นิพนธ์ต้นฉบับ

ระดับกรดอะมิโนในพลาสม่าด้วยการตรวจโดยวิธีโครมาโตกราฟีแบบแลกเปลี่ยนไอออน ในเด็กทารกแรกเกิดไทยในภาคตะวันออกเฉียงเหนือ

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บทคัดย่อ

ความเป็นมา: ค่าปกติของกรดอะมิโนในพลาสม่าของทารกมีความสำคัญในการวินิจฉัยโรคพันธุกรรมเมแทบอลิก อย่างไรก็ตามข้อมูลค่าปกติของกรดอะมิโนในพลาสม่าในทารกแรกเกิดในภาคตะวันออกเฉียงเหนือยังจำกัด

วัตถุประสงค์: การศึกษานี้ต้องการหาค่าปกติสำหรับกรดอะมิโนในพลาสม่าของทารกแรกเกิดครบกำหนดชาวไทยที่มีสุขภาพปกติในภาคตะวันออกเฉียงเหนือ

วิธีการศึกษา: ทารกแรกเกิดครบกำหนดสุขภาพปกติในภาคตะวันออกเฉียงเหนือเข้าร่วมในการศึกษา จะได้รับการเจาะเลือด 2 มล.ในหลอดที่มีเฮพารินพร้อมกับการเจาะตรวจคัดกรองทารกแรกเกิดด้วยกระดาษซับเลือดที่อายุ 48-72 ชั่วโมง ระดับกรดอะมิโนในพลาสม่าจะตรวจวิเคราะห์ด้วยวิธีโครมาโตกราฟีแบบแลกเปลี่ยนไอออนพร้อมอนุพันธ์นินไฮดรินหลังคอลัมน์ ข้อมูลที่ได้มีการแจกแจงไม่ปกติจึงใช้เปอร์เซ็นไทล์ที่ 2.5 และ 97.5 ในการกำหนดช่วงค่าปกติของระดับกรดอะมิโนในพลาสม่าของทารกแรกเกิด

ผลการศึกษา: ทารกแรกเกิดครบกำหนดสุขภาพปกติ 42 ราย (มัธยฐานอายุครรภ์ 38 สัปดาห์) เป็นชาย 22 ราย (ร้อยละ 52.4) เข้าร่วมการศึกษานี้ ระดับกรดอะมิโนในพลาสม่าเปรียบเทียบกับการศึกษาในอดีตของประเทศไทยและค่าปกติของรายงานอื่นพบว่าบางชนิดมีความแตกต่างกันซึ่งอาจจะเป็นผลจากวิธีการวิเคราะห์ และกลุ่มอายุที่แตกต่างกันของแต่ละการศึกษา ค่ากรดอะมิโนที่ไม่จำเป็นที่วัดได้ในการศึกษานี้มี

ความแปรปรวนมากกว่าชนิดอื่น การเปรียบเทียบกับช่วงค่ามาตรฐานจากรายงานอื่น ๆ ชี้ให้เห็นว่ากรดอะมิโนในพลาสมาในการศึกษานี้มีความจำเพาะกับกลุ่มประชากร

สรุป: ค่าปกติสำหรับกรดอะมิโนที่ได้จากการศึกษานี้มีความสำคัญสำหรับใช้วินิจฉัยและดูแลรักษาโรคพันธุกรรมเมแทบอลิกในทารกแรกเกิดชาวไทย ความแตกต่างของระดับกรดอะมิโนในการศึกษานี้เมื่อเปรียบเทียบกับการศึกษาในอดีตสะท้อนให้เห็นผลของความแตกต่างจากวิธีการวิเคราะห์ และกลุ่มอายุ ผลการศึกษานี้ชี้ให้เห็นถึงความสำคัญที่จะต้องมีข้อมูลระดับกรดอะมิโนในพลาสม่าที่เป็นค่าปกติที่จำเพาะกับกลุ่มพื้นที่ที่มีลักษณะประชากรและปัจจัยแวดล้อมที่จำเพาะ

คำสำคัญ: กรดอะมิโน, ทารกแรกเกิด, โรคพันธุกรรมเมแทบอลิก, โครมาโตกราฟีแบบแลกเปลี่ยนไอออน

Plasma amino acid profiles by using ion-exchange chromatography in neonates from northeastern Thailand

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Abstract

Background: The reference values for plasma amino acids in neonates are essential for making a diagnosis of inborn errors of metabolism (IEM). However, data on plasma amino acid reference ranges for northeastern Thai neonates remain limited.

Objectives: This study aims to establish reference intervals for plasma amino acids in healthy full-term neonates from northeastern Thailand.

Methods: Healthy full-term neonates from northeastern Thailand were enrolled. Two mL of heparinized blood was collected along with newborn screening blood spots during 48-72 hours of age. Plasma amino acids were analyzed using ion-exchange chromatography with post-column ninhydrin derivatization. Due to non-normal distribution, reference intervals were determined using the non-parametric percentile method, defined by the 2.5th and 97.5th percentiles. Results were compared with previous Thai studies.

Results: There were 42 full-term newborns (median gestational age: 38 weeks), of whom 22 were male (52.4%). Plasma amino acid levels were compared with those from previous Thai studies and other reference ranges. Some amino acid levels differed from previous studies, likely due to differences in analytical methods and age groups. Greater variability was observed in non-essential amino acids. When compared to other published reference values. The plasma amino acid profiles were specific to this population.

Conclusions: The reference ranges established in this study provide valuable data for the diagnosis and management of IEMs in Thai neonates. Differences from previous studies may reflect variations in measurement techniques and age groups. These findings emphasize the importance of region-specific reference data, particularly in populations with distinct demographic and environmental factors.

Keywords: Plasma amino acids, neonate, inborn errors of metabolism, ion-exchange chromatography

Introduction

Plasma amino acid analysis is an essential tool for making diagnosis and monitoring patients with inborn errors of metabolism (IEM).[1] Most patients present nonspecific clinical symptoms during the neonatal period, leading to diagnostic problems due to these ambiguous clinical features.[2] Since 2022, the National Health Security Office of Thailand (NHSO) has approved a benefit package covering the diagnosis and treatment of 24 rare groups of IEMs, providing universal coverage for Thai citizens.[3] The package encompasses various amino acid metabolism disorders such as maple syrup urine disease (MSUD), tyrosinemia types 1, 2, and 3, phenylketonuria, hypermethioninemia, and urea cycle defects, including argininemia and citrullinemia type 1 and 2. Responsibility for the diagnosis and treatment of these diseases falls under the Rare Diseases Centers. Srinagarind Hospital is one of the seven Rare Diseases Centers, and the only one located outside Bangkok, in the northeastern part of Thailand.

Plasma amino acid profile plays a crucial role in the diagnosis of IEM. However, it is essential that these laboratory findings are interpreted in conjunction with the patient's clinical manifestations. Although ion-exchange chromatography (IEC) with post-column ninhydrin derivatization and high-performance liquid chromatography (HPLC) are commonly used for plasma amino acid analysis, normal reference ranges vary across laboratories, age groups, and ethnicities.[4–7] Only three studies have documented normal plasma amino acid levels in the Thai population. Sirichakwal et al.[8] provided data on normal plasma amino acid levels in 136 Thai individuals aged 1 to 45 years, while Svasti et al.[9] reported plasma amino acid levels in 15 infants under six months of age. Additionally, Uaariyapanichkul et al.[10] examined plasma amino acid levels in 277 healthy infants and children aged 0 to 12 years. Despite previous studies, reference ranges of plasma amino acid levels in neonates remain limited. Establishing normal reference values for Thai neonates is crucial, as this age group is most commonly diagnosed with IEM. This study focuses on neonates from northeastern Thailand, as the reference values will be clinically applied at Srinagarind Hospital, the designated IEM referral center for the region under NHSO policy. Therefore, the objective of this study is to determine plasma amino acid levels and establish the reference intervals for neonates in northeastern Thailand.

Methods

Participants:

The inclusion criteria were northeastern Thai neonates with a gestational age between 37 to 42 weeks and a birth weight of 2,000 to 4,000 g. All participants with clinical sepsis, hypoglycemia,

intrauterine growth retardation, small or large for gestational age, birth asphyxia, receiving total parenteral nutrition, not receiving milk or infant formula, or a family history of IEM were excluded from this study. The current CLSI-approved guideline on Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory (C28-A3) recommends a non-parametric method for estimating reference intervals (RIs) with a minimum of 120 samples for optimal statistical confidence. Due to budget constraints and in alignment with previous studies, we enrolled 42 participants, which meets the CLSI minimum requirement of 39 samples for determining the 2.5th and 97.5th percentiles with 90% confidence.[11]

Laboratory method:

Two mL heparinized blood samples were collected from the neonates simultaneously with the acquisition of blood filter paper during newborn screening (48-72 hours after birth). If the sample could not be analyzed on the day of collection, the plasma was separated by centrifugation and stored at -20°C. The blood samples were centrifuged to separate plasma from cellular components. The samples were analyzed according to the protocol for plasma amino acids analysis by Bichrom30+ (Biochrom Ltd., Cambridge Science Park, England). Plasma samples were first deproteinized using 5% sulfosalicylic acid and then centrifuged to remove precipitated proteins. The supernatant was loaded onto a cation-exchange resin column, where amino acids were separated based on their ionic properties using a series of lithium buffer gradients. Post-column, amino acids were derivatized with ninhydrin reagent, producing colored complexes that were detected photometrically at 440 nm (for proline and hydroxyproline) and 570 nm (for primary amino acids). The results were quantified by comparing peak areas to known amino acid standards as shown in Figure 1. Each amino acid peak in the chromatogram amino acid was separated based on its retention time. The individual amino acid concentrations were calculated using the EZChrom Elite Data Handling 21 CFR part 11 compliant software (version 3.3.2), based on their peak areas, which are directly proportional to the amino acid concentration in the sample. Intra- and inter-assay coefficient of variation (CV) assessments were not performed due to the system's throughput limitation of 8 samples per run and budget constraints. However, the instrument's performance was regularly monitored using internal quality control samples to ensure analytical reliability.

Statistical analysis:

Descriptive statistics, including mean, median, standard deviation (SD), and range, were used to summarize plasma amino acid levels. Reference intervals were determined using the non-parametric percentile method, with the 2.5th and 97.5th percentiles defining the lower and upper limits, following CLSI C28-A3 guidelines. Due to the non-normal distribution of data, we applied non-

parametric methods where appropriate. We compared plasma amino acid levels with previously published studies to evaluate variability across populations.

Ethical considerations:

This study was approved by the Khon Kaen University Ethics Committee for Human Research, Panel 1, under approval number HE601103. This work was supported by the Faculty of Medicine, Khon Kaen University, Thailand [Grant Number IN63245]. We obtained consent forms from the participants' parents after providing them with full information.

Results

Participant Characteristics:

A total of 42 neonates participated in this study, 22 of whom were males (52.4 %). The median gestational age was 38 weeks. The mean and median birth weights were 3,083 and 3,070 g, respectively (range: 2,220-4,010 g.).

Plasma Amino Acid Levels:

The dual-wavelength amino acid chromatograms were demonstrated in Figure 1. The amino acid profiles were displayed as a box plot in Figure 2. The mean, median, standard deviation (S.D.) range and 95% confidence interval (95% CI) were presented in Table 1. The most abundant amino acids were glutamine, glycine, alanine, and proline, with glutamine showing the widest range (125.0–1059.8 μmol/L), followed by glutamic acid and glycine, indicating high inter-individual variability. Non-essential amino acids exhibited broader variability compared to essential amino acids, while branched-chain amino acids (BCAAs) such as leucine, isoleucine, and valine showed more consistent levels. This study confirmed that glutamine had the highest plasma concentration, while cystine and 3-methylhistidine were the least abundant amino acids.

When compared to previously published reference ranges, this study found notable age-related and methodological differences. Amino acid levels in neonates differed significantly from older infants, as observed in previous studies. Several amino acids, including glutamine, glycine, and proline, were higher in younger neonates, consistent with previous findings that amino acid concentrations decreased with age.[10] Methodological variations also contributed to discrepancies, as this study used IEC, while previous studies employed MS/MS, HPLC, or other techniques. The levels of BCAA and aromatic amino acids were comparable to those reported by Wu et al.[7], while citrulline and arginine levels showed considerable variation across studies, likely due to differences in fasting status, sample collection timing, and analytical

methods. The reference plasma amino acid ranges in our study compared with other references were exhibited in Table 2.

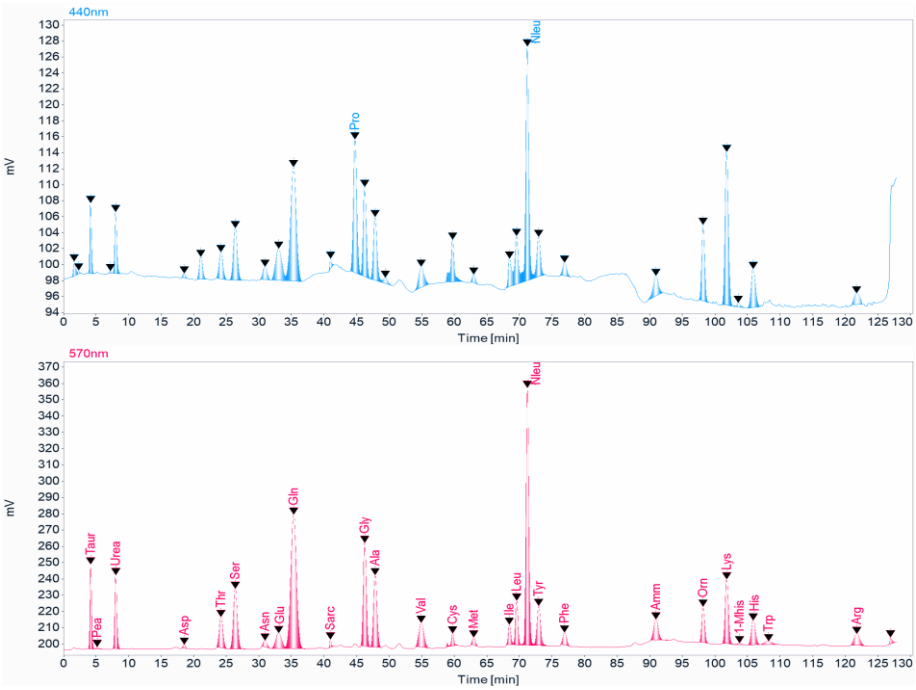

Figure 1. The Dual-wavelength amino acid chromatograms were shown in different retention times.

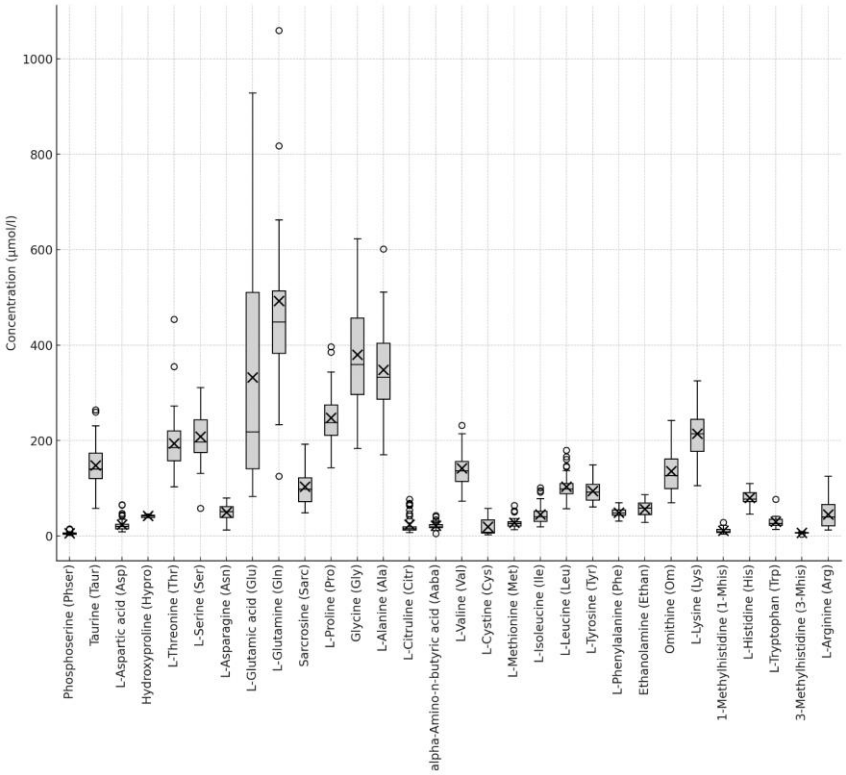

Figure 2. Box plot of amino acid analysis in this study

Table 1. Plasma amino acid profiles in this study (n = 42)

Amino acid (µmol/L)	Mean	Median	Std. Deviation	range	95% CI
Phosphoserine (Phser)	6.0	5.6	3.3	1.6-14.9	4.8-7.2
Taurine (Tau)	148.4	140.3	47.1	57.9-264.3	133.7-163.0
L-Aspartic acid (Asp)	24.0	19.9	14.3	9.0-66.0	19.0-29.0
Hydroxyproline (Hypro)	42.0	42.1	3.5	38.0-46.9	37.7-46.3
L-Threonine (Thr)	193.9	185.8	65.2	103.7-454.0	173.6-214.3
L-Serine (Ser)	208.2	197.4	54.5	57.9-311.6	191.2-225.2
L-Asparagine (Asn)	50.0	50.9	16.5	12.6-79.9	44.8-55.1
L-Glutamic acid (Glu)	332.3	218.4	243.0	82.8-928.4	244.7-419.9
L-Glutamine (Gln)	492.9	449.1	242.3	125.0-1059.8	346.6-639.3
Sarcosine (Sarc)	103.2	97.8	35.5	49.0-192.8	89.7-116.7
L-Proline (Pro)	238.7	232.1	50.5	143.1-396.6	222.0-257.9
Glycine (Gly)	380.3	359.3	115.9	183.9-623.8	343.9-416.1
L-Alanine (Ala)	348.6	333.3	88.6	170.7-601.7	321.0-376.2
L-Citrulline (Cit)	24.0	15.7	19.5	7.7-77.0	17.3-30.6
alpha-amino-n-butyric acid (Aaba)	21.8	19.4	8.3	5.4-42.9	18.7-25.0
L-Valine (Val)	141.6	136.9	36.0	72.9-232.0	130.4-152.8
L-Cystine (Cys)	19.5	8.5	18.0	3.1-57.7	13.9-25.2
L-Methionine (Met)	27.5	26.4	9.8	13.8-64.4	24.4-30.5
L-Isoleucine (Ile)	45.1	40.4	19.8	19.8-101.2	39.0-51.3
L-Leucine (Leu)	103.6	99.2	26.9	57.4-179.7	95.2-112.0
L-Tyrosine (Tyr)	94.4	92.1	23.3	61.0-149.3	87.1-101.7
L-Phenylalanine (Phe)	49.1	48.5	9.1	31.8-69.9	46.2-51.9
Ethanolamine (Ethan)	56.4	58.9	15.4	28.5-86.7	50.7-62.2
Ornithine (Orn)	136.3	126.5	44.4	70.3-242.8	122.4-150.1
L-Lysine (Lys)	214.2	214.3	52.0	105.9-325.6	198.0-230.4
1-Methylhistidine (1-Mhis)	11.6	9.0	6.4	4.4-28.2	9.3-13.9
L-Histidine (His)	80.0	77.6	13.9	46.1-110.0	75.7-84.3

Amino acid (µmol/L)	Mean	Median	Std. Deviation	range	95% CI
L-Tryptophan (Trp)	30.1	26.0	14.5	14.0-76.9	22.6-37.6
3-Methylhistidine (3-Mhis)	6.7	6.7	1.5	3.6-8.8	5.5-8.0
L-Arginine (Arg)	45.4	39.7	29.0	12.6-125.7	36.2-54.7

Table 2. Reference ranges of plasma amino acids (µmol/L) compared with previously published data

	Present study (2.5–97.5th percentile)	**Shih, et al.**[4] (Reference Interval)	**Shapira, et al.**[5] (Reference Interval)	**Sickkids**[6] (Reference Interval)	**Wu et al.**[7] (Reference Interval)	**Svasti et al.**[9] (Mean ± SD, 95% CI)	**Uaariyapanichkul et al.**[10] (2.5–97.5th percentile)
Number of participants	n=42	n=17	NA	NA	n=16	n = 15	n = 85
Age group	48-72 hours	<3 months	0-1 month	0-6 days	28-32 days	0-6 months	<4 days
Phosphoserine (Phser)	2.1-14.2	NA	NA	0-3	NA	18.9±11.4 (12.6-25.3)	NA
Taurine (Tau)	74.4-258.8	10–167	46-492	87-375	1.1-166.6	96.1±57.5 (64.2-128.0)	NA
L-Aspartic acid (Asp)	10.0-65.3	0-31	20-129	19-121	4.6-50.6	8±4.9 (5.2-10.7)	102-216
Hydroxyproline (Hypro)	38.2-46.5	NA	0-91	28-104	NA	28.9±13.6 (21.3-36.5)	NA
L-Threonine (Thr)	105.8-353.3	46-222	90-329	81-313	69.8-197.1	135.9±104.3 (78.1-193.7)	NA
L-Serine (Ser)	131.9-301.8	92-178	99-395	199-843	0-325.6	130.9±56.4 (99.6-162.2)	NA
L-Asparagine (Asn)	22.0-77.9	38-121	29-132	38-91	15.8-80.7	84.2±24.2 (70.8-97.7)	NA
L-Glutamic acid (Glu)	95.2-793.1	8-179	62-620	91-401	24.3-243.1	199.9±137.5 (123.7-276.0)	485-687.4
L-Glutamine (Gln)	157.6-987.1	402-776	376-709	451-1113	142.3-850.5	421.9±197.3 (312.7-531.2)	NA
Sarcosine (Sarc)	55.6-163.8	NA	0-625	0-4	NA	NA	NA
L-Proline (Pro)	166.7-384.4	97-254	110-417	127-292	82.5-319.3	198.3±73.8 (157.5-239.2)	97-150

	Present study (2.5–97.5th percentile)	**Shih, et al.**[4] (Reference Interval)	**Shapira, et al.**[5] (Reference Interval)	**Sickkids**[6] (Reference Interval)	**Wu et al.**[7] (Reference Interval)	**Svasti et al.**[9] (Mean ± SD, 95% CI)	**Uaariyapanichkul et al.**[10] (2.5–97.5th percentile)
Glycine (Gly)	209.0-597.5	154-338	232-740	299-782	76.6-376.3	191.1±90.5 (141.0-241.3)	300-414
L-Alanine (Ala)	218.7-511.2	142–421	131-710	175-427	124.6-647.2	278.7±101.6 (222.4-334.9)	424-633
L-Citrulline (Cit)	10.3-69.7	8-36	10-45	9-44	5.2-23.6	33.7±35.8 (13.9-53.6)	13.8-23
alpha-amino-n-butyric acid (Aaba)	9.8-41.6	3-24	8-24	7-42	NA	13.3±12.8 (6.2-20.4)	NA
L-Valine (Val)	93.6-214.5	79-217	86-190	87-326	88.4-221.9	160.3±53.7 (130.5-190.1)	89.1-179.2
L-Cystine (Cys)	3.1-53.5	6-43	17-98	16-53	34.8-69.0	NA	NA
L-Methionine (Met)	15.7-52.5	9-44	10-60	13-44	21.6-50.0	32±11.5 (25.6-38.4)	10.7-17.7
L-Isoleucine (Ile)	22.4-94.9	12-77	26-91	25-129	26.5-89.9	44.3±16.4 (35.1-53.4)	NA
L-Leucine (Leu)	68.1-165.7	46-147	48-160	46-165	53.2-169.4	91.1±27.2 (76.0-106.2)	511-703*
L-Tyrosine (Tyr)	61.3-140.6	13-91	55-147	27-182	38.3-119.4	72.9±29.9 (56.3-89.4)	74.1-114.4
L-Phenylalanine (Phe)	33.1-63.8	25–74	38-137	49-107	21.7-69.8	53.6±13.3 (46.2-61.0)	50.3-70.1
Ethanolamine (Ethan)	31.4-81.4	NA	0-115	23-110	NA	NA	NA
Ornithine (Orn)	79.3-224.6	41-129	48-211	82-365	0-157.2	53.1±32.8 (34.9-71.3)	124-185
L-Lysine (Lys)	115.7-305.9	69-200	92-325	90-319	80.2-231.5	109.6±55.1 (79.1-140.2)	NA
1-Methylhistidine (1-Mhis)	4.9-23.8	NA	0-43	0-20	NA	NA	NA

	Present study (2.5–97.5th percentile)	**Shih, et al.**[4] (Reference Interval)	**Shapira, et al.**[5] (Reference Interval)	**Sickkids**[6] (Reference Interval)	**Wu et al.**[7] (Reference Interval)	**Svasti et al.**[9] (Mean ± SD, 95% CI)	**Uaariyapanichkul et al.**[10] (2.5–97.5th percentile)
L-Histidine (His)	57.6-105.0	37-83	30-138	76-215	33.6-118.7	27±17.5 (17.2-36.7)	NA
L-Tryptophan (Trp)	14.6-62.7	21-75	0-60	22-59	19.4-100.0	1.68±7.4 (12.7-20.9)	NA
3-Methylhistidine (3-Mhis)	4.1-8.6	NA	NA	6-21	NA	NA	NA
L-Arginine (Arg)	14.1-103.4	7-128	6-140	2-118	42.3-148.2	66±30.7 (47.4-84.6)	5.6-14.7

*Leucine, isoleucine, alloisoleucine, and hydroxyproline could not be separated.

Discussion

Various techniques can measure plasma amino acid levels, including HPLC, IEC with post-column ninhydrin derivatization, and tandem mass spectrometry (MS/MS). Previous studies in Thailand used different analytical techniques. Sirichakwal et al.[8] (1999) employed IEC, Svasti et al.[9] (2001) utilized HPLC, and Uaariyapanichkul et al.[10] (2018) applied MS/MS. A confirmation test following a positive newborn screening, using a highly precise and reliable method, is essential. This study employed IEC with post-column ninhydrin derivatization, which was a widely used technique in clinical laboratories.[12] This method offers high precision, reliable quantification, and effective separation of structurally similar amino acids, such as leucine, isoleucine, and hydroxyproline, making it suitable for diagnostic applications. It is also easier to use in clinical settings than HPLC because it follows standard procedures and doesn't need as much method optimization. However, the key limitation of IEC is its lower throughput and longer analysis time per sample. Although HPLC is highly precise, it requires extensive method optimization and prolonged sample preparation. Therefore, it is less practical for routine use in confirmatory testing. In contrast, MS/MS offers rapid analysis, high throughput, and minimal steps of sample preparation, making it ideal for large-scale newborn screening programs. However, a limitation of MS/MS is its inability to fully resolve structurally similar amino acids. Therefore, it is not suitable for confirmatory testing, where precise differentiation is critical. Thus, IEC remains a robust choice for confirmation testing, ensuring accurate amino acid quantification while addressing the limitations of alternative techniques.

Differences in study populations further complicate comparisons. Our study focused exclusively on neonates aged 48-72 hours, whereas Svasti et al.[9] included infants up to six months old, and Sirichakwal et al.[8] examined individuals aged 1 to 45 years. Only Uaariyapanichkul et al.[10] included a subgroup matching the age range of our study and reported that several amino acid levels, including leucine/isoleucine, glutamic acid, alanine, and glycine, were highest in neonates under four days old and declined over time. This was a result of changes in hepatic enzyme activity, metabolic maturation, and dietary intake, leading to significant variability in plasma amino acid levels with age. These age-related metabolic changes highlighted the importance of using age-specific reference intervals for accurate interpretation.

Several confounding factors might influence plasma amino acid levels in newborns, including sample collection timing, dietary intake, and regional differences. Without standardized fasting protocol, we collected our samples simultaneously with newborn screening, potentially affecting amino acids sensitive to dietary intake. Similarly, the exact time of sample collection since the last feeding was not mentioned in prior studies, contributing to variability in the results. Additionally, geographic and demographic differences must be considered. While Svasti et al. and Uaariyapanichkul et al. conducted their previous studies in Bangkok (Central Thailand), our study participants were from northeastern Thailand.[9,10] Factors such as genetics, ethnicity, socioeconomics, maternal nutrition, and breastfeeding practices could contribute to regional variations in plasma amino acid levels. These confounding factors emphasized the necessity for population-specific reference intervals to improve diagnostic accuracy in newborn screening programs.

Comparing our reference ranges with previously published data, branched-chain amino acids (BCAAs), including leucine, isoleucine, and valine, as well as aromatic amino acids (AAAs), such as tryptophan, phenylalanine and tyrosine showed minor differences in values. These amino acids are crucial for the diagnosis of specific amino acid disorders. Among the essential amino acids, only the reference range for threonine exceeded the ranges reported in other published studies.[4–7] In contrast, the reference ranges of nonessential amino acids showed more variability between studies, as these amino acids were synthesized internally by the body rather than solely obtained from the diet. Glutamine, the most abundant amino acid in humans[13], exhibited the highest levels and widest reference range. Glutamine played a crucial role in various metabolic processes, including protein synthesis, immune function, and serving as a nitrogen donor in various biosynthetic pathways. These roles were particularly important during the early stages of life because of rapid growth and cellular proliferation.[13] The glutamine level was significantly

higher in small for gestational age infants due to increased energy consumption in low-birth-weight infants.[13] This phenomenon was also observed in one of our participants, a full-term neonate with a birth weight of 2,200 g, who demonstrated the highest glutamine level.

This study provided essential reference values for plasma amino acid levels specifically in Thai neonates from the northeastern region. The findings serve as a critical resource for the diagnosis and management of IEMs in this region, particularly at Srinagarind Hospital, the only Rare Diseases Center in the region. Importantly, these data align with the recent expansion of the national health benefit package, which now covers 24 groups of IEMs. However, it is important to note that in clinical practice, these findings should always be interpreted in conjunction with the patient's clinical presentation rather than being relied on solely.

The limitations of this study included a small sample size, a non-standardized fasting protocol, and methodological differences that complicated comparisons with previous studies. Future studies should include larger sample sizes, standardized fasting protocols, and longitudinal data to enhance the applicability of reference values in clinical practice.

Conclusion

The reference values established in this study contributed significantly to the understanding of normal plasma amino acid levels in northeastern Thai neonates and emphasized the necessity for localized data in the diagnosis and management of IEMs. Additionally, longitudinal studies that track amino acid levels beyond the neonatal period could provide insights into the metabolic adjustments that occurred as infants grow, further refining the reference ranges for clinical use.

Conflict of interests

No potential conflict of interest relevant to this article was reported.

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