

Clinical and Biochemical Profiles of Filipino Patients with Distal Urea Cycle Disorders Detected by Abnormal Expanded Newborn Screening

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ABSTRACT

Objective. The study is a retrospective review which provides preliminary data on the correlation between biochemical profiles and initial clinical manifestation of patients diagnosed to have argininosuccinate synthetase deficiency (ASSD) and argininosuccinate lyase deficiency (ASLD) detected by expanded newborn screening (ENBS).

Methods. This is a study of five distal UCD patients initially detected by elevated citrulline on ENBS. Medical charts of the patients were reviewed. The initial clinical manifestations of the patients were correlated with results of biochemical tests.

Results. There were four cases of ASLD and one case of ASSD reviewed in this study. All cases of ASLD were confirmed by the presence of argininosuccinic acid (ASA) in the urine metabolic screen (UMS). The plasma citrulline level of the ASSD patient is significantly elevated as compared to the ASLD patients (2,690 $\mu\text{mol/L}$; NV: 10-45 $\mu\text{mol/L}$). The ASSD patient and one ASLD patient were symptomatic within the first six days of life. Both presented with significantly elevated plasma ammonia, citrulline and glutamine levels. Three ASLD patients were asymptomatic on initial screening.

Conclusion. ENBS has shown importance in the early detection and management of ASSD and ASLD. Early initiation of management may prevent hyperammonemic crises. Long term outcome studies are needed to look into the correlation of neurodevelopmental outcome with lifelong accumulation of citrulline and glutamine in ASSD and ASA in ASLD.

Key Words: expanded newborn screening (ENBS), argininosuccinate synthetase deficiency (ASSD), argininosuccinate lyase deficiency (ASLD), argininosuccinic acid (ASA)

INTRODUCTION

Urea cycle disorders (UCD) comprise a rare group of inborn error of metabolism resulting from a defect in the nitrogen waste-disposal system responsible for the conversion of ammonia to urea.¹ Defects in this pathway result in hyperammonemia that will result into neurologic sequelae.^{1,2,3} Acute hyperammonemia presents with encephalopathy that may progress to brain edema and death.⁴ In a multicenter study of UCD patients, it was found that intellectual and developmental disabilities were reported the most, followed by learning disability.⁵

Urea cycle disorders may be categorized as either proximal or distal (Figure 1). Proximal UCDs is the absence or lack of mitochondrial enzymes involved in the cycle. Distal UCDs, on the other hand, is the deficiency of cytosolic enzymes involved in the urea cycle. This includes

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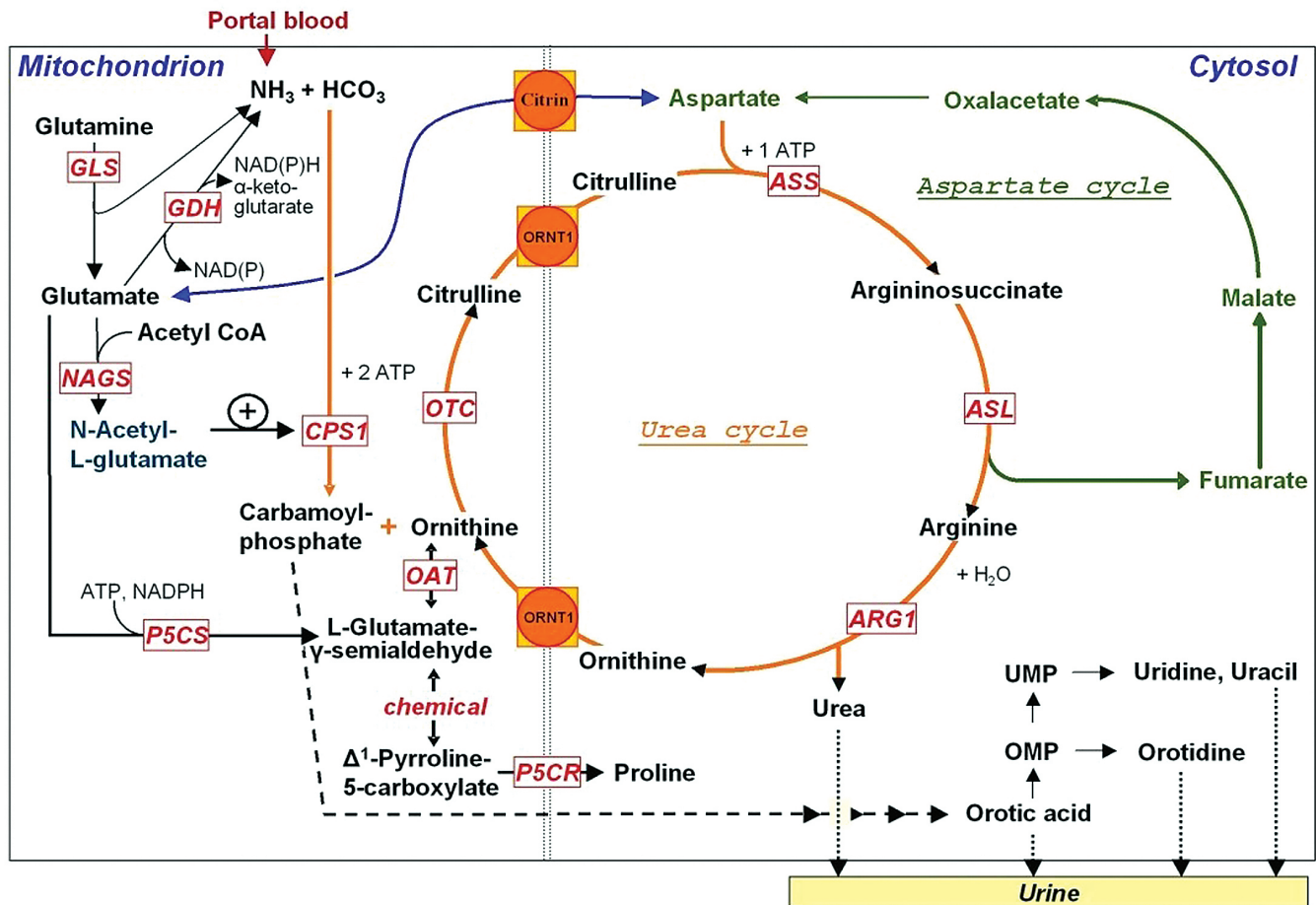


Figure 1. The urea cycle and associated pathways. Urea cycle disorders (UCDs) result from inherited deficiency in any one of the six enzymes or transporters of the urea cycle pathway. Proximal UCDs is the lack or absence of mitochondrial enzymes. This include N-acetyl-L-glutamate synthetase (NAGS), carbamoylphosphate synthetase 1 (CPS1), and ornithine transcarbonylase (OTC). Distal UCDs, on the other hand, is the lack or absence of enzymes in the cytosol. This include argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL) and arginase 1 (ARG1). In an enzyme deficiency, there is an increase in the metabolite proximal to the enzyme.

Figure reprinted from Häberle J, Boudaert N, Burlina A, Chakrapani A, Dixon M, Huemer M, et al. Suggested guidelines for the diagnosis and management of urea cycle disorders. *Orphanet J Rare Dis.* 2012 May; 7:32. Article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>).

argininosuccinate synthetase deficiency (ASSD) (OMIM #603470) and argininosuccinate lyase deficiency (ASLD) (OMIM #608310). Studies have shown that patients with ASSD and ASLD are equally or more severely impaired as compared to the proximal UCDs. In the case of ASLD, the clinical phenotype appears to be unrelated to the severity and duration of hyperammonemic episodes.^{6,7}

Prior to 2014, the newborn screening panel in the Philippines was limited to six disorders – congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH), phenylketonuria (PKU), glucose-6-phosphate dehydrogenase deficiency (G6PD) and maple syrup urine disease (MSUD).⁸ Expanded newborn screening (ENBS) has been available in the Philippines since 2014 and has allowed screening for 28 disorders. ASSD was the first urea cycle disorder included in the panel.⁹ Citrulline is used as a primary marker for ASSD

and ASLD in Tandem Mass Spectrometry.^{10,11,12} ASLD was recently included in the ENBS panel of the Philippines.

In the study by Padilla and de la Paz,¹³ there was one ASLD patient in the metabolic registry of the Biochemistry laboratory, Institute of Human Genetics. This patient was diagnosed before 2014, the year ENBS was initiated. Currently, there are five ASLD patients and one ASSD patient in the registry (Personal communication, Biochemistry laboratory). In the Philippine setting, plasma amino acid profile and comprehensive urine metabolic screen (UMS) are done to confirm a UCD diagnosis. The presence of argininosuccinic acid (ASA) in the UMS is the biochemical hallmark of ASLD.^{7,14} On the other hand, plasma amino acid levels of patients with ASSD reveal an ammonia of $\geq 1,000 \mu\text{mol/L}$, glutamine $>1,000 \mu\text{mol/L}$ and citrulline often $> 2,000 \mu\text{mol/L}$.^{3,15}

Table 1. Summary of biochemical findings of patients diagnosed with distal urea cycle disorders

Patient number	ENBS Collection (Day of life)	Expanded Newborn Screening				Plasma Ammonia μmol/L
		Citr μmol/L	Citr/Phe	Citr/Arg	Citr/Tyr	
1	1	84.3	1.48	NA	1 (≤0.45)	53
2	4	87.25	1.76	8.5	NA	31
3	6	186.12	6.32	21.31	NA	500
4	1	74.4	1.37	5.93	NA	94
5	5	3,123.49	38.47	243.83	NA	745
Reference value		<60	<1.1	<19.61	≤0.45	9 to 33
Mean		3.4	711.11	9.88	69.89	284.6
Standard Deviation		2.30	1349.32	16.12	116.15	321.19

*Reference range values for plasma amino acid for age group 31 days to 23 months. Sample was taken on the 33rd day of life.

The Urea Cycle Disorders longitudinal study, a multicenter investigation, has shown that 25% of ASSD was detected by ENBS as compared to 29% of ASLD.¹⁶ A 27-year follow up study done in Austria showed a favorable outcome of ASLD patients identified by NBS. Patients were asymptomatic until 4 weeks of life or later when diagnosis and treatment was initiated.¹⁷ In another study done in Massachusetts, 13 patients diagnosed to have ASLD by newborn screening had a relatively mild clinical course due to early diagnosis and treatment.¹⁸

This study reviewed the clinical and biochemical profile of Filipino patients diagnosed to have argininosuccinate synthetase deficiency (ASSD) and argininosuccinate lyase deficiency (ASLD) detected by ENBS and confirmed by biochemical investigations. This can be used as preliminary data on the correlation between biochemical findings and initial clinical manifestations of the two mentioned disorders in the Filipino population.

METHODS

Description of Study Setting

The Institute of Human Genetics, National Institutes of Health (IHG-NIH), University of the Philippines, Manila is the unit that co-manages and receives referrals from the different newborn screening centers. Cases were identified from the records of confirmed cases of the Biochemistry Laboratory, IHG-NIH.

Subjects

This study involved a chart review of five Filipino patients confirmed to have argininosuccinate synthetase deficiency (ASSD) and argininosuccinate lyase deficiency (ASLD) from December 2014 to September 2018. The diagnosis was based on elevated citrulline in ENBS and confirmed by plasma and urine amino acids and urine organic acid. This study was reviewed and approved by the University of the Philippines Manila Research Ethics Board (UPMREB Number 2018-459-01).

Clinical and Biochemical Characteristics

Data on the patient's date of birth, day of collection of samples for ENBS and initial presenting symptoms were obtained from the Clinical Genetics Unit, IHG-NIH and records of attending metabolic specialist and clinical geneticist. Initial plasma ammonia, plasma amino acid profile, urine organic acid, and/or UMS were reviewed. For all patients, results of the plasma amino acid and UMS were obtained from the Biochemistry laboratory, IHG-NIH.

Clinical and biochemical data collected were transferred in a password-protected Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) spreadsheet file. Mean and standard deviation were obtained for the plasma ammonia and plasma amino acid values.

RESULTS

Demographic and Biochemical Findings

There were five patients diagnosed to have a distal urea cycle disorder through ENBS: one argininosuccinate synthetase deficiency (ASSD) and four argininosuccinate lyase deficiency (ASLD). The five patients were distributed in the islands of Luzon and Visayas. The case of ASSD was from Guimaras, an island in Western Visayas. Three of the ASLD patients were from Luzon (one from Ilocos Norte, one from Bulacan and one from Manila). The fourth ASLD patient was from Capiz, a province in Visayas.

Table 1 shows the biochemical findings of the five patients with distal UCD. ENBS was done on an average of 3.4 days (range one to six days). In two patients, 6-test newborn screening (NBS) was initially done which yielded normal results. ENBS was only done when they already presented with signs and symptoms of UCD. The patients' citrulline levels on ENBS ranged from 74.4 to 3,123.49 (NV <60 μmol/L). All patients had elevations in at least one secondary analyte (citrulline/phenylalanine ratio). Three patients had elevations in secondary analytes (citrulline/phenylalanine and citrulline/arginine ratios).

Plasma Amino Acid				Urine Metabolic Screen	Diagnosis
Citrulline μmol/L	Arginine μmol/L	Ornithine μmol/L	Glutamine μmol/L		
235	15	13	693	Argininosuccinic acid and anhydrides	ASLD
95*	43*	63*	397*	Argininosuccinic acid and anhydrides	ASLD
115	16	18	1120	Argininosuccinic acid and anhydrides	ASLD
				Argininosuccinic acid and anhydrides	ASLD
2690	28	24	1917	Increased intensity of bands at levels of glutamine and citrulline is suggestive of citrullinemia	ASSD
*3 to 45 10 to 45	*12 to 133 6 to 140	*22 to 103 48 to 211	*246 to 1182 340 to 940		
783.75	25.5	29.5	1031.75		
1272.34	13.08	22.78	660.58		

Table 2. Summary of clinical findings of patients diagnosed with distal urea cycle disorders

Patient number	Sex	Pediatric Aging	Ethnicity	Consanguinity	Initial Clinical Presentation and Age of Presentation	Management	Outcome and current status	Current age	Diagnosis
1	Male	38	Filipino	non-consanguineous	Asymptomatic	a, b, c	Alive; Developmentally at par	11 months	ASLD
2	Male	40	Filipino	non-consanguineous	Asymptomatic	a, b	Alive; Developmentally at par	9 months	ASLD
3	Male	38	Filipino	non-consanguineous	Poor feeding Irritability hyperthermia on the 6 th day of life	a, d, e, f	Died at 11 months for metabolic decompensation secondary to acute gastroenteritis		ASLD
4	Male	39	Filipino	non-consanguineous	Asymptomatic	a, b, c	Alive; Ongoing physical therapy for gross motor delay	1 year 9 months	ASLD
5	Male	38	Filipino	non-consanguineous	Poor feeding Increased sleeping time on the 5 th day of life	d*	Died during the neonatal period		ASSD

a - Protein limitation; b - Oral sodium benzoate; c - Oral L-arginine; d - High dextrosity fluid and/or lipid emulsion; e - Intravenous sodium benzoate; f - Intravenous L-arginine. * - Peritoneal dialysis, protein limitation, intravenous sodium benzoate and L-arginine were recommended for the patient.

Initial plasma ammonia levels for the patients ranged from 31-745 μmol/L (NV: 9-33 μmol/L). Citrulline levels were elevated for four patients, with an average level of 783.75 μmol/L (Range: 95-2,690 μmol/L; NV: 10-45 μmol/L). The citrulline of the patient with ASSD is significantly elevated at 2,690 μmol/L. Glutamine values were also elevated for patients 3 and 5. Patient 5 had the highest glutamine value of 1,917 μmol/L (NV: 340-910 μmol/L). Ornithine and arginine levels were within reference range values; with average values of 25.5 μmol/L (NV: 48-210 μmol/L) and 29.5 μmol/L (NV: 6-140 μmol/L), respectively.

Comprehensive urine amino and organic acid profiles are part of the confirmatory test for urea cycle disorders. Patient 5, a case of ASSD, showed presence of orotic acid in urine organic acid analysis. The four patients with ASLD had non-specific findings. For all patients, urine amino acids were analyzed by high volume electrophoresis. The presence of ASA and its anhydrides confirmed the diagnosis of ASLD

in the four patients. For patient 5, increased intensity of bands at levels of glutamine and citrulline was suggestive of the diagnosis. This was consistent with the patient's elevated plasma citrulline and glutamine levels.

Clinical Presentation

Table 2 shows the clinical presentation of patients with distal UCD. All patients in this study were males. All were full-term and were born to non-consanguineous unions of Filipino descent. Patient 3 and patient 5 were symptomatic within the first six days of life with elevated plasma ammonia at 500 μmol/L and 745 μmol/L, respectively. Patient 3 presented with poor feeding, irritability and hyperthermia on the 6th day of life. Patient 5, on the other hand, exhibited poor feeding and increased sleeping time on the 5th day of life.

Medical management for acute metabolic decompensation was initiated for patient 3, a case of ASLD. This included initially placing the patient on protein rest and

increasing total caloric intake. Total caloric intake was increased with high dextrosity intravenous fluid and lipid emulsion. Patient was also started on intravenous sodium benzoate and L-arginine. He was discharged after 31 hospital days. Medical management and peritoneal dialysis were recommended for patient 5 on the basis of significantly elevated plasma ammonia level and deteriorating clinical status. However, caregivers opted to bring the patient home instead. The patient died during the neonatal period.

Patients 1, 2 and 4 were asymptomatic on initial referral. Initial plasma ammonia values of the 3 patients ranged from 31-94 $\mu\text{mol/L}$ (NV: 9-33 $\mu\text{mol/L}$). Medical management for urea cycle disorder was initiated on outpatient basis. Initial management included protein restriction at 1-1.5 mg/kg/day. L-arginine was started at 92-100 mg/kg/dose. For patients 1 and 4, sodium benzoate was initiated at 100 mg/kg/day. Patients 1 and 2 are on regular follow-up with the metabolic service of the Philippine General Hospital (UP-PGH). Patient 4 is on regular follow up with a metabolic specialist. The oldest living patient is now one year and nine months, while the youngest patient is nine months old. Since diagnosis, the three patients never had an episode of metabolic crisis. Despite the absence of a metabolic crisis, patient 4 is ongoing physical therapy for gross motor delay. He is also being seen by a Developmental Pediatrician for co-management.

DISCUSSION

This is the first review of diagnosed cases of distal urea cycle disorders initially detected by expanded newborn screening (ENBS) in the Philippines. The national coverage of ENBS in the Philippines is only 12.8% as of December, 2018.¹⁹ Since ENBS was initiated in 2014, there have been four diagnosed cases of ASLD and one diagnosed case of ASSD out of 665,578 babies screened.²⁰

Urea cycle disorders may present as a neonatal onset or late onset form. Patients with the neonatal onset form appear normal for 24-48 hours and then exhibit non-specific signs and symptoms such as progressive lethargy, hypothermia and apnea. The signs and symptoms are related to elevated plasma ammonia levels.^{1,3} Ammonia toxicity is thought to cause cytotoxic brain edema and astrocyte swelling through a variety of mechanisms.²¹ Hyperammonemia may result into the perturbation of potassium buffering in the astrocytes, altered γ -aminobutyric acid (GABA)-mediated transmission causing excitotoxicity, and toxicity caused by oxidative stress due to increased free radical formation and increased nitric oxide synthesis.^{1,22,23,24}

In this study, two patients presented with the neonatal onset form. Plasma ammonia level of 150 $\mu\text{mol/L}$ or higher in neonates with associated neurologic signs and symptoms is a strong indication for the presence of a UCD.²¹ As compared to ASSD, hyperammonemia is usually less severe in ASLD. Argininosuccinate is freely excreted in the urine,

therefore disposing 4 nitrogen molecules.²¹ It was also previously reported that no UCD patient having had >300 $\mu\text{mol/L}$ initial or >480 $\mu\text{mol/L}$ peak ammonia exhibited normal psychomotor development.^{24,25}

Three patients (patients 1, 2 and 4) were asymptomatic on initial presentation and never had an episode of metabolic decompensation. All three had an initial plasma ammonia < 150 $\mu\text{mol/L}$ (Range: 31-94 $\mu\text{mol/L}$). Based on the patients' clinical presentation, they may have the late onset or partial enzyme deficiency form. For the late onset form, elevation in ammonia is brought about at any age by stressors, such as infection, post-operative state, and high protein diet, that overwhelms the individual's capacity to convert ammonia to urea.^{1,2,3,21}

ENBS and associated biochemical tests were helpful in coming up with a diagnosis and initiating appropriate management. As with the Philippine ENBS program,⁸ citrulline is used as the primary metabolite for distal urea cycle disorders in other programs worldwide.^{10,11,12,26} As the metabolite proximal to enzymes argininosuccinate synthetase and argininosuccinate lyase, citrulline is elevated in cases of ASSD and ASLD. In ASLD, the expected range of plasma citrulline is 100-300 $\mu\text{mol/L}$.^{11,14} However, deficiency of argininosuccinate synthetase in ASSD leads to extreme elevation of citrulline in plasma with expected range of 2,000-5,000 $\mu\text{mol/L}$.²⁷

An increase in plasma citrulline may also induce elevation of glutamine and ammonia, depletion of arginine, and enhanced excretion of orotic acid in urine.^{3,27} Excretion of orotic acid in the urine was seen in patient 5. Glutamine levels are considered tolerable when they do not exceed 1000 $\mu\text{mol/L}$.³ It can be noted that the symptomatic patients in this study had plasma glutamine levels of more than 1000 $\mu\text{mol/L}$. Rising plasma glutamine levels may indicate pending hyperammonemia.³

UMS has allowed the differentiation between ASSD and ASLD in our laboratory. The presence of ASA and its metabolites as an incidental finding in the UMS warranted the inclusion of ASLD in the newborn screening panel. The diagnostic criteria for ASLD is the presence of ASA in the blood or urine.⁵ Argininosuccinate lyase is the enzyme responsible of breaking down argininosuccinate to arginine and fumarate. ASA is not routinely detected in plasma amino acid analysis and can easily be misidentified since it may co-elute with other amino acid, especially leucine. Since ASA is markedly excreted in urine, UMS is helpful in detecting ASLD.^{28,29} Quantitative measurement of urine ASA may be done by column chromatography.¹⁷

Reversal of catabolic state is one of the cornerstones in the management of ASLD and ASSD.^{3,21} Catabolism of protein stores triggers hyperammonemia. An affected child will continue to produce ammonia and will not stabilize until catabolism is reversed.²¹ Catabolism is addressed by increasing the total caloric intake. Caloric supplementation include intravenous high dextrosity fluid and lipid emulsion.

Cessation of protein intake is done for maximum of 48 hours.³ Pharmacologic intervention include administration of ammonia scavenger and L-arginine. Sodium benzoate is an ammonia scavenger that conjugates benzoate with glycine to produce hippurate; urea cycle is bypassed.³ L-arginine, on the other hand, is used to replace arginine not produced by the urea cycle.²¹ Extracorporeal detoxification of ammonia is done for levels >500 µmol/L.³ It can be done for lower ammonia levels if affected individual is grossly encephalopathic and refractory to medical therapy.^{3,21}

ENBS has helped in the early detection and management of distal urea cycle disorders. This is particularly true for cases of late onset UCDs where neonates are asymptomatic due to partial enzyme activity. A study had shown that patients detected to have distal UCD by newborn screening have higher scores for neuropsychological tests as compared to clinically identified cases.³⁰

Despite early intervention for distal UCD cases detected by newborn screening, gross motor delay, expressive language delay and decreased performance IQs are prominent for both ASSD and ASLD.^{17,31} The presence of ASA and its metabolites in ASLD has been implicated in the higher incidence of neurocognitive symptoms, liver fibrosis, renal impairment and systemic hypertension.^{7,14} This will explain the presence of gross motor delay in patient 4 despite early intervention and absence of metabolic crises. It was also noted that for both distal urea cycle disorders, cognitive impairment is present even in the absence of hyperammonemic crises.

In this study, patients were confirmed to have distal UCD through biochemical testing correlated with clinical presentation. However, mutation studies would be ideal since specific mutations are associated with the neonatal and late onset forms.^{30,32} While biochemical studies can establish the diagnosis of the two mentioned disorders, molecular investigations help understand the phenotypic variations and prognosis of the disease. While genetic counselling was documented for the patients, molecular investigations would have provided crucial supporting information regarding pathogenesis and outcome.³

CONCLUSION

ENBS, along with plasma amino acid profile and urine metabolic screen results, have shown importance in the early detection and management of ASSD and ASLD in the local setting. The physician should correlate the patient's clinical presentation and laboratory results to immediately start intervention. Early initiation of management may contribute in the prevention of hyperammonemic crises for late-onset cases of ASSD and ASLD. Long term studies are needed to look into the correlation of neurodevelopmental outcome with lifelong accumulation of ammonia, citrulline and glutamine in our patients with both ASLD and ASSD and ASA in ASLD. Molecular testing is recommended to help understand the phenotypic variation of

the disease and its prognosis. It can also provide information needed for genetic counselling for the families affected.

Statement of Authorship

All authors have approved the final version submitted.

Author Disclosure

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