Heliyon 10 (2024) e36003

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

A preliminary retrospective evaluation of screening and diagnosis of ornithine transcarbamylase deficiency in high-risk patients at a referral center in Vietnam

Dien Minh Tran [a], Trang Thi Thu Tran [a], Quyen Hue Luong [a], Mai Thi Chi Tran [a,b,*]

[a] National Children's Hospital, Hanoi, Viet Nam
[b] Hanoi Medical University, Hanoi, Viet Nam

ARTICLE INFO

Keywords:
Ornithine transcarbamylase deficiency
Urea cycle disorders
Hyperglutaminemia
Hypocitrullinemia
High-risk screening

ABSTRACT

Introduction: To date, newborn screening (NBS) for proximal urea cycle disorders, including Ornithine transcarbamylase deficiency (OTCD), was not recommended due to the lack of appropriate tests and insufficient evidence of the benefits. This study aimed to investigate the potential of tandem mass spectrometry (MS/MS) for OTCD screening and its value in guiding further investigation to obtain a final diagnosis in high-risk patients.

Methods: The study included patients with OTCD referred to the National Children's Hospital between April 2020 and November 2023. A retrospective evaluation of amino acid concentrations measured by MS/MS and their ratios in patients with early-onset and late-onset OTCD was conducted.

Results: While all ten early-onset cases had glutamine concentrations above the upper limit, only five of them had citrulline concentrations below the lower limit of the reference interval. Only two late-onset cases had elevated glutamine levels, while all had citrulline within reference intervals. The Cit/Phe ratio was decreased, and the Gln/Cit and Met/Cit ratios were increased in all early-onset OTCD cases, while they were abnormal in only one late-onset case.

Conclusions: The preliminary results suggest that hyperglutaminemia, in combination with low or normal citrulline concentrations and specific ratios (Gln/Cit, Met/Cit, and Cit/Phe), can serve as reliable markers for screening early-onset OTCD in high-risk patients. However, these markers proved less sensitive for detecting the late-onset form, even in symptomatic patients.

1. Introduction

Urea cycle disorders (UCDs) are a group of inborn errors of metabolism, resulting from deficiency in any one of the six enzymes or two transporters of the urea cycle pathway [1]. The proximal urea cycle disorders are defined as the consequence of defects in the first three steps of the urea cycle (N-acetylglutamate synthase, carbamyl phosphate synthase and ornithine transcarbamylase), while the distal disorders include the final three steps of the urea cycle (arginosuccinate synthase deficiency, also called citrullinemia type 1; arginosuccinate lyase deficiency; and arginase deficiency) [2]. Ornithine transcarbamylase deficiency (OTCD), the most common urea cycle disorder, is an X-linked inherited disease, accounting for about half of the reported patients [3]. Population estimates for OTCD

* Corresponding author. National Children's Hospital, Hanoi, Viet Nam.
E-mail address: tranchimai@hmu.edu.vn (M.T.C. Tran).

https://doi.org/10.1016/j.heliyon.2024.e36003
Received 19 February 2024; Received in revised form 4 August 2024; Accepted 7 August 2024
Available online 8 August 2024

range from 1 in 14,000 to 1 in 80,000 [4,5], and more recently, about 1 in 56,500 live births [6].

OTC catalyzes the combination of ornithine and carbamoyl phosphate to form citrulline, which is crucial for the clearance of ammonia resulting from protein turnover [7].Consequently, OTCD results in hyperammonaemia, decreased production of citrulline, elevated glutamine and high excretion of urinary orotic acid, which are the biochemical indications of this disorder [3]. OTCD exhibits wide phenotypic heterogeneity, ranging from asymptomatic to severely symptomatic cases. The neonatal-onset phenotype is usually seen in affected males [8], while the late-onset form occurs in approximately 20 % of heterozygous female cases, with highly variable age of onset and clinical features [9]. Although the neonatal-onset form is the most common, OTCD is prevalent in infants, adolescents and even adults [2]. While clinical presentation may appear in the first week of life, even in the first 2 or 3 days of life, newborn screening (NBS) if performed will assist in detecting the disease early and providing appropriate treatment to prevent disease morbidity and mortality.

NBS for distal UCDs have been implemented in some countries; however, NBS for proximal UCDs has not been recommended due to the lack of appropriate tests and evidence of benefit [2]. The sensitivity of NBS for the proximal UCDs remains a major technical challenge [10]. Blood glutamine and citrulline are commonly considered primary markers to screen for proximal UCDs. However, glutamine is unstable, and low citrulline alone is not specific enough to detect proximal UCDs [11]. Alternative strategies for proximal UCD-NBS have been developed. The Region 4 Stork Project, an international collaboration in the US and 45 other countries, conducted a study to obtain the clinical validation of cutoff values for NBS by tandem mass spectrometry (MS/MS) [12]. This study included participants with CPS1 and OTCD. In addition to amino acid concentrations, their ratios (citrulline to arginine (Cit/Arg), citrulline to phenylanaline (Cit/Phe), glutamine to citrulline (Gln/Cit), glutamate to citrulline (Glu/Cit), and methionine to citrulline (Met/Cit)) were also used as markers for proximal UCDs screening [12]. This screening tool detected known early-onset and late-onset proximal UCD patients except for one asymptomatic late-onset OTCD patients and second patient with incomplete set of analytes, both of whom had normal citrulline levels [2].

NBS for inborn errors of metabolism by tandem mass spectrometry is not mandatory in Vietnam. Since 2018, the National Children's Hospital has been using tandem mass spectrometry for screening inborn errors of metabolism in high-risk patients. This study investigates the possibility of MS/MS for OTCD screening and its value in guiding further investigation to get the final diagnosis of OTCD in high-risk patients. We retrospectively evaluated amino acid concentrations measured by MS/MS and their ratios in patients with early-onset and late-onset OTCD. Additionally, the effectiveness of combining amino acid analysis in dried blood spot (BDS) by MS/MS and urine organic acid analysis by gas chromatography mass spectrometry (GC/MS) for OTCD diagnosis in high-risk patients was evaluated.

2. Methods

2.1. Subjects

We conducted a retrospective study through identifying 21 patients who were diagnosed with OTCD based on biochemical features, including urine organic acid analysis at Biochemistry Department of the National Children's Hospital. This study was approved by the Ethics Committee at National Children's Hospital, Hanoi, Vietnam with the approval number VNCH-TRICH-2023-31A, dated 19th July 2023. Following Institutional Review Board approval, all procedures followed were in accordance with the ethical standards. Informed consent was obtained from the patients' parent for being included in the study.

Patients with OCTD were referred to the National Children's Hospital between April 2020 and November 2023 and were included in the study. Early-onset OTCD is defined as the onset of symptoms occurs in the newborn period, while late-onset OTCD occurs when the clinical presentation appears after 30 days of life [11]. Among the 21 OTCD patients in this study, ten males suffered from early-onset form and eleven (3 males and 8 females) had late-onset form.

2.2. Biochemical analysis

Routine biochemical tests, including blood ammonia, were performed on the AU5800 automated chemistry analyzer (Beckman Coulter). Hyperammonemia is defined as plasma ammonium levels exceeding 150 μmol/L in newborns and 100 μmol/L in infants.

2.2.1. MS/MS analysis of dried blood spots

Blood samples taken from high-risk patients were generally spotted on a filter paper. Amino acids in dried blood samples were analyzed by tandem mass spectrometry (MS/MS) (Shimadzu LCMS/MS 8040) using the NeoMass AAAC kit from Labsystems Diagnostics Oy, Finland. Quality control samples were analyzed each time patient samples were run. We participate in the US CDC external quality assurance program to compare our results with other laboratories and to ensure consistency of test results.

2.2.2. Plasma amino acid quantitation

All venous blood samples for analysis were collected into lithium heparin containers and transported to the laboratory at room temperature. Plasma was separated from cells upon arrival at the laboratory within 1 h following centrifugation at 1500*g* and stored at −20 °C until analysis. Amino acid measurement was carried out using an Acquity™ Ultraperformance® Liquid Chromatography (UPLC) system with an integrated TUV detector and MassTrak™ AAA Solutions Kit (Waters Corporation, Milford, MA). The MassTrak™ kit utilizes pre-column derivatization of amino acids with a 6-aminoquinolyl-N-hydroxysuccinimdyl carbamate tag (AccQTag®) followed by reversed-phase UPLC on a C18 column (1.7 μm; 2.1 × 150 mm) and UV detection at 260 nm. The modified

UPLC method, validated by Roy W.A. Peake, was used [13]. Quality control materials were analyzed at two concentration levels for each analyte to assess the accuracy and precision of the method. To ensure testing quality, we participate in the ERNDIM scheme for quantitative amino acid analysis.

2.2.3. Urine organic acid analysis

Urinary organic acid analysis was performed using gas chromatography mass spectrometry (GC/MS; QP-2020 plus, Shimadzu) according to the method of professor Seijy Yamaguchi [14]. A classical method for profiling urinary organic acids-ethyl acetate extraction/oxime-trimethylsilyl derivatization/gas chromatography mass spectrometry - was used. Briefly, a volume of urine containing 0.2 mg creatinine was used for extraction. Two internal standards, namely margaric acid and tropic acid, were used. Tetracosane was added, serving as internal standard for retention times. Oximes were formed by reaction with hydroxylammonium chloride at pH14. After the reaction, the mixture was extracted with ethyl acetate under the condition of pH1. The ethyl acetate layer was then pipetted to another vial and dried under nitrogen stream. The extraction step was repeated once, combining the extracts into the same vial. The extracted organic acids were then derivatized by adding N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1 % trimethylchlorosilane (TMCS) and putting into 80°C oven for 30 min. The separation, identification, and semi-quantitative analysis of organic acids were done by GC/MS. To ensure testing quality, we participate in ERNDIM scheme for qualitative urinary organic analysis.

Due to financial and personal issues, as well as the unavailability of genetic testing for OTCD during the retrospective period, none of the patients in this study underwent mutation analysis at our hospital.

3. Results

The ten early-onset OTCD patients, all males aged 2–5 days, were referred to the hospital in severe condition, with notably high blood ammonia concentrations. Dried blood spots and urine samples were collected for laboratory investigation to detect inborn errors of metabolism. Urine organic acid analysis revealed elevated excretion of orotic acid and uracil (Fig. 1 showed the typical pattern total ion chromatogram of an OCTD patient detected by urine organic acid analysis). In the late-onset form, nine had hyperammonemia, while two females presented with ammonia concentrations within the normal range (Table 1). Despite the absence of characteristic symptoms of these two patients (OTCD 16 and OTCD20), OTCD was detected through urine organic acid analysis due to their family history with previously diagnosed brothers. In the present study, almost all OTCD patients exhibited hyperammonemia, in which early-onset form had higher blood ammonia values than late-onset form (Table 1).

Table 2 presents patient blood amino acid concentrations in dried blood spots measured by MS/MS and in plasma measured by UPLC. In dried blood spots, arginine concentrations for eight early-onset OTCD patients fell within reference interval, with only two slightly exceeding the upper limit. This reference interval, locally established from 23,389 healthy newborns, represented the 99 % central value. No patient had arginine concentration below the lower limit. Contrary to expectations, arginine levels in symptomatic early-onset OTCD patients were not low. Among the ten early-onset patients, only five had citrulline concentrations below the lower limit of reference interval, suggesting that low citrulline concentration may not be a reliable marker for OTCD. While only four patients had glutamate concentrations above the upper limit of reference interval, all ten early-onset cases had glutamine concentrations above the upper limit, indicating high glutamine concentration as a sensitive screening marker for OTCD in newborns with the early-onset form. Six early-onset patients had ornithine concentrations above the upper limit of reference interval. Among the eleven late-onset patients, only 2 had elevated glutamine levels compared to reference interval established in our laboratory, and 3 had elevated ornithine concentrations. The other amino acids, such as arginine, citrulline, and glutamate, were within reference intervals (Table 2). As seen in Table 2, plasma amino acids in early-onset cases exhibited a pattern quite similar to that in dried blood spots: all showed very high plasma glutamine concentrations. However, nine of them had low plasma citrulline levels, while only five had low DBS citrulline levels. Similarly, among the seven late-onset cases in which plasma amino acid analysis was performed, three had high

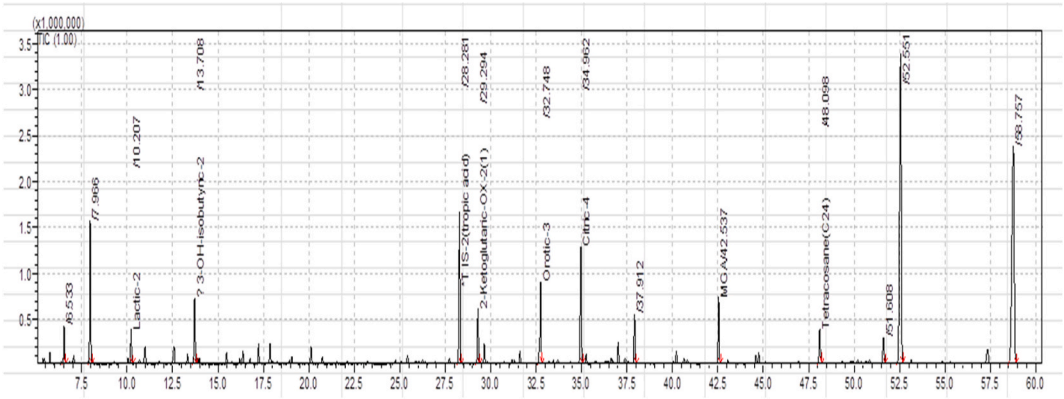

Fig. 1. Total ion chromatogram of urine organic acid analysis of an OTCD patient.

Table 1
Clinical manifestation, blood ammonia concentrations and time of blood and urine sampling in OTCD patients.

Patient ID	Symptoms	Age	Sex	Time of sample collection			Plasma ammonia concentrations (μmol/L)	
				DBS for AA & AC analysis by MS/MS	Plasma for AA quantitation	Urine for organic acid analysis	At hospitalization	Peak
OTCD1	Newborn, hyperammonemia	4 days	Male	At hospitalization	2 days after DBS collection	2 days after DBS collection	154.4	1119.8
OTCD2	Newborn, lethargy, hyperammonemia	2 days	Male	At hospitalization	1 day after DBS collection	1 day after DBS collection	341.6	862.2
OTCD3	Newborn, lethargy, hyperammonemia	3 days	Male	At hospitalization	At hospitalization	At hospitalization	900.2	1423.4
OTCD4	Newborn, hyperammonemia	2 days	Male	At hospitalization	1 day after DBS collection	1 day after DBS collection	1724.6	1724.6
OTCD5	Newborn, hyperammonemia	5 days	Male	At hospitalization	At hospitalization	At hospitalization	754.6	754.6
OTCD6	Newborn, respiratory failure	2 days	Male	At hospitalization	At hospitalization	At hospitalization	230.8	1160.4
OTCD7	Newborn, lethargy, hyperammonemia	3 days	Male	At hospitalization	At hospitalization	At hospitalization	743.4	1405.2
OTCD8	Newborn, respiratory failure, hyperammonemia	2 days	Male	At hospitalization	At hospitalization	At hospitalization	1000.6	1000.6
OTCD9	Newborn, hyperammonemia	5 days	Male	At hospitalization	1 day after DBS collection	1 day after DBS collection	749.8	1435.7
OTCD10	Newborn, lethargy, hyperammonemia	4 days	Male	At hospitalization	At hospitalization	At hospitalization	1096.3	1775.7
OTCD11	Elevated liver enzymes, hyperammonemia	5 years	Female	At hospitalization	2 month after DBS collection	2 month after DBS collection	297.7	297.7
OTCD12	Encephalitis, hyperammonemia	21 months	Male	At hospitalization	1 day after DBS collection	1 day after DBS collection	274.5	308.2
OTCD13	Infant, mild hyperammonemia	21 months	Female	At hospitalization	At hospitalization	At hospitalization	115.7	115.7
OTCD14	Infant, mild hyperammonemia	2 months	Male	At hospitalization	NA	At hospitalization	187.9	187.9
OTCD15	Nausea, vomiting, hyperammonemia	24 months	Female	At hospitalization	At hospitalization	At hospitalization	453.4	453.4
OTCD16	Brother diagnosed with OCTD	18 months	Female	At clinic	21 days after DBS collection	One month after DBS collection	**34.2**	**44**
OTCD17	Encephalitis, hyperammonemia	8 years	Female	At hospitalization	NA	At hospitalization	110.9	208.5
OTCD18	Lethargy, mild hyperammonemia	21 months	Female	At hospitalization	NA	13 days after DBS collection	112.2	186.8
OTCD19	Coma	13 years	Male	At hospitalization	At hospitalization	At hospitalization	167.9	301.6
OTCD20	Abdominal pain, her brother had UCD	8 years	Female	At clinic	NA	At clinic	**38.2**	**65.8**
OTCD21	Liver failure	3 months	Female	At hospitalization	5 days after DBS collection	5 days after DBS collection	201.5	201.5

glutamine and three had low citrulline concentrations. Quantitative plasma amino acid analysis is a crucial tool for investigating metabolic disorders. Alongside organic acid analysis, it is mainly utilized in clinical laboratories for diagnosing and monitoring inborn errors of metabolism [13]. However, in comparison to amino acid measurement in DBS by MS/MS, achieving high throughput in NBS is not possible. The findings of this study further elucidated the efficacy of the Labsystems Neomass AAAC kit in assessing markers pertinent to OTCD. The data obtained emphasized the kit's reliability in analyzing these markers, providing valuable insights into the screening of both early and late-onset OTCD patients.

In Table 3, two amino acid ratios, including Gln/Cit and Met/Cit, were higher than the cut-off value, and Cit/Phe was lower than the cut-off value in all ten early-onset OTCD cases. In the early-onset form, the Cit/Arg ratio was below the cut-off value in only seven patients, while the ratio of Glu/Cit was above the cut-off value in eight patients. In contrast, abnormal Cit/Phe, Gln/Cit, and Met/Cit ratios were observed in only one late-onset OTCD case. The Cit/Arg and Glu/Cit ratios remained within reference intervals in all eleven late-onset patients. Abnormal amino acid ratios were predominantly found in early-onset patients, who also exhibited elevated glutamine concentrations. Utilizing these ratios did not increase the sensitivity of OTCD screening for the early-onset form and offered no additional benefit for late-onset detection.

4. Discussion

While NBS for distal UCDs has been implemented within the last two decades following the introduction of tandem mass

Table 2
Amino acid concentrations in dried blood spots and plasma in OTCD patients.

Patient ID	Amino acid concentrations in dried blood spots (μmol/L)					Amino acid concentrations in plasma (μmol/L)				
	Arginine	Citrulline	Glutamate	Glutamine	Ornithine	Arginine	Citrulline	Glutamate	Glutamine	Ornithine
OTCD1	11.50	1.54	640.79	1317.01	66.70	13.3	12.7	182.8	1675.8	13.3
OTCD2	15.87	1.76	668.19	1352.03	84.45	43.9	1.00	209.0	5212.0	43.9
OTCD3	21.23	5.63	992.14	1594.73	114.98	13.8	ND	585.9	4646.3	13.8
OTCD4	14.04	3.85	1185.03	1353.23	63.29	22.8	4.5	333.4	2169.4	22.8
OTCD5	26.20	7.76	587.87	1948.68	151.82	36.4	4.2	47.6	1502.2	36.4
OTCD6	16.91	4.90	1153.02	2481.29	123.83	44.7	3.5	120.4	5549.5	44.7
OTCD7	6.82	3.55	670.66	941.27	58.16	ND	ND	723.5	2068.4	ND
OTCD8	9.36	5.33	1403.71	1124.43	62.37	ND	ND	216.9	6605.3	ND
OTCD9	12.84	4.46	464.51	592.63	47.49	14.9	4.3	709.7	3890.9	14.9
OTCD10	2.37	1.40	1343.14	1122.00	80.90	17.6	ND	515.4	2116.2	17.6
OTCD11	7.03	20.93	477.97	492.58	20.08	23.5	24.3	130.1	1250.0	23.5
OTCD12	9.64	5.25	410.38	622.81	22.68	13.4	2.5	27.4	695.4	13.4
OTCD13	6.55	10.02	328.86	403.65	34.62	21.9	7.1	31.1	603.9	21.9
OTCD14	22.13	14.62	259.71	287.77	206.74	NA	NA	NA	NA	NA
OTCD15	8.46	7.22	212.87	270.05	20.00	30.6	6.5	47.1	955.8	30.6
OTCD16	15.50	19.76	248.76	172.42	193.70	38.1	30.7	77.6	653.5	38.1
OTCD17	8.14	8.06	217.08	242.33	20.04	NA	NA	NA	NA	NA
OTCD18	10.16	17.54	244.97	284.30	27.71	NA	NA	NA	NA	NA
OTCD19	13.35	20.82	169.34	267.89	25.07	42.1	ND	80.9	255.0	42.1
OTCD20	7.25	12.20	203.39	188.41	41.70	NA	NA	NA	NA	NA
OTCD21	7.03	27.19	275.48	240.00	155.38	19.1	35.0	51.9	772.0	19.1
Reference interval	0.71–20.02	4.11–19.20	245.70–1042.70	57.79–422.55	14.32–97.88	6–140	10–45	62–620	376–709	48–221

NA: Not available, ND: Not detected.

Table 3
Amino acid ratios in dried blood spot of OTCD patients.

Patient ID	Cit/Arg	Cit/Phe	Gln/Cit	Glu/Cit	Met/Cit
OTCD1	0.13	0.02	855.20	416.10	48.61
OTCD2	0.11	0.04	768.20	379.65	30.73
OTCD3	0.27	0.06	283.26	176.22	13.92
OTCD4	0.27	0.07	351.49	307.80	14.80
OTCD5	0.30	0.05	251.12	75.76	10.19
OTCD6	0.29	0.03	506.39	235.31	27.81
OTCD7	0.52	0.06	265.15	188.92	15.77
OTCD8	0.57	0.04	210.96	263.36	31.18
OTCD9	0.35	0.06	132.88	104.15	11.32
OTCD10	0.59	0.04	801.43	959.39	52.25
OTCD11	2.98	0.46	23.53	22.84	2.64
OTCD12	0.54	0.13	118.63	78.17	7.03
OTCD13	1.53	0.38	40.28	32.82	1.78
OTCD14	0.66	0.32	19.68	17.76	1.37
OTCD15	0.85	0.20	37.40	29.48	4.16
OTCD16	1.27	0.79	8.73	12.59	0.87
OTCD17	0.99	0.32	30.07	26.93	4.29
OTCD18	1.73	0.64	16.21	13.97	3.45
OTCD19	1.56	0.83	12.87	8.13	1.55
OTCD20	1.68	0.37	15.44	16.67	2.26
OTCD21	0.61	0.06	8.83	10.13	9.85
Reference interval	0.51–12.92	0.09–0.49	6.02–55.01	22.63–144.21	0.41–4.67

spectrometry, NBS for proximal UCDs were not recommended due to the lack of an adequate biochemical markers, evidence of early detection's impact on patient outcome, and the situation of UCD with mild disease [2,11]. The use of low citrulline as a marker can result in high false positive rates due to prematurity, low protein intake, and intestinal dysfunction [15]. The late-onset form of UCDs may exhibit normal citrulline levels, leading to false negative results [10]. Recently, there are some evidence supporting the inclusion of proximal UCD in NBS [2,11,16]. In this study, we retrospectively evaluated several amino acid concentrations measured by MS/MS in 21 OTCD patients to investigate whether amino acids alone and/or their ratios can be used as reliable markers for OTCD screening in newborn and high-risk patients.

Citrulline is a metabolic intermediate of the urea cycle, synthesized mainly in the mitochondrial matrix of hepatocytes, and in the small intestine and kidney [16]. Hypocitrullinemia may be seen in infants on protein-restricted diets, infants with mitochondrial disease, premature infants, infants with enteropathy, and other inborn disorders of metabolism [16–25]. In this study, although ten early-onset OTCD patients exhibited symptoms, only five demonstrated low blood citrulline concentrations in DBS. This finding indicates that citrulline levels alone are not a sensitive marker for early-onset OTCD screening in high-risk patients. Moreover, hypocitrullinemia, as noted, can occur in various pathological conditions during the newborn period, further reducing its reliability as a sole marker for OTCD-NBS.

Among eleven late-onset OTCD cases, none had citrulline concentration in DBS below the lower limit of reference interval, although most of them presented clinically at the hospital, except for two asymtommatic individuals who had a family history. This indicates that low citrulline level alone cannot be a reliable marker for the detection of late-onset OTCD even in high-risk patients. A similar result was also reported in the outcome of 6-years Tuscany newborn screening program where out of six newborns with low blood citrulline concentration, only one suffered with late-onset OTCD [15]; thus indicating that "hypocitrullinemia alone may not be a reliable marker for OTCD, especially for late-onset forms" [15]. Though one retrospective study suggested that hypocitrullinemia detected by the MS/MS-NBS can be used as a screening marker for some patients with late-onset OTCD, further evaluation is recommended. This includes retrospective evaluation of pre-symptomatic citrulline levels in patients with late-onset form [16].

Glutamine is the most abundant and versatile amino acid in the body, and is of fundamental importance to intermediary metabolism, inter-organ nitrogen exchange via ammonia (NH_3) transport between tissues, and pH homeostasis [26]. High blood glutamine is an indicator of increased blood ammonia, which can be due to inherited or acquired metabolic disorders. Hyperamonemia can be seen in fatty acid oxidation disorders, organic aciduria and urea cycle disorders. However, in MS/MS-NBS, abnormal patterns of acylcarnitines will detect disorders of fatty acid oxidation disorders or organic aciduria, differentiating them from UCDs. The presence of hyperglutaminemia in hyperammonemic patients with UCDs has been known since the earliest case reports and is another manifestation of the disorder of nitrogen homeostasis [17]. In our retrospective study, among the 10 children with early-onset OTCD, all had glutamine concentrations in DBS higher than the cutoff value. Meanwhile, only 2 of 11 children with late-onset type had hyperglutaminemia, the rest had glutamine concentrations within the reference interval. Thus, glutamine may be a better marker than citrulline in screening for OTCD, but it is only sensitive to early-onset form, and it is almost impossible to detect patients with late-onset OTCD despite clinical manifestation. However, hyperglutaminemia, a sign of hyperammonenia and screening marker for all UCDs, cannot differentiate between OTCD and other UCDs. Therefore, the pattern of hyperglutaminemia and hypocitrulinemia/normal citrullinemia should be used to differentiate proximal UCDs from distal UCDs. Our retrospective data demonstrates that high blood glutamine was sensitive in detecting early-onset OTCD in high-risk patients. However, it may be not appropriate for detecting late-onset OTCD, as its concentration usually fell within reference interval even when the patient had clinical symptoms. We should be

aware of that scenario in high-risk screening, and make further investigations such as urine organic acid and/or mutation analysis when clinical manifestation or family history suggests OTCD. The combination of glutamine and citrulline concentrations helps distinguish between proximal and distal UCDs. High blood glutamine levels combined with high citrulline or arginine or argininosuccinate can detect distal urea cycle disorder. Conversely, increased blood glutamine levels and decreased or normal blood citrulline levels are a pattern suggestive of proximal urea cycle disease including CPS1, NAGS and OCTD. If screening can measure orotic acid in dried blotted blood samples, OCTD can be determined directly from the screening results [27]. However, most current newborn screening programs do not include measurement of orotic acid in dried blood samples, so urinary organic acid analysis is necessary for this differentiation. Increased urinary excretion of orotic acid and uracil aids in distinguishing OCTD from CPS1 and NAGS.

To investigate whether amino acid ratios can increase the sensitivity of OTCD detection, five amino acid ratios recommended by R4S were calculated for 21 OTCD patients [12]. The result showed that Cit/Phe ratio decreased, Gln/Cit and Met/Cit ratios increased in all early-onset OTCD cases. Two other ratios (i.e., Cit/Arg and Glu/Cit) overlapped between the disease and control groups. It seems that ratios do not improve the possibility of using amino acids alone in screening OTCD in high-risk patients, even in early-onset form. However, three of them (Cit/Phe, Gln/Cit and Met/Cit) had similar sensitivity to glutamine level in early-onset OCTD detection. Through retrospective evaluation, we found that among several amino acids, the glutamine level had the highest possibility in early-onset OTCD detection in high-risk patients. However, the combination of abnormal amino acid pattern and ratios may provide more evidence suggesting proximal UCDs such as CPS1, OTCD, and NAGS and differentiate them from distal UCDs.

Although there is controversy regarding the clinical outcomes of NBS for proximal UCDs in the general population [10], MS/MS screening remains valuable for high-risk patients, especially in the early-onset form. Screening using MS/MS can provide results within 24–48 h of sample collection. For infants with early-onset genetic metabolic disorders, screening results guide further investigations to quickly obtain an accurate diagnosis, from which clinicians can take appropriate treatment for the patient. With UCDs, MS/MS screening in early-onset patients will distinguish proximal and distal UCDs, thereby guiding more investigations such as urinary organic acid analysis, determination of blood amino acid levels, and especially gene mutation analysis or enzyme activity measurements to confirm the diagnosis. Even if the patient's treatment outcome is not as expected, early definitive diagnosis as well as future genetic counseling for the child's parents will also benefit the family and community.

One limitation of our study is that DBSs from early-onset and late-onset OTCD cases were collected when the patients were symptomatic, not during the newborn period before the onset of symptoms. However, most NBS programs currently recommend blood collection at 24–72 h of age. Newborns with neonatal-onset OTCD often develop hyperammonemia 2–4 days after birth. In fact, it is not easy to ensure that blood samples are collected in early-onset cases before the onset of symptoms. Our preliminary results provide evidence that amino acid and acylcarnitine analysis by MS/MS in symptomatic early-onset OTCD cases can be an effective tool for screening this disease and guiding further investigation to quickly obtain a final diagnosis. In our hospital, the effective tool for diagnosing OTCD in positive cases detected by high-risk screening is organic acid analysis using GC/MS. Our results also emphasize that symptomatic late-onset OTCD cases might not be detected by DBS analysis using MS/MS. Therefore, it can be extrapolated that OTCD-NBS for late-onset cases in the general neonatal population may not be feasible and should not be recommended.

Another limitation of our study is the lack of genetic mutation analysis for all OTCD patients. Currently, genetic testing for OTCD is available in our hospital. However, the cost of genetic diagnosis remains a challenge for most patients in developing countries as it is not covered by health insurance. Compared to MS/MS and urine organic acid analysis used for screening and diagnosing OTCD, the cost of genetic diagnosis is about three times higher. Additionally, the turnaround time for gene mutation analysis is approximately three weeks, much longer than that of MS/MS and urine organic acid analysis, which have turnaround times of about 2–3 days and less than 7 days, respectively. While mutation analysis plays an important role in confirming diagnosis and genetic counseling for inherited diseases, improvements are needed for timely diagnosis to start proper treatments.

5. Conclusion

Through a preliminary retrospective evaluation of amino acids and their ratios measured by MS/MS in ten early-onset and eleven late-onset OTCD patients at the onset time, we found that hyperglutaminemia in combination with low or normal citrulline concentrations and Gln/Cit, Met/Cit, Cit/Phe ratios can be reliable markers for screening early-onset proximal UCDs in high-risk patients. However, they were not sensitive enough to screen for the late-onset form, even in symptomatic patients. Therefore, further investigations such as urine organic acid and/or mutation analysis should be performed when clinical manifestations or family history suggest OTCD.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Dien Minh Tran: Writing – review & editing, Supervision, Resources, Project administration. **Trang Thi Thu Tran:** Writing –

review & editing, Investigation, Formal analysis, Data curation. **Quyen Hue Luong:** Writing – review & editing, Investigation, Data curation. **Mai Thi Chi Tran:** Writing – review & editing, Writing – original draft, Validation, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are indebted to the patients and their families for their consent to participate in the study. The authors would also like to thank the Labsystems Diagnostics team for their support in English editing.

References

[1] Mew A., Simpson K.L., Gropman A.L., Lanpher B.C., Chapman K.A., Summar M.L., Urea Cycle Disorders Overview, 2003 Apr 29 [Updated 2017 Jun 22]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1217/2017.
[2] J.L. Merritt II, L.L. Brody, G. Pino, P. Rinaldo, Newborn screening for proximal urea cycle disorders: current evidence supporting recommendations for newborn screening, Mol. Genet. Metabol. 124 (2) (2018) 109–113.
[3] B. Seker Yilmaz, J. Baruteau, N. Arslan, H.I. Aydin, M. Barth, A.E. Bozaci, A. Brassier, E. Canda, A. Cano, E. Chronopoulou, Three-country snapshot of ornithine transcarbamylase deficiency, Life 12 (11) (2022) 1721.
[4] S.W. Brusilow, N.E. Maestri, Urea cycle disorders: diagnosis, pathophysiology, and therapy, Adv. Pediatr. 43 (1) (1996) 127–170.
[5] N. Nagata, I. Matsuda, K. Oyanagi, Estimated frequency of urea cycle enzymopathies in Japan, Am. J. Med. Genet. 39 (2) (1991) 228–229.
[6] M.L. Summar, S. Koelker, D. Freedenberg, C. Le Mons, J. Haberle, H.-S. Lee, B. Kirmse, T.E. Registry, M.o.t.U.C.D. Consortium, The incidence of urea cycle disorders, Mol. Genet. Metabol. 110 (1–2) (2013) 179–180.
[7] M. Couchet, C. Breuillard, C. Corne, J. Rendu, B. Morio, U. Schlattner, C. Moinard, Ornithine transcarbamylase–From structure to metabolism: an update, Front. Physiol. 12 (2021) 748249.
[8] S. Matsumoto, J. Häberle, J. Kido, H. Mitsubuchi, F. Endo, K. Nakamura, Urea cycle disorders—update, J. Hum. Genet. 64 (9) (2019) 833–847.
[9] S. Gobin-Limballe, C. Ottolenghi, F. Reyal, J.B. Arnoux, M. Magen, M. Simon, A. Brassier, F. Jabot-Hanin, P.D. Lonlay, C. Pontoizeau, OTC deficiency in females: phenotype-genotype correlation based on a 130-family cohort, J. Inherit. Metab. Dis. 44 (5) (2021) 1235–1247.
[10] V. Rüfenacht, J. Häberle, Mini-review: challenges in newborn screening for urea cycle disorders, Int. J. Neonatal Screen. 1 (1) (2015) 27–35.
[11] T. Vasquez-Loarte, J.D. Thompson, J.L. Merritt, Considering proximal urea cycle disorders in expanded newborn screening, Int. J. Neonatal Screen. 6 (4) (2020) 77.
[12] D. McHugh, C.A. Cameron, J.E. Abdenur, M. Abdulrahman, O. Adair, S.A. Al Nuaimi, H. Åhlman, J.J. Allen, I. Antonozzi, S. Archer, Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: a worldwide collaborative project, Genet. Med. 13 (3) (2011) 230–254.
[13] R.W. Peake, T. Law, P.N. Hoover, L. Gaewsky, A. Shkreta, M.D. Kellogg, Improved separation and analysis of plasma amino acids by modification of the MassTrak™ AAA Solution Ultraperformance® liquid chromatography method, Clin. Chim. Acta 423 (2013) 75–82.
[14] M. Kimura, T. Yamamoto, S. Yamaguchi, Automated metabolic profiling and interpretation of GC/MS data for organic acidemia screening: a personal computer-based system, Tohoku J. Exp. Med. 188 (4) (1999) 317–334.
[15] C. Cavicchi, S. Malvagia, G. La Marca, S. Gasperini, M. Donati, E. Zammarchi, R. Guerrini, A. Morrone, E. Pasquini, Hypocitrullinemia in expanded newborn screening by LC–MS/MS is not a reliable marker for ornithine transcarbamylase deficiency, J. Pharmaceut. Biomed. Anal. 49 (5) (2009) 1292–1295.
[16] T. Lee, K. Yoshii, S. Yoshida, T. Suga, K. Nakamura, H. Sasai, K. Murayama, H. Kobayashi, Y. Hasegawa, Y. Takeshima, Retrospective evaluations revealed pre-symptomatic citrulline concentrations measured by newborn screening were significantly low in late-onset ornithine transcarbamylase deficiency patients, Clin. Chim. Acta 510 (2020) 633–637.
[17] N.E. Maestri, K.D. McGowan, S.W. Brusilow, Plasma glutamine concentration: a guide in the management of urea cycle disorders, J. Pediatr. 121 (2) (1992) 259–261.
[18] A. Pandya, R. Koch, F. Hommes, J. Williams, N-acetylglutamate synthetase deficiency: clinical and laboratory observations, J. Inherit. Metab. Dis. 14 (5) (1991) 685–690.
[19] A. Morris, S. Richmond, S. Oddie, M. Pourfarzam, V. Worthington, J. Leonard, N-acetylglutamate synthetase deficiency: favourable experience with carbamylglutamate, J. Inherit. Metab. Dis. 21 (8) (1998) 867–868.
[20] A. Naini, P. Kaufmann, S. Shanske, K. Engelstad, C. Darryl, E.A. Schon, Hypocitrullinemia in patients with MELAS: an insight into the “MELAS paradox”, J. Neurol. Sci. 229 (2005) 187–193.
[21] B. Parfait, P. De Lonlay, J. von Kleist-Retzow, V. Cormier-Daire, D. Chretien, A. Rötig, D. Rabier, J. Saudubray, P. Rustin, A. Munnich, The neurogenic weakness, ataxia and retinitis pigmentosa (NARP) syndrome mtDNA mutation (T8993G) triggers muscle ATPase deficiency and hypocitrullinaemia, Eur. J. Pediatr. 158 (1999) 55–58.
[22] M.R. Baumgartner, C.-a.A. Hu, S. Almashanu, G. Steel, C. Obie, B. Aral, D. Rabier, P. Kamoun, J.-M. Saudubray, D. Valle, Hyperammonemia with reduced ornithine, citrulline, arginine and proline: a new inborn error caused by a mutation in the gene encoding Δ1-pyrroline-5-carboxylate synthase, Hum. Mol. Genet. 9 (19) (2000) 2853–2858.
[23] P. Crenn, C. Coudray–Lucas, F. Thuillier, L. Cynober, B. Messing, Postabsorptive plasma citrulline concentration is a marker of absorptive enterocyte mass and intestinal failure in humans, Gastroenterology 119 (6) (2000) 1496–1505.
[24] J.M. Rhoads, E. Plunkett, J. Galanko, S. Lichtman, L. Taylor, A. Maynor, T. Weiner, K. Freeman, J.L. Guarisco, G.Y. Wu, Serum citrulline levels correlate with enteral tolerance and bowel length in infants with short bowel syndrome, J. Pediatr. 146 (4) (2005) 542–547.
[25] G. Wu, L.A. Jaeger, F.W. Bazer, J.M. Rhoads, Arginine deficiency in preterm infants: biochemical mechanisms and nutritional implications, J. Nutr. Biochem. 15 (8) (2004) 442–451.
[26] V. Cruzat, M. Macedo Rogero, K. Noel Keane, R. Curi, P. Newsholme, Glutamine: metabolism and immune function, supplementation and clinical translation, Nutrients 10 (11) (2018) 1564.
[27] O. Staretz-Chacham, S. Daas, I. Ulanovsky, A. Blau, N. Rostami, T. Saraf-Levy, N. Abu Salah, Y. Anikster, E. Banne, D. Dar, The role of orotic acid measurement in routine newborn screening for urea cycle disorders, J. Inherit. Metab. Dis. 44 (3) (2021) 606–617.