원 저

요소회로대사 질환 환자들의 장기적인 임상 경과에 대한 단일 기관 경험

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Long-term Clinical Consequences in Patients with Urea Cycle Disorders in Korea: A Single-center Experience

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Purpose: Urea cycle disorder (UCD) is an inherited inborn error of metabolism, acting on each step of urea cycle that cause various phenotypes. The purpose of the study was to investigate the long-term clinical consequences in different groups of UCD to characterize it.

Methods: Twenty-two patients with UCD genetically confirmed were enrolled at Pusan National University Children's hospital and reviewed clinical features, biochemical and genetic features retrospectively.

Results: UCD diagnosed in the present study included ornithine transcarbamylase deficiency (OTCD) (n=10, 45.5%), argininosuccinate synthase 1 deficiency (ASSD) (n=6, 27.3%), carbamoyl-phosphate synthetase 1 deficiency (CPS1D) (n=3, 13.6%), hyperornithinemia-hyperammonemia-homocitrullinuria syndrome (HHHS) (n=2, 9.1%), and arginase-1 deficiency (ARG1D) (n=1, 4.5%). The age at the diagnosis was 32.7±66.2 months old (range 0.1 to 228.0 months). Eight (36.4%) patients with UCD displayed short stature. Neurologic sequelae were observed in eleven (50%) patients with UCD. Molecular analysis identified 37 different mutation types (14 missense, 6 nonsense, 6 deletion, 6 splicing, 3 delins, 1 insertion, and 1 duplication) including 14 novel variants. Progressive growth impairment and poor neurological outcomes were associated with plasma isoleucine and leucine concentrations, respectively.

Conclusion: Although combinations of treatments such as nutritional restriction of proteins and use of alternative pathways for discarding excessive nitrogen are extensively employed, the prognosis of UCD remains unsatisfactory. Prospective clinical trials are necessary to evaluate whether supplementation with BCAAs might improve growth or neurological outcomes and decrease metabolic crisis episodes in patients with UCD.

Key words: Urea cycle disorder, Clinical consequences, Neurologic outcomes, Branched-chain amino acid

Introduction

The urea cycle, first described by Krebs and Henseleit in 1932,[1,2] is the main pathway to convert toxic ammonia to non-toxic urea and excrete from the body through the urine. It is important to maintain normal ammonia because that the hyperammonemia causes cerebral edema, and if prolonged, neurologic injury[2]. The urea cycle

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disorder (UCD) includes seven different groups acting on each step of urea cycle and cause various phenotypes. The phenotypic spectrum is wide ranging from severe life-threatening hyperammonemic decompensations within the first 28 days of life (early onset) to mild or moderate chronic hyperammonemic conditions reflected by a heterogeneous clinical manifestation such as lethargy, headache, hepatological, gastrointestinal and neurological or psychiatric symptoms any time after the newborn period (late onset)[3].

Although the mechanism of UCD and proper management have been known it still has growth failure and poor neurological outcome even after early diagnosis and treatment in early onset UCD. Of particular, appropriate medical management is considered important of normal growth and also critical for good neurologic outcomes in patients with UCD[4]. However, clinical trials investigating dietary treatment outcomes between different severity-adjusted UCD subgroups concerning clinical endpoints, such as growth or metabolic stability, are still lacking[5,6]. Up to date, current recommendations for dietary and pharmacological long-term management remain inconclusive due to missing longitudinal studies investigating the adverse effects of current treatment modality[3,5,7]. In the present study, the clinical features and long-term consequences in different groups of UCD were evaluated including the relationship the plasma branched-chain amino acid (BCAA) levels and growth and neurological outcomes.

Materials and Methods

1. Patients

The twenty-two patients genetically confirmed with UCD were enrolled in this study from November 2009 to September 2021 in Pusan National University Children's hospital, Yangsan, Korea. We retrospectively reviewed clinical spectrums, biochemical and genetic findings of those patients. Short stature is defined as height below the third percentile or greater than two standard deviations (SD) below the mean height for chronological age. The definition of failure-to-thrive (FTT) includes cases where weight-only catch-up growth is not possible, or weight and height are both out of catch-up growth, or weight, height, and head circumference are out of catch-up growth, respectively. Written informed consent was obtained from all participants who provided identifiable samples.

2. Statistical analyses

All the analyses were performed in SPSS version 20 (SPSS Inc, Chicago, IL, USA). We compared BCAA concentrations (L-valine, L-leucine, L-isoleucine), glutamate and glutamine between the normal growth (non-FTT) group and FTT group, also between the normal neurological outcome group and poor neurological outcome group by Mann-Whitney's U test. And we considered that P-value less than 0.05 means having statistical significance.

Results

Of the total 22 patients with UCD, ornithine transcarbamylase deficiency (OTCD) showed the highest frequency with 10 (45.5%), followed by argininosuccinate synthetase 1 deficiency (ASSD) with 6 (27.3%), carbamoyl phosphate synthetase 1 deficiency (CPS1D) with 3 (13.6%), hyperornithinemia-hyperammonemia-homocitrullinuria syndrome (HHHS) with 2 (9.1%), and arginase-1 deficiency (ARG1D) with 1 (4.5%). Table 1 shows

demographic and clinical courses among patients with UCD.

The age at the diagnosis was 32.7±66.2 months old (range 0.1 to 228.0 months). The mean plasma peak ammonia level was 895.8±1033.0 μM/L. At the time of diagnosis, the initial symptoms of UCD were various, such as vomiting (n=8), drowsiness (n=6), seizure (n=4), jaundice (n=3), irritability (n=2), feeding difficulty (n=2), cyanosis (n=1), hypoglycemia (n=1), and dystonia (n=1). Eight (36.4%) patients with UCD displayed short stature which was frequent in order of CPS1D (66.7%), HHHS (50%), ASSD (33.3%), and OTCD (30%). Neurologic sequelae were observed in eleven (50 %) patients with UCD. There were 9 patients who had neurologic symptoms at the time of diagnosis and remained as neurologic sequelae. Two patients who developed neurologic sequelae after the metabolic crisis didn't have neurologic symptoms at the time of diagnosis. Neurological sequelae were accompanied by global developmental delays in 10 patients, epilepsy in 6 patients, and motor paralysis in 4 patients. One patient had dystonia in the lower legs and showed mild and diffuse atrophic changes in brain MR image.

A poor neurologic outcome was shown in CPS1D (n=3, 100%), HHHS (n=2, 100%), ARG1D (n=1, 100%), OTCD (n=4, 40%), and ASSD (n=1, 16.7 %). Three (13.6%) of total patients died of metabolic crisis, of which 2 (66.7%) patients died of CPS1D and 1 patient (33.3%) died of OTCD. There was no patient who has experience with liver transplantation.

All patients had protein restriction (<2.0 g/kg/day) and the dose of nitrogen scavengers (sodium benzoate and sodium phenylbutyrate) was about 250 mg/kg per day, respectively. The average treatment duration was 115.5±73.4 months (range 4 to 276 months).

Table 2 shows the genetic features of patients with UCD. The missense mutation (37.8%, 14/37) was the most common mutation, followed by nonsense mutation (16.2%, 6/37), splicing mutation (16.2%, 6/37), deletion (16.2%, 6/37), delins (8.1

Table 1. Comparison of Gender, Age, Biological Findings, and Prognostic Outcome among UCD Patients

	OTCD (n=10)	ASSD (n=6)	CPS1D (n=3)	HHHS (n=2)	ARG1D (n=1)	Total (n=22)
M:F	3:7	3:3	1:2	2:0	1:0	10:12
Age at dx (m, range)	65.9±87.7 (0.1 to 228.0)	0.4±0.4 (0.1 to 1.0)	0.1±0.0 (0.1 to 0.1)	1.0±1.3 (0.1 to 2.0)	120	32.7±66.2 (0.1 to 228.0)
Current age (y, range)	15.5±9.8 (1.3 to 32.0)	7.0±6.3 (1.0 to 19.0)	8.0 (2.2 to 8.7)	11.5±7.7 (6.0 to 17.0)	22.0	12.8±8.9 (1.3 to 32.0)
Plasma peak ammonia (μM/L,range)	427.5±343.3 (69.7 to 1,000)	1,073.4±1281.3 (23.0 to 3,386.5)	2,639.3±276.3 (2,325.0 to 2,844.0)	517.5±682.4 (34.0 to 1000.0)	40.0	895.8±1,033.0 (23.0 to 3,386.5)
Short stature (n, percent)	3 (30.0%)	2 (33.3%)	2 (66.7%)	1 (50.0%)	None	8 (36.4%)
Neurologic sequelae (n, percent)	4 (40.0%)	1 (16.7%)	3 (100.0%)	2 (100.0%)	1 (100.0%)	11 (50.0%)
Death (n, percent)	1 (10.0%)	None	2 (66.7%)	None	None	3 (13.6%)

Abbreviations: M. male; F, female; dx, diagnosis; m, months; n, numbers; y, years; SD, Standard deviation; OTCD, ornithine transcarbamylase deficiency; ASSD, argininosuccinate synthase 1 deficiency; CPS1D, carbamoyl phosphate synthetase 1 deficiency; HHHS, hyperornithinemia-hyperammonemia-homocitrullinuria syndrome; ARG1D, arginase-1 deficiency.

%, n=3), insertion (2.7%, n=1), and duplication (2.7%, n=1). Of particular, novel variants accounted for 53.8% (n=14) of all variants. Comparing the difference of clinical and biochemical findings between the normal growth (non-FTT) group and FTT group, there was a statistical significance in plasma isoleucine level comparing the difference from normal values and current height SDS between two groups (P=0.005, P=0.000, respectively) (Table 3). In addition, there was a statistical significance of leucine level and glutamate between normal neurologic outcome group and poor neurologic outcome group (P=0.005, P=0.021, respectively) (Table 4).

Discussion

In the present study, we evaluated the long-term clinical consequences in different groups of UCD to characterize it. As usual, the most common disease among UCD was OTCD, followed by ASSD. And three major results were drawn: 1) Patients with CPS1D and HHHS had poor growth and neurological outcome. 2) Plasma isoleucine levels were lowered in FTT group than in non-FTT group. 3) Poor neurological outcome group had lower plasma leucine levels and higher glutamate levels than normal neurological outcome group.

In particular, CPS1D and HHHS diseases have poor neurological prognosis in most patients ex-

Table 2. Genetic Features of Patients with UCD

	OTCD (n=10)	ASSD (n=6)	CPS1D (n=3)	HHHS (n=2)	ARG1D (n=1)	Total (n=22)
Exon:Intron	6:2	5:2	5:1	1:1	2:0	19:6
Missense (n)	3	7	1	1	2	14/37 (37.8%)
Nonsense (n)	2	None	4	None	None	6/37 (16.2%)
Deletion (n)	2	None	3	1	None	6/37 (16.2%)
Splicing (n)	2	2	1	1	None	6/37 (16.2%)
Delins (n)	3	None	None	None	None	3/37 (8.1%)
Insertion (n)	None	1	None	None	None	1/37 (2.7%)
Duplication (n)	None	1	None	None	None	1/37 (2.7%)
Novel variants (n)	3/7 (42.8%)	5/9 (55.5%)	3/5 (60%)	2/3 (66.7%)	1/2 (50%)	14/26 (53.8%)

Abbreviations: n, numbers; OTCD, ornithine transcarbamylase deficiency; ASSD, argininosuccinate synthase 1 deficiency; CPS1D, carbamoyl phosphate synthetase 1 deficiency; HHHS, hyperornithinemia-hyperammonemia-homocitrullinuria syndrome; ARG1D, arginase-1 deficiency.

Table 3. Comparison of Clinical and Biochemical Findings between Non-failure-to-thrive (non-FTT) Group and Failure-to-thrive (FTT) Group

	Non-FTT group (n=14)	FTT group (n=8)	P-value
Current height SDS (range)	−0.78±−1.01 (−2.39 to 0.91)	−3.40±−1.76 (−6.30 to −1.88)	0.000
Plasma glutamate* (nM/mL) (range)	1.1±3.3 (0.00 to 12.10)	6.8±10.4 (−2.30 to 29.3)	0.072
Plasma glutamine* (nM/mL) (range)	44.2±105.3 (0.00 to 400.90)	66.0±97.0 (−63.90 to 242.50)	0.636
Plasma leucine* (nM/mL) (range)	−0.7±3.4 (−8.80 to 4.40)	−4.1±5.8 (−12.80 to 6.90)	0.090
Plasma isoleucine* (nM/mL) (range)	−0.8±1.4 (−3.30 to 1.50)	−3.5±2.6 (−7.50 to −0.70)	0.005
Plasma valine* (nM/mL) (range)	0.3±3.9 (−6.10 to 11.30)	−0.2±4.8 (−10.60 to 6.20)	0.801

*The average of the difference values from the normal values according to age at the time of measuring the plasma amino acid test.
Abbreviations: FTT, failure-to-thrive; SDS, standard deviation score.

Table 4. Comparison of Biochemical Findings between Normal Neurological Outcome Group and Poor Neurological Outcome Group

	Normal neurologic outcome group (n=11)	Poor neurologic outcome group (n=11)	*P*-value
Plasma glutamate* (nM/mL) (range)	−0.2±0.7 (−2.3 to 0.0)	6.6±8.9 (0.0 to 29.3)	0.021
Plasma glutamine* (nM/mL) (range)	49.2±119.0 (0.0 to 400.9)	55.0±84.0 (−63.9 to 242.5)	0.898
Plasma leucine* (nM/mL) (range)	0.6±2.7 (−2.4 to 6.9)	−4.5±4.7 (−12.8 to 4.3)	0.005
Plasma isoleucine* (nM/mL) (range)	−1.1±1.7 (−5.4 to 0.0)	−2.4±2.6 (−7.5 to 1.5)	0.172
Plasma valine* (nM/mL) (range)	0.3±1.9 (−3.8 to 4.2)	0.0±5.7 (−10.6 to 11.3)	0.878

*The average of the difference values from the normal values according to age at the time of measuring the plasma amino acid test.

periencing metabolic crises due to hyperammonemia occurring at a young age, and the severity of symptoms is determined mainly by genotypes and residual enzyme function. Interestingly, all 7 female patients out of 10 patients with OTCD were diagnosed with UCD due to clinical symptoms such as recurrent vomiting, altered mentality, and seizure. On the other hand, a patient with ARGID first developed dystonia in the lower legs with normal levels of ammonia around the age of 4.

In the present study, isoleucine levels showed a statistically significant difference between the non-FTT group and the FTT group, but other BCAA levels also showed a lower tendency in the FTT group. Previous studies have reported some evidence that BCAA levels are low in patients with UCD displaying growth impairment. Molema F, et al.[8] suggested that the decreased plasma BCAA levels in UCD can be a potential cause of growth impairment. Posset R, et al.[9] reported that growth impairment was associated with reduced or low normal plasma BCAA concentrations. Importantly, in catabolic states, BCAA are normally oxidized to generate ATP. Carbon originating from leucine enters the tricarboxylic acid cycle as acetyl-CoA for complete disposal as CO_2, whereas isoleucine and valine mainly provide carbon for anaplerotic conversion of propionyl-CoA to succinyl-CoA[10]. L-isoleucine and L-valine have been shown to be the only amino acids with significant cerebral uptake in patients with fulminant hepatic failure, and cerebral BCAA transaminases (BCAT) are stimulated under hyperammonemic conditions, thereby enhancing the consumption of L-isoleucine and L-valine for anaplerotic reactions as well as transamination processes for the generation of L-glutamate and L-glutamine via activities of BCAT1 and BCAT2, respectively[9]. Diseases characterized by a prominent catabolic state show decreased plasma BCAA levels[11]. In this study, the normal neurological outcome group showed higher plasma leucine levels and lower glutamate levels than the poor neurological outcome group. A recent study suggested that leucine is associated with neurodevelopmental disorders [12]. Concerning the effect of supplementation of leucine on the neurologic outcome, Shih YT, et al.[12] reported that leucine supplementation ameliorates synaptic and memory defects of Nf1+/− mice which alter interneuron circuits resulting in promoting neuronal activation of the dentate gyrus and CA1 projection neurons[12]. Looking at the literature report on the supplementing BCAA in UCD, Häberle J, et al.[4] recommended it should be considered essential amino acids or BCAA supplementation as an acute treatment of UCD. However, the current recommendations for dietary and pharmacological management remain inconclusive

due to missing longitudinal studies evaluating the adverse effects of current treatment principles[4,5]. In the present study, combination of treatments such as nutritional restriction of protein and use of alternative pathways for discarding excessive nitrogen are extensively employed, but the prognosis of UCD remains unsatisfactory. In the future, it needs more study about that BCAA supplements can help patients with UCD have good prognosis in growth and neurologic outcomes. The limitation is that this study is too small group, so it needs prospective study in large groups. Secondly, we have measured BCAA values several times whenever metabolic crisis occurred, but some measurements were omitted. Therefore, it may be necessary to reduce the bias of the study by continuously measuring BCAA without omitting it in the future.

BCAA results were usually investigated and analyzed at the time of diagnosis and at the time of the most recent visit, but it will be necessary to serially measure and analyze BCAA values whenever metabolic risk occurs. Lastly, while the UCD registry contains detailed information on dietary prescriptions, we could not verify and describe the actual daily intake by participating patients with UCD or adequately control for patient compliance.

We identified 22 patients with UCD, of which OTCD was the most common followed by ASSD, and poor neurological outcome were found in all patients with CPS1D, HHHS, and ARG1D. Genetically, a total of 37 different mutations were detected, of which 14 novel variants were discovered. Growth impairment and poor neurological outcomes were associated with reduced plasma BCAA concentration such as isoleucine or leucine. A prospective study is required to evaluate whether supplementation with BCAA might decrease the risk of metabolic crisis and improve growth or neurologic outcomes in patients with UCD.

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Conflicts of Interest

The authors have nothing to declare.

요 약

목적: 요소회로대사 질환은 선천성 대사이상질환으로, 요소 회로 각 단계의 특정 효소의 결핍에 따라 다양한 질환과 임상적 증상이 나타난다. 본 연구의 목적은 요소회로대사 질환의 다양한 종류에 따른 장기적 임상경과를 조사하는 것이다.

방법: 부산대학교 어린이병원에 등록된 22명의 요소회로대사 질환 환자들의 임상적 양상과 생화학적, 유전학적 검사 결과들을 포함한 의무기록을 후향적으로 조사하였다.

결과: 본 연구에서 진단된 요소회로대사 질환은, OTCD 10명(45.5%), ASSD 6명(27.3%), CPS1D 3명, HHHS 2명 ARG1D 1명으로 확인되었다. 진단 시 평균연령은 32.7±66.2개월(범위 0.1–228.0개월) 이었다. 요소회로대사 질환 환자 8명(36.4%)에서 성장장애가 동반되었다. 또한 요소회로대사 질환 환자11명(50%)에서는 신경학적 후유증이 관찰되었다. 분자유전학적 분석 결과 새로운 돌연변이 14개를 포함해 37개의 서로 다른 돌연변이 유형(과오돌연변이 14개, 넌센스 6개, 결실변이 6개, 스플라이싱변이 6개, 결실삽입변이 3개, 삽입변이 1개, 중복변이 1개)이 확인됐다. 진행성 성장 장애와 나쁜 신경학적 결과는 혈장 이소루이신과 루이신 농도와 각각 관련이 있었다.

결론: 단백의 제한과 같은 조치나 과다한 질소를 제거하는 약제의 복용에도 불구하고, 요소회로대사 질환의 예후는 아직까지 만족스럽지 못하다. 가지사슬아미

노산의 보충이 요소회로대사 질환 환자들의 성장부전과, 대사성 위기 또는 신경학적 예후에 효과를 보일지에 관해서는 전향적인 추가 연구가 필요하겠다.

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