated in a prospective study of 60 NPH patients, with the authors suggesting that the method may have significant therapeutic value, if performed correctly [31]. The mechanism for KYNA alterations after lumbar puncture is unclear because it is not yet known whether the elevation of KYNA levels in the periphery and CNS is involved in the development of dementia. In a previous paper, we suggested a possible influence of CSF flow on lumbar CSF protein concentration [10]. In addition, the lower CSF:serum IgG ratio after lumbar puncture suggested altered blood–CSF barrier permeability due to high protein levels in the CSF. It is debatable whether the protein levels are responsible for the elevated KYNA synthesis because lowering of protein levels was accompanied by lowering of KYNA levels in the CSF. There are some indications that lumbar puncture applied to He-v patients is not successful as a therapy [authors' observation]. It is likely that an alteration of blood–CSF barrier permeability might be a critical event in patients with NPH and that it might lead to neurochemical alterations in both the CSF and serum, but this hypothesis needs further investigation.

A notable observation is the significantly higher level of KYNA in the CSF of He-v patients. Although only a moderate increase in CSF KYNA was seen in NPH patients, they showed comparable increases in serum KYNA content. The KYNA CSF/serum ratio changes showed a tendency for an increase in He-v patients compared with the corresponding controls, whereas the ratio was moderately reduced in NPH patients. L-KYN levels in the CSF were moderately increased in NPH patients, whereas L-KYN and L-TRP levels in CSF showed a

tendency for a decrease in He-v patients but were significantly reduced in serum. These data suggest significant differences between the two forms of hydrocephalus with respect to L-KYN and L-TRP, but these findings need to be confirmed.

It is reasonable to believe that age plays a role in the differences between the two forms of hydrocephalus because the age of the NPH patients was lower than 50 years, whereas that of He-v patients varied from 51 to 80 years. Because the choroid plexus secretes CSF, it is likely that it plays a major role in the homeostasis of factors in the extracellular fluid [19, 32, 33]. Speculatively, a reduction in glia depressing factor activities in the CNS might play a pivotal role in the increased KYNA formation in the CNS, as we have observed in He-v patients and reported for patients with neuroborreliosis [21]. The choroid plexus, a part of the blood–CSF barrier, controls the entry of nutrients, including amino acids such as L-TRP and nucleosides and peptide hormones, from the periphery into the brain [33, 34] and choroid plexus activities might contribute to pathologies with advancing age [19].

Due to the differences in the ages of the hydrocephalus patients, the data were evaluated by comparison with those of corresponding control subjects, and we dichotomized the controls and the hydrocephalus patients according to age (≤ 50 vs. ≥ 51 years). Such data were useful for analyses and better evaluation of diagnostic conditions. The clinical parameters of control subjects identified in the present study were comparable with those of a previous study [6]. We extended the investigation of clinical parameters in the serum by determining CRP levels and found elevated CRP levels in the serum of older control subjects (≥ 51 years). These data suggest that inflammatory activities increase with aging, which is in line with previous results [22]. We observed a marked increase in CRP levels in He-v patients and a moderate increase in NPH patients compared with the corresponding controls. Tang et al. [22] commented that there is increasing evidence showing that CRP is not only an inflammatory biomarker, but also an important risk factor associated with aging-related diseases, including cardiovascular disease, hypertension, diabetes mellitus, and kidney disease. We believe that it may also be associated with hydrocephalus. In line with the authors' comments, our data indicate that the elevated CRP levels, particularly in He-v patients, are due to increased occurrence of inflammation during the aging process and due to the development of disease.

An article published by Hsu et al. [23] described an association between the baseline serum CRP level and the future development of vascular dementia, but not Alzheimer disease, after adjustment for common cardiovascular risk factors, stroke, and competing risk of death. The authors also pointed out that many studies have investigated the association between markers of peripheral inflammation and risk of dementia but that the results have been conflicting. Elevated CSF KYNA levels and serum CRP levels are markers significantly associated with the aging process; our He-v patients subsequently showed major changes representing additive effects involving the aging process and hydrocephalus. It is clear that evaluation of clinical parameters is an important step to better differentiate these forms of hydrocephalus, but the measurement of KYNA content might help to identify the best choice of therapy to ameliorate the increased levels of KYNA in the CSF and/or serum and thereby combat dementia.

Conclusion

In summary, the levels of KYNA, L-KYN, and L-TRP increase in the CSF during the aging process and the biochemical environment in the CNS might thus partly contribute to the development of dementia. Furthermore, patients with either form of hydrocephalus—NPH or He-v—show increased KYNA levels in the CSF and serum. The impaired cognition and memory seen in patients with either form of hydrocephalus might be related to the increased KYNA metabolism. We suggest that implantation of a permanent CSF shunting system in NPH patients followed by measurement of KYNA, L-KYN, and L-TRP levels in the CSF/serum would be an important step to confirm the action of KYNA. In addition, anti-

dementia drugs such as Cerebrolysin [28] and other drugs able to lower KYNA formation could help to combat dementia in He-v patients. However, this therapeutic approach needs further evaluation.

Abbreviations

ANOVA (analysis of variance); CRP (C-reactive protein); CSF (cerebrospinal fluid); CT (computed tomography); HPLC (high-performance liquid chromatography); IDO (indoleamine-2, 3-dioxygenase); He-v (hydrocephalus e-vacuo); KYNA (kynurenic acid); L-KYN (L-kynurenine); L-TRP (L-tryptophan); NPH (normal pressure hydrocephalus); SEM (standard error of the mean).

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Disclosure Statement

The authors declare no conflict of interests.

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