Zinc concentrations in the cerebrospinal fluid of normal adults and patients with neurological diseases

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SUMMARY Zinc concentrations in CSF were determined with flame atomic absorption spectrophotometry in patients assumed to have a normal CSF. No sex difference was found. There was a correlation between zinc, protein and albumin concentrations in CSF. In patients with increased protein levels in CSF or subarachnoid haemorrhage increased zinc concentrations were found.

Zinc (Zn) is a necessary component of many metalloenzymes,¹ some of which are known to be of importance for the function of CNS for example, RNA and DNA polymerase and carbonic anhydrase. Zinc deficiency in rats may lead to malformations of the CNS.² Zinc deficiency has been suggested as an explanation for the CNS symptoms in liver cirrhosis, foetal alcohol syndrome, malabsorption and acrodermatitis enteropathica.³ Low serum or plasma concentrations of Zn have been found in multiple sclerosis^{4,5} and in chronic alcoholics with liver disease.⁶ High concentrations have been found in multiple sclerosis⁷ and Pick's disease.⁸ There are few reports of Zn concentrations in normal CSF and in CSF from patients with neurological diseases.⁹ Low CSF Zn concentrations have been found in patients with alcohol withdrawal seizures¹⁰ and increased concentrations have been reported in patients on oestrogen therapy.¹¹ Increased CSF Zn levels also were noted in patients with increased CSF protein concentrations.¹² Other authors¹⁵⁻¹⁶ have not found any relation between CSF Zn and CSF protein concentrations.

In a previous study Palm *et al.*⁹ described a method for the determination of CSF Zn by flame atomic absorption spectrophotometry (AAS) utilising a pulse nebuliser technique. Lower CSF Zn levels were found compared with earlier reports. The aim of the present study was to investigate the CSF Zn concentrations in patients assumed to have a normal CSF, and in patients with increased CSF protein concentrations, brain tumours, zinc deficiency, subarachnoid haemorrhage and in patients

on oestrogen and corticosteroid treatment. These drugs are known to induce low serum Zn concentration.

Patients and methods

Patients Controls

The controls were 18 healthy volunteers, nine males, mean age 28 ± 4 (SD) years, and nine females, mean age 30 ± 4 years and 34 patients assumed to have a normal CSF. Of the patients 14 were males, mean age 38 ± 11 years, and 20 females, mean age 42 ± 11 years. They had attended the neurological clinic because of headache, dizziness, vasovagal syncope, neuralgic pain and other pain syndromes or psychoneurosis; a lumbar puncture was performed for diagnostic reasons. The neurological examination did not reveal any objective signs of a lesion in the CNS. They had no signs of acute or chronic infection, liver disease or alcoholism and were not being treated with corticosteroids, ACTH, oral contraceptives or chelating drugs. None of the women was pregnant. The ESR was below 25 mm/h and the Hb was more than 115 g/l in all cases. The CSF was macroscopically clear and uncoloured, had a leucocyte count of less than $5/\mu\text{l}$ and the CSF protein was less than 500 mg/l. These controls were used as age- and sex-matched controls to the other patients.

Patients with increased CSF protein concentrations ($n = 11$; table 3) Six of the patients were males, five were females. All had a CSF protein level over 750 mg/l. In this group there were patients with untreated ($n = 5$) or corticosteroid treated ($n = 2$) Guillain-Barré syndrome, patients with myelopathy of unknown cause ($n = 2$), one patient with cervical disc herniation and a partial spinal block and one patient with increased CSF protein concentration two years after an intracerebral bleed.

Patients with brain tumours ($n = 12$; table 3) Ten patients, five males and five females, suffered from malignant brain tumours. Eight had malignant gliomas and two had cerebral metastasis. Two patients, one of each sex, had meningiomas.

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Patients with zinc deficiency ($n = 5$; table 3) Four patients with chronic alcoholism, three males and one female, and a female with malabsorption after a jejuno-ileal shunt were included in this group. All had a serum Zn level below $10.0 \mu\text{mol/l}$.

Patients treated with oestrogens and corticosteroids ($n = 12$; table 3) Six females were on oral contraceptives (oestrogen + progestogen). Four had had symptoms of transitory ischaemic attacks, one had lumbago and one suffered from paresthesias. Two females were on oestrogens, one of them showed torticollis, while the other had multiple sclerosis. Four patients, three males and one female, were on corticosteroid therapy with prednisolone because of polyneuropathy ($n = 2$) or optic neuritis ($n = 2$).

Patients with subarachnoid haemorrhage ($n = 6$) Five patients were males and one a female. The mean age of the group was 57 ± 11 years. The bleeding source in all cases was a ruptured arterial aneurysm. Four of the patients were seriously ill and required parenteral nutrition. Samples were obtained within three days of the subarachnoid haemorrhage from five patients. The CSF sample from the sixth patient was obtained on the 18th day after the initial subarachnoid haemorrhage when a second bleed occurred.

Methods

The CSF samples were taken after overnight fast at 08.00–09.00 except in the patients with subarachnoid haemorrhage. The lumbar puncture was performed in the lateral recumbent position after 10–30 min rest. The skin was anaesthetized with one ml of Xylocain 10 mg/ml (Astra, Sweden) and the lumbar puncture performed with a sterile disposable hypodermic needle 0.9×90 mm (Mediplast, Sweden). The first two ml were taken for cell count. CSF samples for Zn, protein and albumin analysis were taken from the 10th to the 17th ml in the healthy volunteers and in the patients from the fifth to the 20th ml. CSF was allowed to drop directly into acid washed plastic tubes, which were immediately sealed with Parafilm (American Can Company, USA) and frozen at -20°C until analysed. The CSF samples were not centrifuged or transferred to other tubes. CSF samples with signs of traumatic spinal tap were rejected.

CSF samples from the patients with subarachnoid haemorrhage were taken through a catheter in the lateral ventricle. The samples were aspirated with a zinc free syringe. The first two ml were rejected and the following eight ml transferred to an acid washed glass tube and centrifuged at 5000 rpm for 15 minutes. The supernatant was then transferred to acid washed plastic tubes by zinc free Pasteur pipettes and frozen at -20°C .

Blood samples were taken immediately after the lumbar puncture from an antecubital vein after minimal stasis. The blood was collected in acid washed glass tubes, allowed to clot for two hours, centrifuged at 5000 rpm for 10 minutes and the serum transferred to acid washed plastic tubes with Pasteur pipettes. The tubes were sealed with Parafilm and frozen at -20°C . Serum samples were not obtained from the patients with subarachnoid haemorrhage.

The CSF Zn analysis was performed with flame AAS.¹⁶ Standards in 0.150 mol/l NaCl were used. The samples for serum Zn analysis were diluted eleven times with 0.1 mol/l HCl and the Zn concentrations determined with flame

AAS.¹⁶ The CSF protein concentrations were determined according to Lowry *et al*¹⁷ with tyrosine as the standard. The concentrations of CSF albumin and serum albumin were determined by electroimmuno-assay according to Laurell¹⁸ with human albumin (Kabi, Sweden) as the standard.

Statistics

The differences between group means in the controls for different variables were tested using Student's *t* test. The test was modified if the variances were significantly different ($p < 0.01$; F-test). Product moment correlation coefficients (*r*) were calculated for selected variables and tested using Student's *t* test. In the patients with neurological diseases Wilcoxon matched-pairs signed-ranks test was used and Spearman correlation coefficients determined. $p < 0.05^x$, $p < 0.01^{xx}$, and $p < 0.001^{xxx}$ were chosen as levels of statistical significance.

Results

CONTROLS

The frequency distribution of the CSF Zn concentrations is seen in fig 1. There were no sex differences in CSF Zn, CSF protein, CSF albumin concentrations or in the CSF/serum albumin ratio (table 1). Higher serum Zn levels were found in the males. When CSF Zn was correlated with different parameters, positive correlations were found between CSF Zn and CSF protein, CSF Zn and CSF albumin and CSF Zn and CSF/serum albumin ratio (table 2). The best correlation was found between CSF Zn and CSF albumin (fig 2). No correlation was found between CSF Zn and serum Zn. Serum Zn was negatively correlated to age for males + females ($r = 0.33$, $p = 0.021$).

Fig 1 The frequency distribution for the CSF Zn concentrations in the controls.

Table 1 CSF Zn, CSF protein, CSF albumin and serum Zn concentrations and CSF/serum albumin ratio in controls. The results are given as the mean $\pm$ SD. There is a significant sex difference only for the serum Zn concentrations ($p = 0.024$).

	Mean age years	CSF Zn $\mu\text{mol/l}$	CSF protein mg/l	CSF* albumin mg/l	CSF/serum† albumin ratio $\times 10^3 \mu\text{mol/l}$	Serum Zn‡ $\mu\text{mol/l}$
Males (n = 23)	34 $\pm$ 10	0.15 $\pm$ 0.04	349 $\pm$ 72	182 $\pm$ 43	4.4 $\pm$ 1.2	14.4 $\pm$ 1.7
Females (n = 29)	38 $\pm$ 11	0.17 $\pm$ 0.04	323 $\pm$ 72	161 $\pm$ 56	4.0 $\pm$ 1.2	13.3 $\pm$ 1.6
Males + Females (n = 52)	36 $\pm$ 11	0.16 $\pm$ 0.04	335 $\pm$ 72	170 $\pm$ 52	4.1 $\pm$ 1.2	13.8 $\pm$ 1.7

*n = 47 (19 males, 28 females)

†n = 44 (18 males, 26 females)

‡n = 48 (22 males, 26 females)

Table 2 Correlation coefficients between CSF Zn and CSF protein, CSF albumin, CSF/serum albumin ratio, and serum Zn in the controls.

	CSF protein— CSF Zn	CSF albumin— CSF Zn	CSF/serum albumin— CSF Zn	Serum (s) Zn— CSF Zn
Males (n = 23)	r = 0.41 p = 0.049	r = 0.65 p = 0.003	r = 0.74 p = 0.001	r = 0.20 p = 0.377
Females (n = 29)	r = 0.38 p = 0.038	r = 0.44 p = 0.018	r = 0.23 p = 0.257	r = -0.30 p = 0.137
Males + Females (n = 52)	r = 0.35 p = 0.011	r = 0.47 p = 0.001	r = 0.40 p = 0.007	r = -0.12 p = 0.427

Fig 2 CSF albumin and CSF Zn concentrations in 47 controls.

PATIENTS WITH NEUROLOGICAL DISEASES

Increased CSF Zn levels were noted in patients with Guillain-Barré syndrome as well as in those with other causes of blood-brain-barrier damage (table 3). The two patients with Guillain-Barré syndrome on corticosteroid therapy did not differ from the other such patients in CSF Zn or CSF protein parameters.

Patients with malignant brain tumours had increased CSF Zn concentrations, especially those with increased CSF protein levels (table 3; fig 3). Four had CSF protein concentrations over 0.750 g/l

and their CSF Zn was $0.42 \pm 0.12 \mu\text{mol/l}$. In the remaining six patients the CSF Zn level was 0.24 ± 0.16 . The two patients with meningiomas had CSF Zn levels ($0.14 \pm 0.02 \mu\text{mol/l}$) that did not differ from the controls.

In the zinc deficient patients there was no difference in CSF Zn or protein parameters compared with the controls (table 3). The serum Zn levels were low in the patients.

Neither oral contraceptives, oestrogen therapy nor corticosteroid therapy altered CSF Zn or CSF protein concentrations (table 3).

The patients with subarachnoid haemorrhage had considerably increased CSF Zn levels ($1.68 \pm 1.22 \mu\text{mol/l}$) (fig 3). Their CSF protein concentration was also increased ($2133 \pm 1052 \text{ mg/l}$).

In the patients with increased CSF protein levels there was no correlation between CSF Zn and the protein parameters in CSF.

Discussion

Considerable lower "normal" CSF Zn concentrations have been found with the present analytical method compared to those found in most other laboratories (for reference see ref. 9). Only Kjellin¹⁹ using neutron activation analysis reported CSF Zn concentrations almost in the same range as in the present study. Contamination problems,²⁰ non-atomic absorption,²¹ and different analytical methods may explain the scattered high levels

Table 3 CSF Zn, CSF protein, CSF albumin, serum Zn concentrations and CSF/serum albumin ratio in some neurological diseases. The results are given as the mean $\pm$ SD and are compared with the controls (Wilcoxon matched-pairs signed-ranks test).

Patient group	N	Mean age years	CSF Zn $\mu\text{mol/l}$	CSF protein mg/l	CSF albumin mg/l	CSF/serum albumin ratio $\times 10^3$	Serum Zn $\mu\text{mol/l}$
GBS	7	44 $\pm$ 12	0.35 $\pm$ 0.09 ^x	1739 $\pm$ 859 ^x	1290 $\pm$ 608 ^x	28.9 $\pm$ 10.1 ^x	11.4 $\pm$ 1.6 ^x
Controls	7	44 $\pm$ 14	0.18 $\pm$ 0.04	351 $\pm$ 45	175 $\pm$ 18	4.0 $\pm$ 0.5	13.2 $\pm$ 2.4
Other BBBB	4	62 $\pm$ 20	0.36 $\pm$ 0.09	2081 $\pm$ 279	1417 $\pm$ 339	35.1 $\pm$ 4.2	12.6 $\pm$ 0.8
Controls	4	54 $\pm$ 9	0.16 $\pm$ 0.04	334 $\pm$ 48	170 $\pm$ 32	4.2 $\pm$ 0.6	13.2 $\pm$ 1.7
Malignant brain tumour	10	55 $\pm$ 8	0.31 $\pm$ 0.15 ^x	1030 $\pm$ 989 ^x	709 $\pm$ 848 ^x	16.7 $\pm$ 20.4	12.4 $\pm$ 1.9
Controls	10	52 $\pm$ 9	0.17 $\pm$ 0.04	348 $\pm$ 66	180 $\pm$ 51	4.5 $\pm$ 1.4	12.6 $\pm$ 1.7
Zinc deficiency	5	36 $\pm$ 10	0.18 $\pm$ 0.04	366 $\pm$ 89	201 $\pm$ 52	5.3 $\pm$ 1.5	8.8 $\pm$ 0.8 ^x
Controls	5	36 $\pm$ 11	0.17 $\pm$ 0.05	340 $\pm$ 86	155 $\pm$ 42	4.1 $\pm$ 1.2	14.8 $\pm$ 1.8
Oestrogen treated	8	35 $\pm$ 12	0.16 $\pm$ 0.05	341 $\pm$ 132	152 $\pm$ 44	3.9 $\pm$ 1.3	10.9 $\pm$ 2.2
Controls	8	36 $\pm$ 8	0.17 $\pm$ 0.05	299 $\pm$ 55	147 $\pm$ 41	3.5 $\pm$ 0.8	13.6 $\pm$ 2.1
Cort. ster. treated	4	35 $\pm$ 18	0.13 $\pm$ 0.04	320 $\pm$ 38	179 $\pm$ 51	3.9 $\pm$ 1.2	11.3 $\pm$ 2.2
Controls	4	32 $\pm$ 12	0.15 $\pm$ 0.02	345 $\pm$ 50	180 $\pm$ 26	4.4 $\pm$ 0.7	14.9 $\pm$ 1.7

GBS = Guillain-Barré syndrome, BBBB = Blood brain barrier damage.

Fig 3 CSF Zn and CSF protein concentrations in patients with Guillain-Barré syndrome ($n = 7$), malignant brain tumours ($n = 10$), subarachnoid haemorrhage ($n = 6$), and patients with other causes for blood-brain-barrier damage ($n = 4$). The scale on the y-axis is a log scale.

obtained by others. In the present study the normal CSF Zn levels showed small variations but the sex difference found in the serum Zn levels was not noted in CSF Zn. The CSF protein, CSF albumin and serum Zn concentrations and the CSF/serum albumin ratio in the controls were within the normal range.²²⁻²⁵ The CSF/serum albumin ratio is consi-

dered to be a good test of blood brain barrier function.²² A sex difference was not found in the protein parameters in CSF as has been reported by some authors.²³ Increased protein parameters in CSF with increasing age has been reported.^{22, 26} In the present study no conclusions could be drawn concerning the age dependency of CSF protein parameters or CSF Zn.

The positive correlation between CSF Zn and protein parameters in the normal patients has been reported previously for healthy volunteers,⁹ where the best correlation, however, was found between CSF Zn and CSF protein. It is not known if the Zn in CSF is bound to proteins and amino acids. In serum Zn is bound mainly to albumin (about 80%) and to α_2 -macroglobulin (20%).²⁷ A small part is also bound to amino acids such as histidine and cysteine.²⁸ CSF albumin derives from serum albumin²⁹ and the concentration of α_2 -macroglobulin in CSF is low.³⁰ The serum to CSF ratio for albumin is about 200:1,²² for histidine 6:1³¹ and for Zn 90:1 (present study). The good correlation between CSF Zn and CSF albumin in the controls indicates that most of the Zn in CSF is bound to albumin. The proportionally high CSF levels of amino acids and low level of α_2 -macroglobulin suggests that relatively more Zn is bound to amino acids in CSF than in serum.

The increased CSF Zn concentrations in patients with high CSF protein levels reflects blood brain barrier damage with leakage of Zn-binding proteins from serum to CSF. These increased CSF Zn levels are in accordance with Kjellin,¹² but not with other reports.¹³⁻¹⁵

In the patients with brain tumours high CSF Zn levels were found in those with increased CSF protein concentrations, which probably is a result of a blood brain barrier damage, but also in some patients with normal CSF protein levels. An explanation to the latter finding may be that small bleedings occur in some of the malignant tumours. Erythrocytes have a high content of Zn³² and Zn from haemolysed erythrocytes may reach the CNS to increase the CSF Zn concentrations. In spite of the correlations between CSF Zn and protein parameters in CSF in the controls, no correlation was found in the patients with increased CSF protein concentrations, perhaps because there were few patients.

Bleeding causes the high CSF Zn levels in patients with subarachnoid haemorrhage. The CSF samples in such patients were obtained from the lateral ventricle. This can not explain, however, the great differences in CSF Zn concentrations compared to normal CSF. Ventricular CSF has a lower protein concentration than lumbar CSF³³ and because of the correlation between Zn and proteins in CSF a low CSF Zn concentration should be found normally in ventricular CSF. The high CSF Zn levels found in the patients with subarachnoid haemorrhage are in contrast to the reports by Bogden *et al*¹⁴ who found normal CSF Zn levels in nine patients with subarachnoid haemorrhage.

In the healthy volunteers⁹ a positive correlation was found between CSF Zn and serum Zn concentrations in the males. In the normal patients, in zinc deficient patients or in those with drug induced low serum Zn concentrations this correlation was not found. One reason to the divergence may be that the CSF sampling was more standardised in the volunteers. In oral contraceptives, the oestrogen component reduces the serum Zn levels.³⁴ Females on oral contraceptives and oestrogens consequently were grouped together. Increased CSF Zn concentrations during oestrogen therapy as reported by Bogden and Troiano¹⁰ could not be verified in the present study. Corticosteroids also decrease the serum Zn concentrations.³⁵ In the present study there were few patients on corticosteroid therapy and the serum concentrations in this group did not differ significantly from that in the control group. The lack of correlation between CSF Zn and serum Zn levels was also reported earlier.^{13 14}

In conclusion, CSF Zn concentrations normally are correlated with the CSF protein and CSF albumin concentrations, as well as to the CSF/serum albumin ratio, but not to the serum Zn levels. In patients with the blood brain barrier damage and subarachnoid haemorrhage the CSF Zn concentrations increase. Increased concentrations were also found in some patients with malignant brain

tumours with normal CSF protein levels. The importance of CSF Zn determinations in other neurological diseases should be evaluated.

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