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CONGENITAL HYPERAMMONEMIA: SYMPTOMATIC CARRIER GIRL PATIENT AND HER ASYMPTOMATIC HETEROZYGOUS MOTHER FOR ORNITHINE TRANSCARBAMYLASE (OTC) DEFICIENCY: SPECIFIC ENZYME DIAGNOSTIC AND KINETIC INVESTIGATIONS FOR THE DETECTION OF HETEROZYGOUS GENOSTATUS

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Activities of the specific enzymes of the inherited hyperammonemic syndromes (carbamoyl-phosphate synthetase CPS), ornithine transcarbamylase (OTC), arginine-succinate-synthetase (ASS), arginine-succinate-lyase (ASL) and arginase (ASE) were measured in a liver biopsy specimen of a 2 years-old girl suffering from chronic hyperammonemia and in the erythrocyte- and leukocyte-homogenisate of her parents. The activity of OTC in liver homogenisate of the patient was 62.9 percent; in the leukocytes of the parents it was 78.5 percent (in mother) and 102 per cent (in the father) as compared to the controls.

Our patient proved to be a symptomatic carrier of OTC deficiency and her mother proved to be an asymptomatic carrier.

INTRODUCTION

Ornithine transcarbamylase (OTC) deficiency is an X-linked recessive disorder, usually with lethal hyperammonemia in OTC homozygous males. OTC, one of the five enzymes required for ureagenesis, catalyzes the synthesis of citrulline from carbamyl phosphate and ornithine. Female heterozygotes, however, have variable phenotypic expression depending on the inactivation of the X-chromosomes containing the normal and mutant gene. Some females have hyperammonemic episodes that result in mental retardation, but others may never show the manifestation of this disease, however mild form /1,4,5/.

Batshaw /2/ detected cerebral dysfunction in asymptomatic carriers of OTC deficiency.

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Carrier detection in OTC deficiency: Palmer /12/ has cited studies in which protein or ammonium chloride loads were used as a test for heterozygosity in kindreds with OTC deficiency. Hyperammonemia was not noted after the protein load in many of these studies. Hokanson /9/ detected hyperammonemia in only three of four obligate OTC heterozygotes after a protein challenge. The orotate excretion in the heterozygote group was at least by three standard deviations greater than in the control group. Batshaw /2/ investigations confirmed that urinary excretion of orotic acid is a more sensitive indicator of the OTC heterozygotes than is hyperammonemia.

We report here on specific enzyme investigations of urea cycle and K_m values for OTC of a symptomatic carrier girl patient and her asymptomatic carrier for OTC deficiency.

CASE REPORT

A. T. a 20 month-old girl patient was admitted to our clinic with detected hyperammonemia (311 gamma %), hepatomegaly, elevated liver enzyme activities (SGOT 49 U/l, SGPT 134 U/l). the EEG showed encephalopathic signs.

The perinatal anamnesis was uneventful, familial anamnesis: her twin-brother is healthy, as her 5 years-old sister, too. Birth weight was 3200 g. Her motoric and mental development have become slow from the age of 13 month, she was found to be clumsy, she was noted to fall down frequently, generalized muscle hypotonia and ataxia developed.

Neurological investigation revealed right side spastical hemiparesis, progressive neurological deterioration was established.

Subdural hematoma was suspected according to the cerebral scintigraphy, but carotis angiography was negative.

Since her early infancy she has vomited easily and had intestinal symptoms like diarrhoeic episodes.

Alfa-1-antitrypsin deficiency, morbus Wilson, galactosemia, tyrosinemia, lysinuric protein intolerancy and glycogenosis had been excluded.

She proved to be OTC heterozygote according to our specific enzyme analysis.

After the introduction of the low protein diet (1.5-1.0 g/kg/day), and of the sodium- benzoate (250 mg/kg/day), folic acid, vitamine B_6 therapy the plasma ammonia level decreased to 45 gamma %.

Urinary amino acid chromatography, purine and pyrimidine metabolites and orotic acid were normal. During the protein

TABLE I

Activities of the specific enzymes of the urea cycle of T. family and controls

Liver tissue of patient T.A. and controls
Enzymes in the case of CPS and OTC in citrulline μmol, in ornithine in the cases of ASS, ASL and ASE μmol/h/g liver

	Normal value	T.A. patient	%
1. Carbamoyl-phosphate synthetase (CPS)	264 ± 54	284	107.6
2. Ornithine carbamoyl-transferase (OTC)	6178 ± 1234	3884	62.9
3. Arginine-succinate-synthetase (ASS)	87 ± 18	98	112.6
4. Arginine-succinate-lyase (ASL)	216 ± 32	234	108.3
5. Arginase (ASE)	83000 ± 13000	81200	97.8

Enzyme activities of the homogenisate of the erythrocytes (μmol ornithine/h/gHb)

	Normal value	T.father	%	T.mother	%
3. ASS	1.9 ± 0.7	1.8	94.7	1.6	84.2
4. ASL	12.8 ± 5.3	11.6	90.6	10.8	84.4
5. ASE	1250 ± 445	1106.0	88.5	1176.0	94.0

Enzyme activities of the homogenisate of the leukocytes (1., 2. μmol citrulline 2., 4. and 5. μmol ornithine (h/mg prot.)

	Normal value	T.father	%	T.mother	%
1. CPS	7.7 ± 1.6	6.9	89.6	5.4	70.1
2. OTC	92.4 ± 21.1	94.3	102.0	72.5	78.5
3. ASS	0.48 ± 0.12	0.52	108.3	0.55	114.6
4. ASL	0.42 ± 0.14	0.48	114.3	0.50	119.0
5. ASE	26.85 ± 7.20	25.22	93.2	26.26	97.8

loading the orotic acid urinary excretion has elevated.

She has developed psychosomatically well on MILUPA UCD 2 diet and arginine supplementation. She unexpectedly died of hyperammonemic coma (blood ammonia: 720 µmol/l) against of the introduced peritoneal dialysis at the age of 6 years in April 1990.

METHODS

The activities of specific enzymes of the inherited hyperammonemic syndromes (carbamoyl-phosphate synthetase = CPS, OTC, arginine-succinate-synthetase - ASS, arginine - succinate - lyase = ASL and arginase = ASE were measured in the liver biopsy specimen and in the erythrocyte and leukocyte homogenate of her parents /11/.

The value of the enzyme activities of the urea cycle are given in Table I, from the T. Family K_m /ornithine/ and K_m (carbamyl phosphate) of OTC are summarized in Table II.

TABLE II

K_m values of OTC enzyme

From liver biopsy specimen	Normal values fetal	adult	T.A.patient %
K_m (ornithine /mmol/l	3.5	0.42	4.32
K_m (carbamyl phosphate) (mmol/l)	0.2	0.21	1.28

From leukocytes	Normal value		T.father %	T.mother	%
K_m (ornithine µmol/l	3.88	4.12	106.2	10.25	264.2
K_m carbamyl phosphate (mmol/l)	0.48	0.44	91.7	0.52	108.3

RESULTS

The activities of the above-mentioned enzymes in liver biopsy of the hyperammonemic girl patient was diminished (62.9 %) and the activity of OTC in the homogenate of the mother's leukocyte was 78.5 %. The activity of CPS was diminished too, 70.1 %.

The activities of the urea cycle enzymes proved to be normal in the case of the father.

K_m /ornithine/ and K_m (carbamyl phosphate) of the patient's OTC enhanced similarly as her mother's K_m (ornithine) was increased, too (Table II).

DISCUSSION

Goldstein /6/ demonstrated an excessive urinary excretion of orotic acid after an oral protein load in the obligate heterozygotes for OTC deficiency. Others have confirmed the observation that this non-invasive test identifies heterozygotes more reliably than the measurement of the blood ammonia after a protein or ammonia load /9/. Becroft /3/ discussed the failure of protein loading test to identify heterozygosity for OTC deficiency and of the expected increase of orotic acid excretion and of pyrimidine and purine metabolites in the urine.

Hauser /7/ concluded that measurement of urinary orotidine excretion after the administration of allopurinol is a simple and reliable test for the identification of heterozygous women for ornithine carbamoyltransferase deficiency. This test relies on the allopurinol-induced accumulation of orotidine, whose synthesis is stimulated by carbamoyl phosphate, a substrate that accumulates in ornithine carbamoyltransferase deficiency. The mean plasma glutamine and ammonium levels were significantly higher in the carriers for OTC deficiency than in the controls, while the mean plasma arginine and citrulline levels were significantly lower in carriers /8/.

The identification of the different genostatus was possible due to the determination of the specific enzyme activities of the ureacycle from the homogenate of the peripheral leukocytes.

Kinetic abnormalities from patients with OTC deficiency have been published by Gray /8/. There is a great heterogeneity of mutant enzyme structure or expression as indicated by wide variation in K_m (ornithine) and K_m (carbamyl-phosphate) values. In the case of Qureshi /13/ - a 7 years-old girl, suffering from chronic hyperammonemia and orotic aciduria - the K_m (carbamyl-phosphate) of the mutant enzyme was lower as normal, while the K_m (ornithine) was normal. The activity of OTC was only 17 % of that of a control, pH optimum was 8.1 in the patient and the control.

We have found diminished affinity of the OTC enzyme for both of different substrates from the liver tissue in the case of our girl patient with OTC heterozygosity. The OTC K_m values for the ornithine and carbamyl phosphate in the leukocytes of the father were normal, while in the case of the mother - as an asymptomatic heterozygous genotype - the K_m (ornithine) value proved to be highly elevated, more than twice of the normal value.

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