

# Fatal cerebral edema associated with serine deficiency in CSF

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**Abstract** Two young girls without a notable medical history except for asthma presented with an acute toxic encephalopathy with very low serine concentrations both in plasma and cerebrospinal fluid (CSF) comparable to patients with 3-phosphoglycerate dehydrogenase (3-PGDH) deficiency. Clinical symptoms and enzyme measurement (in one patient) excluded 3-PGDH deficiency. Deficiencies in other serine biosynthesis enzymes were highly unlikely on clinical grounds. On basis of the fasting state, ketone bodies and lactate in plasma, urine and CSF, we speculate

that reduced serine levels were due to its use as gluconeogenic substrate, conversion to pyruvate by brain serine racemase or decreased L-serine production because of a lack of glucose. These are the first strikingly similar cases of patients with a clear secondary serine deficiency associated with a toxic encephalopathy.

## Introduction

L-Serine, a nonessential amino acid, plays an important role in cellular proliferation (Snell 1984). In addition, L-serine is the precursor of the neurotransmitters D-serine and glycine and is involved in one-carbon metabolism. L-Serine can be derived from different sources, such as dietary intake, degradation of proteins and phospholipids, and glucose via the glycolytic intermediates 3-phosphoglycerate and 3-phosphohydroxypyruvate (de Koning et al. 2003).

Three enzymes are involved in serine biosynthesis: 3-phosphoglycerate dehydrogenase (3-PGDH), 3-phosphohydroxypyruvate aminotransferase, and phosphoserine phosphatase. Genetic 3-PGDH deficiency (Jaeken et al. 1996; de Koning et al. 2000) is associated with congenital microcephaly, severe psychomotor retardation, and intractable seizures (de Koning et al. 2004). The biochemical hallmark of this disorder is a significantly reduced concentration of L-serine and, to a variable degree, glycine, in cerebrospinal fluid (CSF) and plasma (de Koning et al. 2000). Deficiencies of the other two enzymes involved in the L-serine biosynthesis show clinical phenotypes (severe neurological symptoms, severe psychomotor retardation, and seizures) similar to that found in 3-PGDH deficiency (Hart et al. 2007; Jaeken et al. 1997; Veiga-da-Cunha et al. 2004). Decreased serine in CSF has also been described in cases in which the biochemical defect

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**Table 1** Summary of cerebral spinal fluid (CSF) and plasma amino acids in the two patients dying from brain-stem compression after acute cerebral edema

Amino acid		Measurement			Reference values		
		CSF ( $\mu\text{mol/l}$ )	Plasma	Ratio	CSF ( $\mu\text{mol/l}$ )	Plasma	Ratio
Serine	Patient 1	8	31	0.258	19–38	95–166	0.276 $\pm$ 0.053
	Patient 2	7	33	0.242			
Serine (3-PGDH def) <sup>a</sup>		<b>6–8</b>	<b>28–64</b>				
Glycine	Patient 1	5	107	0.047	0.7–15	139–317	<0.0400
	Patient 2	6	185	0.032			
Glycine (3-PGDH def) <sup>a</sup>		<b>1–4</b>	<b>128–190</b>				
Threonine	Patient 1	17	61	0.279	22–47	49–183	0.291 $\pm$ 0.053
	Patient 2	11	29	0.379			
Alanine	Patient 1	14	72	0.194	8–42	153–560	0.112 $\pm$ 0.028
	Patient 2	37	181	0.204			

CSF cerebral spinal fluid, 3-PGDH 3-phosphohydroxypyruvate aminotransferase

<sup>a</sup> de Koning et al. 2004

remained unsolved. One of these patients showed growth retardation, progressive polyneuropathy, and ichthyosis; supplementation with L-serine produced clinical improvement (Catsman-Berrevoets et al. 1997).

Here we describe two patients with severe encephalopathy associated with a viral infection and serine deficiency in plasma and CSF. In the medical histories, asthma is the only notable clinical condition. Possible mechanisms for the extremely low serine concentrations in CSF are discussed.

### Patient 1

A 7-year old, normally developing girl was diagnosed with moderately severe asthma for which she used inhalation therapy with combination of salmeterol/fluticasone. The day before her death, she presented with a mild febrile condition, including malaise, headache, and nausea. Medical examination showed no abnormality, and domperidone was prescribed. The morning before her death, her mother found her unresponsive in bed, probably following a seizure. At that time, blood glucose, as measured by a bed-side apparatus, was 2.1 mmol/l. In the emergency room, the patient had convulsions, which responded to diazepam. Physical examination showed expiratory wheezing, normal blood pressure and capillary refill time, a relatively low heart rate (100/min), no hepatosplenomegaly, normal pupils, and no signs of trauma or bleeding. Hypoglycemia or electrolyte disturbances were excluded. Liver enzymes and bilirubin were slightly elevated, and blood ammonia was normal (15  $\mu\text{mol/l}$ ). She regained consciousness for 1.5 h but became progressively dyspneic and presented suddenly with apnea and desaturation, decortication rigidity, bilateral fixed mydriatic pupils, and

deep coma. She was intubated and treated with dexamethasone and mannitol. A brain computed tomography (CT) scan showed bilateral central herniation and cerebral edema. There were indisputable signs of brain death, and she died 24 h after the first symptoms.

Amino acid analysis of both plasma and CSF revealed strongly decreased serine concentrations (Tables 1, 2, 3)

**Table 2** Plasma concentrations of all amino acids ( $\mu\text{mol/l}$ ) in the two patients

Amino acid	Patient 1	Patient 2	Reference values
Taurine	21	72	9–130
Threonine	61	29	49–183
<b>Serine</b>	<b>31</b>	<b>33</b>	<b>95–166</b>
Asparagine	18	6	18–69
Glutamic acid	23	28	1–105
Glutamine	301	262	317–757
Proline	70	149	26–412
Glycine	107	185	139–317
Alanine	72	181	153–560
Citrulline	15	4	10–57
Valine	158	73	120–323
Cystine	6	18	0–42
Methionine	9	7	13–38
Isoleucine	59	13	24–100
Leucine	120	48	49–179
Tyrosine	36	43	30–109
Phenylalanine	70	46	28–92
Ornithine	34	12	30–96
Lysine	96	47	81–217
Histidine	89	47	55–104
Arginine	38	31	29–124

**Table 3** CSF concentrations of all amino acids ( $\mu\text{mol/l}$ ) in the two patients

Amino acid	Patient 1	Patient 2	Reference values
Taurine	10	4	2–12
Threonine	1	11	22–47
<b>Serine</b>	<b>8</b>	<b>7</b>	<b>19–38</b>
Asparagine	4	6	2–17
Glutamic acid		1	0–16
Glutamine	380	357	234–689
Proline	1	0	0–2
Glycine	5	6	1–15
Alanine	14	37	8–42
Citrulline	3	1	0–3
Valine	15	17	8–24
Cystine	1	4	0–2
Methionine	3	5	0–7
Isoleucine	5	0	2–11
Leucine	18	9	6–21
Tyrosine	2	6	0–25
Phenylalanine	28	24	6–19
Ornithine	43	4	1–9
Lysine	31	13	8–35
Histidine	32	21	4–23
Arginine	20	5	4–35

comparable with levels observed in patients with 3-PGDH deficiency (de Koning et al. 2004). Enantiomer separation revealed D-serine to be  $0.9 \mu\text{mol/L}$  (ref.  $0.8\text{--}4.3 \mu\text{mol/L}$ ) and L-serine  $4.0 \mu\text{mol/L}$  (ref.  $17.2\text{--}44.0 \mu\text{mol/L}$ ), with slightly elevated D-serine/total serine ratio of 18% in CSF (Fuchs et al. 2006). Concentrations of the other amino acids were normal or decreased according to a nonspecific pattern (data not shown). A deficiency of 3-phosphoglycerate dehydrogenase was excluded in cultured fibroblasts ( $43 \text{ nmol/mg per min}$ ; normal  $29.5 \pm 2.7 \text{ nmol/mg per min}$ ). Further metabolic screening revealed increased ketone bodies and lactate in urine, plasma, and CSF (Table 4). Plasma acylcarnitine profile showed an elevated concentration of OH-C4-carnitine. A fatty acid oxidation defect was excluded. Brain microscopy showed bilateral necrosis in the cerebral tonsils compatible with central

herniation. No signs of encephalitis or meningitis were found, but diffuse interstitial edema was obviously present. Postmortem microbiology sampling yielded a parainfluenza type II virus in the lungs, probably explaining fever onset and asthmatic signs as her presenting condition. The clinical picture probably reflects a toxic encephalopathy.

### Patient 2

A 4-year-old girl with normal mental development suffered from asthma for which she used Flixotide (a steroid, 2 dd  $250 \mu\text{g}$ ) and salmeterol (2 dd  $25 \mu\text{g}$ ). She had normal height but severe malnutrition [weight < 2 standard deviations (SD)] due to feeding difficulties related to behavioral problems. Because of sleep disturbances, the patient had been on treatment with Alimemazine (an antihistaminic sedative agent) for 4 months. Two days before her death (in the evening, day 1), she presented with fever ( $39\text{--}40^\circ\text{C}$ ) without other particularities. The next morning (day 2), she was found in bed with tonic clonic seizures. The seizures were resistant to multiple anticonvulsive drugs. There were no signs of respiratory or circulatory failure. On admission, serum glucose was  $4.0 \text{ mmol/L}$ , with CSF glucose  $1.0 \text{ mmol/L}$ ; serum electrolytes were normal, as was blood ammonia ( $15 \mu\text{mol/l}$ ). There were no signs of a bacterial central nervous system (CNS) infection in CSF, no pleocytosis, and bacterial and viral cultures remained negative; later nasal cultures, however, showed an adenovirus V. Blood C-reactive protein (CRP) was  $>300 \text{ mg/ml}$ .

Physical examination showed a very ill girl with coma scores E1, M4, V1. Intubation and cardiac support were effective for some hours, but a rapid deterioration was seen with an isoelectric electroencephalogram (EEG) pattern and absent stem reflexes. After a repeated isoelectric EEG, the next day, supportive treatment was discontinued. No postmortem examination was performed. Metabolic investigations in plasma and CSF taken at day 2 showed particularly low plasma and CSF serine concentrations. Concentrations of the other amino acids were normal or decreased according to a nonspecific pattern (data not

**Table 4** Plasma, urine and CSF concentrations of lactate and ketone bodies in the two patients with an acute encephalopathy

Patient	Urine lactate ( $\mu\text{mol}/\text{mmol creat}$ )	Urine 3-OHB ( $\mu\text{mol}/\text{mmol creat}$ )	CSF lactate ( $\mu\text{mol/l}$ )	CSF 3-OHB ( $\mu\text{mol/l}$ )	Plasma 3-OHB ( $\mu\text{mol/l}$ )
Patient 1	374 (<56)	22410 (<5)	2308 (<1700)	1731 (<280)	n.d.
Patient 2 <sup>a</sup>	130 (<56)	317 (<5)	7200 (<1700)	n.d.	2200 (<5)

nd, not determined

Urine and plasma from patient 1 were collected the day before death.

shown). Unfortunately, no fibroblasts were available for analysis of 3-PGDH activity. Plasma lactate and 3-hydroxybutyrate were clearly increased, as was CSF lactate (Table 4). Urine organic acid analysis showed a moderate ketonuria as well as a marginally elevated lactate excretion.

## Discussion

Here we present two young female patients with extremely low serine concentrations in CSF and plasma comparable with 3-PGDH-deficient patients without clinical features suggestive for a serine biosynthesis defect. Both patients showed a rapidly progressive toxic encephalopathy and brain edema causing death within 24 h after the first symptoms. Acute toxic encephalopathy has been described in young children (2–3 years of age) without a notable medical history (de Jong-de Vos van Steenwijk et al. 1981), with cerebral edema and fatal brain-stem compression as the major cause of death. Possible triggers include infections by several bacteria or viruses. Our patients suffered from parainfluenza virus type II (patient 1) and a nasal adenovirus 5 (patient 2), respectively. Both viruses have been associated with acute encephalopathy (Hata et al. 2007; Straussberg et al. 2001). The pathogenesis of acute toxic encephalopathy has not been elucidated. Toxins leading to either endothelial cell damage with disruption of the blood–brain barrier (Ashkenazi et al. 1990) or directly affecting cells leading to cytotoxic edema (Kimelberg 1995) might play a role. A relationship between acute encephalopathy and low CSF serine concentration has never been reported.

Both our patients had an overnight (fasting) period before serious neurological symptoms occurred. Low plasma concentrations of (essential) amino acids and increased ketone bodies in urine and plasma reflected this fasting state. In addition, plasma alanine concentrations of 72  $\mu\text{M}$  and 181  $\mu\text{M}$  made a gluconeogenesis defect unlikely. In cases of fasting in combination with fever/illness, a cerebral hypoglycemic state could trigger formation of ketone bodies as an alternative source of energy for the brain. In patient 1, we confirmed an increased concentration of 3-OH-butyrate in CSF. In patient 2, the low CSF glucose concentration relative to plasma concentration might suggest a glucose transporter 1 (GLUT-1) defect (MIM# 606777) despite the absence of clinical symptoms of this genetic defect (Wang et al. 2005); CSF glucose concentration, however, normalized upon glucose bolus.

In addition to ketone bodies, L-serine is a potential energy source under certain conditions in cerebral tissue. L-serine does not easily cross the blood–brain barrier, and glial cells contain enzymes of the complete serine synthesis and

degradation pathways (Furuya and Watanabe 2003; Mitoma et al. 1998). Serine racemase, present in different regions of the brain (de Miranda et al. 2000), can synthesize D-serine, as well as pyruvate, from L-serine (de Miranda et al. 2002; Wolosker et al. 2002). The formed pyruvate may act as a local, emergency energy substrate (Wu et al. 2004). In both patients with a clear cerebral emergency situation, L-serine might have been used as an alternative energy source. The relatively high percentage of D-serine (18% of total serine, ref. 3–16%) (Fuchs et al. 2006) supports this possibility.

In order to study the role of L-serine in energy metabolism in the hypoglycemic brain, we analyzed CSF of four patients diagnosed with a GLUT-1 deficiency. No changes in amino acids in CSF from these patients were observed (results not published). From this observation, we conclude that a chronic low CSF glucose concentration, as present in GLUT-1-deficient patients, does not explain the low CSF serine concentration. An alternative explanation for the low CSF serine concentration is a decreased production of serine from glucose due to an acute hypoglycemic state. The low concentration of plasma L-serine, as observed in our patients, is in line with this idea. In general, fasting patients show mildly decreased plasma and CSF serine concentrations (M. Duran, Amsterdam, personal communication), which are, however, not as low as those observed in our patients. These observations indicate that it is probably the extreme local energy deficit that might contribute to a low cerebral serine concentration. In addition to hypoglycemia, an increased nicotinamide adenine dinucleotide, reduced nicotinamide adenine dinucleotide (NADH/NAD<sup>+</sup>) ratio as seen in anoxia might be considered as a cause of inhibition of serine synthesis. However, we observed increased concentrations of CSF L-serine (threefold) and glycine (tenfold) in patients with asphyxia compared with controls (data not published).

In summary, we observed low serine concentrations in two young girls who died from cerebral edema with brainstem compression. The low serine concentrations were not caused by an inherited disorder of metabolism but probably reflected a pathophysiological state of the brain in which L-serine was either used to produce pyruvate via serine racemase, or L-serine production failed due to a lack of glucose.

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**Competing interest** None declared

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