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CITRULLINEMIA*

Clinical Experience with 23 Cases

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A retrospective study was performed on the clinical outcome of 23 patients with citrullinemia diagnosed during the last 20 years in our clinic. The study group consisted of 13 patients with the neonatal form of the disease, four patients with the subacute form, five patients with the late-onset form and one with the asymptomatic form. All patients were treated with natural protein restriction, sodium benzoate and arginine administration. Almost all of the neonatal-onset patients were treated with exchange transfusions and/or peritoneal dialysis. Fourteen patients died: 11 with the neonatal form, one with the subacute form, and two with the late-onset form. The general neurological outcome of the patients who were alive was not satisfactory. Despite these results, it was concluded that the prognosis and quality of life of patients with citrullinemia might be improved with early diagnosis and appropriate therapy.
Key words: citrullinemia, neonatal, subacute, late-onset, asymptomatic.

Ammonia, coming from protein and amino acid metabolism and from decomposition of urea and other nitrogenous compounds by microorganisms in the intestinal tract, is eliminated from the body through the urea cycle. The major route of ammonia detoxification consists of five steps, each catalyzed by a different enzyme: carbamoyl phosphate synthetase (CPS), ornithine transcarbamylase (OTC), argininosuccinic acid synthetase (AS), argininosuccinic acid lyase (AL) and arginase. A sixth enzyme, N-acetylglutamate synthetase, is also required for synthesis of N-acetylglutamate, which is an activator of the CPS enzyme. Inborn errors are known to occur at each step in the urea cycle[1]. AS catalyzes the production of argininosuccinic acid from citrulline and aspartate. AS deficiency leads to hyperammonemia and the accumulation of citrulline in the blood and spinal fluid[2].

The spectrum of clinical presentation in citrullinemia is so wide that some of the cases may run a rapidly downhill course in the neonatal period while others may show normal or near normal development[3, 4].

In this paper, we describe 23 patients with citrullinemia diagnosed at our clinic so far to draw attention to the variable expression of the disorder.

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Material and Methods

Twenty-three patients who had been diagnosed as having ASS deficiency (citrullinemia) at Hacettepe University Children's Hospital Metabloic Unit over the past 20 years were included in this study. Clinical and laboratory data of patients were obtained from hospital records. Plasma ammonia concentration was determined by colorimetric method[5]. One- and two-dimensional paper chromatography with Ehrlich's stain was used when necessary for serum and urinary amino acid patterns in some patients[6], whereas plasma and urine amino acids were measured by ionexchange chromatography in the rest (Biotronic and LKB amino acid analyzers).

Results

Of the cases with citrullinemia, 13 were male (56.5%) and 10 female (43.5%). Most of the cases (14/23, 60.6%) were in the newborn period, four patients (17.4%) were between one month and two years of age, and five cases (21.8%) were above two years when the diagnosis was made. Some details of patients are shown in Table I and the patients who illustrate the typical manifestation of the different types of citrullinemia are reported.

Table I: Some Clinical Characteristics of Cases with Citrullinemia

					Laboratory Findings			
Case	Age Sex	Consan-guinity	Family History of Sibling Deaths	Outcome	NH_3 (µg/dl)	BUN (mg/dl)	Serum Amino Acid (µmol/L)	Urine Orotic Acid (mmol/mol Cr)
1-Baby Y	3 Days Male	+	+	died at 4th day of life	425	7	citrulline↑↑* glutamine↑	N.D.
2-SB	1 day female	+	+	diet at 1.5 months of age	268	12	citrulline↑ glutamine↑	N.D.
3-BK†	1 day male	+	+	died at 2.5 years	318	9	citrulline↑ glutamine↑	N.D.
4-Baby Ö	3 days female	+	+	diet at 5th day of life	2000	7	citrulline↑↑↑ glutamine↑↑	N.D.
5-EA	2 days male	–	+	died at 7th day of life	600	8	citrulline (15864) glutamine (11729)	↑↑
6-DK	5 days female	+	+	died at 6th day of life	1077	14	citrulline (2333) glutamine (2315)	500↑**
7-Baby Ç	1 day female	+	+	died at 5th day of life	1153	12	citrulline (3544) glutamine (534)	↑↑
8-SÖ	2 days male	+	+	diet at 4th day of life	1913	6	citrulline (1262) glutamine (2284)	53.7
9-TK†	22 days female	+	+	alive MMR‡	180	5	citrulline↑ glutamine↑	N.D.
10-AD	1 day male	–	+	lost to follow-up at 5.5 y	345	8	citrulline↑↑ glutamine↑	N.D.

Table I (Continued)

Case	Age Sex	Consan-Guinity	Family History of Sibling Deaths	Outcome	Laboratory Findings			
					NH_3 (µg/dl)	BUN (mg/dl)	Serum Amino Acid (µmol/L)	Urine Orotic Acid (mmol/mol Cr)
11-Baby U	3 days male	+	+	died at 4th day of life	1020	5	citrulline (3580) glutamine (1570)	500↑
12-EÖ	5 days female	+	+	died at 8th day of life	340	11	citrulline↑↑ glutamine↑	↑
13-Baby Y	1 day female	+	+	died at 5th day of life	1141	9	citrulline↑↑↑ glutamine↑↑	500↑
14-MA	6 mos female	+	+	alive MMR	268	3	citrulline (4916) glutamine (1003)	↑↑
15-YB	22 mos male	+	+	died at 22 months of age	254	8	citrulline↑ glutamine↑	↑↑
16-HY	7 mos male	+	–	alive MMR	275	12	citrulline (2304) glutamine (534)	↑↑
17-CE	2 years male	+	–	lost to follow-up	187	9	citrulline↑↑ glutamine↑↑	N.D.
18-İA	9 years male	+	+	died during the acute attack at 9 years of age	278	10	citrulline↑↑ glutamine↑	N.D.
19-PS	7 years female	+	–	alive	180	7	citrulline (6100) glutamine (1050)	500↑
20-RD	6 years male	–	–	lost to follow-up	473	12	citrulline (10359) glutamine (880)	500↑
21-KT	9 years male	–	–	lost to folow-up	366	9	citrulline↑ glutamine↑	N.D.
22-İZ	15 years male	–	–	died during the first attack	950	24	citrulline (2283) glutamine (1912)	500↑
23-AK	15 days male	+	–	alive 16 year old	65	18	citrulline↑ glutamine↑	N.D.

† siblings.
* by paper chromatography with Ehrlich reagent.
** measurement is out of range.
ND: not done.
‡ MMR: mental-motor retardation.

The Neonatal-Onset Citrullinemia

Thirteen patients with the neonatal form of citrullinemia were admitted to the hospital between the first and twenty-second days of life. Each of them was the healthy product of a term pregnancy with normal anthropometric measurements. All had a positive family history retrospectively; only five of them were symptom free when admitted for further evaluation because of previous sibling deaths. Vomiting, poor feeding, grunting, altered consciousness, convulsion and irregular respiration were common presenting symptoms in this group. Within two to three days, the babies who were symptom free but identified as being at risk on the

basis of sibling deaths became increasingly lethargic and had difficulty in feeding. They were put on a protein-restricted diet along with oral sodium benzoate and arginine supplements. The lethargy progressed to coma. Diagnosis was based on elevated plasma ammonia levels (ranging from 270-2000 µg/dl) and increased plasma citrulline levels. Treatment included peritoneal dialysis and exchange transfusions in addition to sodium benzoate and arginine supplements in symptomatic patients. Nine babies had respiratory arrest requiring mechanical respiration. Ten patients died in the neonatal period and two cases died at 2.5 and 5.5 years of age respectively. Only one patient remained alive. She has been hospitalized several times with hyperammonemic coma and is now three-and-one-half years old and has severe mental retardation.

Case 6 illustrates the typical manifestation of the neonatal-onset citrullinemia.

Case 6 (DK): A five-day-old female infant who weighed 3800 g at birth was the third child of consanguineous healthy parents. The pregnancy was uneventful. The infant was normal at birth. During the first two days of life, the infant seemed to develop normally and was sucking well. She became less alert on the third day and sucking became poor. She became drowsy and hypotonic on the fifth day vomiting and with intermittent depressed breathing and was admitted to hospital, comatose. Physical examination revealed a deeply encephalopathic infant unresponsive to any stimuli. The Moro and sucking reflexes were absent. Her respiration was depressed. The blood pH was 7.02, HCO_3: 12.1 mmol/L, serum urea nitrogen 14 mg/dl, and NH_3: 1077 µg/dl (N: < 120 mg/dl). The serum amino acid analysis showed greatly increased citrulline (2333 µmol/L) and glutamine (2315 µmol/L) levels. The respiration arrest necessitated intubation and artificial ventilation. Nevertheless, her general condition deteriorated rapidly and the infant died on the sixth day of life. There was a family history suggestive of inborn errors of metabolism. The two siblings of the proband had been normal at birth, but had become lethargic on the fourth day of life and died.

The Subacute Form of Citrullinemia

Four patients with citrullinemia in this series had been presented in infancy. Two of them were referred to our hospital for further investigation for developmental delay, while the other two cases were admitted to hospital with hyperammonemic coma. Besides altered consciousness, vomiting, convulsion and developmental delay were presenting symptoms in these patients. In this group, three patients were alive and on a low-protein diet with medication, but they were severely mentally retarded. One died during hyperammonemic coma.

Case 16 shows the typical clinical presentation of the subacute form of citrullinemia.

Case 16 (HY): A seven-month-old boy was referred to our hospital for evaluation of his developmental delay. He was the second child of first degree relative

parents and the product of an uneventful 40-week pregnancy and delivery (birth weight 3170 g). His family history was said to be negative for metabolic disease. At five months of age, he was examined at a local hospital for fever, vomiting, lethargy and irritability, but a specific diagnosis could not be made. He was breast-fed and his body weight did not increase. On admission, his body weight was 6100 g (< 5th percentile) and length was 68 cm (50-75th percentile). He was unable to hold his head up or sit. The postprandial blood ammonia concentration was 275 µg/dl (N: < 120 µg/dl). Blood count and acid-base status were normal. Quantitative estimation of amino acids in plasma demonstrated increased citrulline (2304 µmol/L) and glutamine (534 µmol/L) concentrations. Blood ammonia concentration was reduced by restricting protein intake to 1.5 g/kg/day using a special formula (Milupa UCD-1), and by administration of sodium benzoate 250 mg/kg/day, and L-arginine (4 mmol/kg/day). On this regimen, moderate growth was achieved and blood ammonia levels remained within the normal range. At nine months of age he was able to hold his head up, at 10 months he could sit without aid, and at two years of age he could walk with aid. This case promptly responded to the therapy, but he had permanent neurological sequelae.

The Late-Onset Citrullinemia

Five cases with citrullinemia presented at different ages in late childhood. They were between six and 15 years. Only two patients had positive history for sibling deaths. The presenting symptoms and clinical findings were similar in all but case 19 (PS). Details about this case are given below. Altered consciousness, vomiting and convulsion were presenting symptoms. Protein avoidance and developmental delay were reported retrospectively. Two boys died during the first hyperammonemic attack despite peritoneal dialysis and medication. The other three patients were put on a low-protein diet, and sodium benzoate and arginine were prescribed. Only case 19 (PS) is doing well; the other two cases were lost to follow-up.

Case 19 exemplifies an acute presentation of late-onset citrullinemia as a bizarre behavior.

Case 19 (PS): A seven-year-old girl was brought to the emergency department with the chief complaints of agitation and crying. She was the full-term product of an uneventful pregnancy and delivery and had normal developmental milestones. Her parents were first degree relatives and her younger sister was healthy. At 1.5 and 2.5 years of age, she had been taken to other hospitals with the same symptoms, but no specific diagnosis was reached. The last time, eight days previously, she awoke from a nap and was found to be combative and had no interpretable speech. She spontaneously recovered within 24 hours. On admission, physical examination revealed normal vital signs and a hyperactive confused child with ataxia and no coherent speech. It is significant that she had a dietary protein avoidance, and thus was at the 3rd per centile for height for

which an endocrine evaluation was unrevealing. Routine laboratory findings were all within normal limits. Electroencephalography showed generalized irregularity compatible with a metabolic disease. The first impression was an underlying metabolic disease. Measurements of blood ammonia and amino acids were recommended. Laboratory studies revealed an only slightly high ammonia level, 180 μg/dl (N: < 120 μg/dl); however, her plasma amino acids revealed high citrulline (6100 μmol/L) and glutamine (1050 μmol/L) levels. The orotic acid level in her urine highly increased, the measurement being out of range. She was put on a protein-restricted diet and given sodium benzoate orally. For the last two years, her blood ammonia level has remained within normal limits.

The Asymptomatic Citrullinemia

Case 23 was an example of the asymptomatic variety of citrullinemia. He showed biochemical disturbances of a milder degree without hyperammonemia or any signs of the disease.

Case 23 (AK): A male infant, the first offspring of consanguineous healthy parents, was born after an uncomplicated pregnancy of 40 weeks with a normal birthweight. He was screened for metabolic disease by paper chromatography in the neonatal period. His serum and urinary amino acid patterns showed a high citrulline level, but his repeated serum ammonia levels were within normal limits. His developmental milestones were normal during childhood and he has never experienced hyperammonemia during intercurrent illnesses. At the present time, he is 16 years old and his neurological and psychometric evaluation are normal. His school performance is good. Until now he is asymptomatic, but he was advised to avoid protein loading.

Discussion

Citrullinemia is one of five urea cycle defects and caused by argininosuccinate synthetase deficiency: conversion of citrulline to argininosuccinate is blocked. There is considerable phenotypic variability: 1. a disease which runs a rapid and fatal neonatal course (neonatal form), 2. onset in infancy with clinical symptoms of periodic vomiting, seizure and mental retardation (subacute form), 3. a variant which has a lesser elevation of serum citrulline without clinical symptoms during infancy (late onset form), 4. a variant detected by routine screening and with biochemical disturbances of a milder degree without any signs of disease (asymptomatic form)[7-9].

The neonatal form presents a fatal course with vomiting, feeding difficulties, tachypnea, grunting, irritability proceeding to rigidity (sometimes with opisthotonus), apnea, convulsion, stupor and coma in the first days of life, generally after institution of protein feedings[10, 11]. With aggressive management,

which consists of protein restriction; administration of arginine, sodium benzoate and sodium phenylacetate; and hemodialysis, the acutely ill infant can be rescued, but frequently has permanent neurological sequelae because of the toxic effects of ammonemia. Cases 1-13 can be categorized as having the acute neonatal form of the disorder.

Four patients in whom the subacute form of the disorder was diagnosed presented during infancy. The presentation of this form of citrullinemia may not be so obvious and is characterized by developmental delay, vomiting and failure to thrive. Alternatively, there may be episodic symptoms with mild or severe neurological manifestations[12, 4].

Diagnosis of the late-onset type of citrullinemia was made in five patients. The most characteristic feature of this variant is the late onset of serious and continuing symptomatology, starting from childhood to adulthood. Patients with late-onset urea cycle defects present with episodes of acute metabolic encephalopathy although they may also have chronic symptoms. Usually there is an obvious trigger such as infection or protein loading. Sometimes they present with agitation or irritability or confusion, with or without any apparent cause. Vomiting, headaches, ataxia, fluctuating levels of consciousness, fits, focal neurological findings, learning difficulties and mental retardation are common symptoms and signs. In this series, a 15-year-old boy (Case 22) who was mentally normal died during the first hyperammonemic coma. A seven-year-old girl (Case 19) who was admitted to hospital three times with agitation and crying was also mentally normal. Her verbal and performance IQ were 98 and 105, respectively. The other three cases were retarded. Two patients died under observation, two were lost to follow-up, and one is doing well (Case 19). In this form of citrullinemia, some patients voluntarily restrict their protein intake[2]. This behavior was reported in three patients in this series. Delayed physical development occurred in some. Case 19 (PS) had been evaluated for growth retardation, but her endocrine evaluation was unrevealing. It is noteworthy that growth failure is attributable to a metabolic disease besides endocrinopathy.

There are some cases in literature in whom citrullinemia was detected by routine hospital screening and whose growth and development were normal. In this variety of citrullinemia, citrulline plasma concentrations were always high whether compared with normal controls or with heterozygote relatives of the cases, though for lower than levels in patients with the other types of citrullinemia[13-15]. Case 23 (AK) was detected by newborn screening in Ankara Maternity Hospital in 1980. His development and growth were normal and he never had hyperammonemia despite the constant high citrulline level in his plasma. Therefore, we considered him as having the fourth type of the disorder.

Citrullinemia is reported to be a rare urea cycle disorder[4], but AS deficiency was the most commonly encountered enzymatic defect in Turkey[16], probably due to high rate of consanguineous marriages. The parental consanguinity rate was 78 percent among the cases reported in this paper.

If hyperammonemia is thought to be due to a urea cycle defect in a patient, the enzymatic defect should be localized. Serum and urinary amino acid analysis is of value in this context. In AS deficiency, the plasma citrulline level is found to be markedly increased.

The diagnosis can be confirmed by enzyme activity in the liver[2, 17]. In this study, the diagnoses were based on serum and urinary amino acid patterns except in Case 5 (EA). AS activity in the liver was 11 percent of the control activity in this case; however, enzyme assays were not possible in all patients due to technical limitations.

Recent developments in treatment improved the survival rate of citrullinemic patients on the condition that the effective treatment is commenced before irreversible changes occur in the central nervous system[18]. In addition to a low-protein diet and supplementation with essential amino acids or their nitrogen-free analogues, use of the alternative pathways of waste nitrogen excretion is essential. Peritoneal dialysis and exchange transfusion are utilized to remove the accumulated ammonia from the body[4].

In this series, fourteen cases died during hyperammonemic attack. Ten neonatal cases died despite vigorous therapy; some of them had been admitted to hospital after severe brain damage had occurred. Four patients were lost to follow-up. Five patients, including the asymptomatic case, are alive, but only two are doing well; three of them are mentally retarded. It seems that our experience with infants affected with citrullinemia is discouraging, particularly in some of the neonatal cases in whom treatment was started on the first day of life. But some catabolic processes such as sepsis frequently overwhelm any residual enzyme activity and patients die despite appropriate therapy.

The cases presented here with different ages and symptoms of presentation illustrate the genetic heterogeneity of citrullinemia. Variability in the clinical course of our patients cannot be explained without genetic evaluation.

The authors think that this series is one of the largest studies reported on this subject and is useful in alerting clinicians to the possibility of urea cycle disorders in neonates who present with vomiting and seizure lapsing into lethargy, stupor or coma, especially if there is positive family history of sibling deaths and parental consanguinity. The same holds true for older children who have periodic vomiting, seizure and developmental delay of unknown etiology or who show these signs and symptoms after ingestion of a protein-rich diet or during an intercurrent illness. Awareness of this possibility should lead to a complete diagnostic evaluation and thus early diagnosis which is essential for the success of therapy.

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