Biochemical Findings in the First Filipino Child Confirmed to have Nonketotic Hyperglycinemia: A Case Report

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ABSTRACT

This is a report of the biochemical findings in the first diagnosed case of Nonketotic Hyperglycinemia (NKH) in the Philippines. Urine metabolic screening by high voltage electrophoresis showing grossly increased glycine necessitated confirmation of NKH. Confirmatory analysis was done by paired plasma-cerebrospinal fluid quantitative amino acid analysis using Ultrahigh Performance Liquid Chromatography (UPLC). The result was compatible with the clinical picture of the patient who presented primarily with apnea, seizures, hypotonia and lethargy. This paper emphasizes the importance of locally available biochemical genetic tests in the diagnosis of inborn errors of metabolism.

Key Words: Nonketotic hyperglycinemia, glycine encephalopathy, amino acid analysis

Introduction

Nonketotic hyperglycinemia (NKH), also called glycine encephalopathy, is an autosomal recessive disorder of glycine metabolism resulting to the accumulation of glycine in body tissues and central nervous system.[1] The fundamental defect in NKH is in the glycine cleavage enzyme system, an intramitochondrial enzyme complex made up of four proteins encoded in four different chromosomes, which catalyzes the major reaction of glycine degradation.[2,3,7] In the neonatal phenotype, most patients present with lethargy, muscular hypotonia, myoclonic seizures, progressing to apnea, coma, and often death.[4,5] Glycine concentrations in urine are usually elevated but are not definitive of NKH. The diagnosis of NKH is established by determining the CSF and plasma glycine levels, and by determining the CSF/plasma glycine ratio.[6,7] The collective incidence of NKH worldwide is unknown but it is found to affect about 1 in 55,000 newborns in Finland and 1 in 63,000 newborns in British Columbia and Canada.[8]

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The authors present the first case of nonketotic hyperglycinemia in the Philippines diagnosed through urine metabolic screening and paired plasma-cerebrospinal fluid quantitative amino acid analysis.

Case Presentation

The patient was a 3-day-old male born to a non-consanguineous Filipino couple. He was delivered live, term, via caesarian section with good Apgar scores. However, on the sixth hour of life, the patient became tachypneic with shallow breathing and grunting. Chest x-ray was normal. He was started on Ampicillin and Gentamicin. Patient remained tachypneic and eventually manifested with poor cry and activity on the 2nd day of life. On the third day of life, increasing lethargy, hypotonia and apnea were observed along with jerky movements of the extremities. His family history revealed that an older male sibling died at 9 days of life with similar symptoms.

Urine metabolic screening by High Voltage Electrophoresis

Random urine sample was collected and was blotted on Whatman filter paper. Urine creatinine was determined. Blotter was cut into a section based on 80 nmol/creatinine equivalent. This was later blotted on an electrophoresis paper alongside normal and spiked (i.e., with abnormal compounds added, such as cysteine and S-sulphocysteine) reference urine blotters after elution on distilled water. After subsequent applications, the dry electrophoresis paper was buffered with formic acid/acetic acid buffer then subjected to high voltage electrophoresis (Pharmacia Biotech) for separation of the urinary amino acids. The paper was then dried, stained with Ninhydrin solution, and read semi-quantitatively on a negatoscope by three laboratory staff. The intensity of the color of the urine amino acid bands were compared both vertically (within the same track/sample) and horizontally (per amino acid).

High voltage electrophoresis sheet (Figure 1) showed patient's increased excretion of glycine (track C) compared to spiked and unspiked reference urine (tracks A and B, respectively). The patient's glycine was likewise grossly elevated compared to the other amino acids excreted.

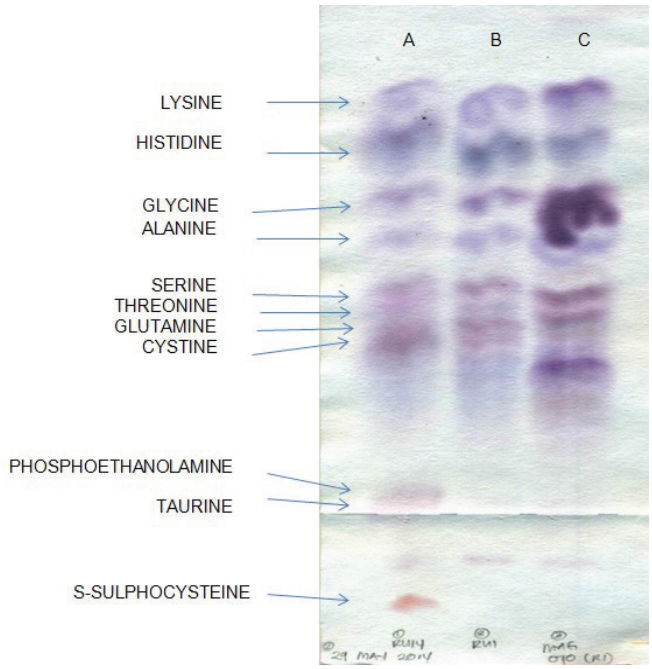

Figure 1. High Voltage Electrophoresis sheet showing tracks A, B, and C as samples of spiked reference urine, unspiked reference urine, and patient's urine (with grossly increased glycine), respectively.

Paired plasma-cerebrospinal fluid quantitative amino acid analysis was suggested for diagnostic confirmation of Nonketotic Hyperglycinemia.

Paired plasma-cerebrospinal fluid quantitative amino acid analysis by Ultrahigh Performance Liquid Chromatography

Plasma and cerebrospinal fluid were collected and were analyzed using Waters Ultrahigh Performance Liquid Chromatography (UPLC)-MassTrak Amino Acid Analysis system. The MassTrak amino acid analysis kit is composed of buffers and derivatizing reagents specific for amino acids.

Blank, amino acid standard, control, and the patient's samples were deproteinized using salicylic acid. Norvaline at 250 μmoL/L was added as the internal standard. The samples were buffered with sodium hydroxide-borate, derivatized, and injected to the UPLC. Results were generated using Waters Empower software. Standard and control results should be satisfactory to accept the results of the batch run.

A diagnosis of NKH is established if the glycine-glycine ratio of CSF/plasma is greater than 0.08.[9] In our patient, the plasma glycine level was 2116 μmoL/L and the CSF glycine was 834.6 μmoL/L giving a ratio of 0.4, thus confirming the diagnosis of nonketotic hyperglycinemia (Figures 2 and 3; Tables 1 and 2).

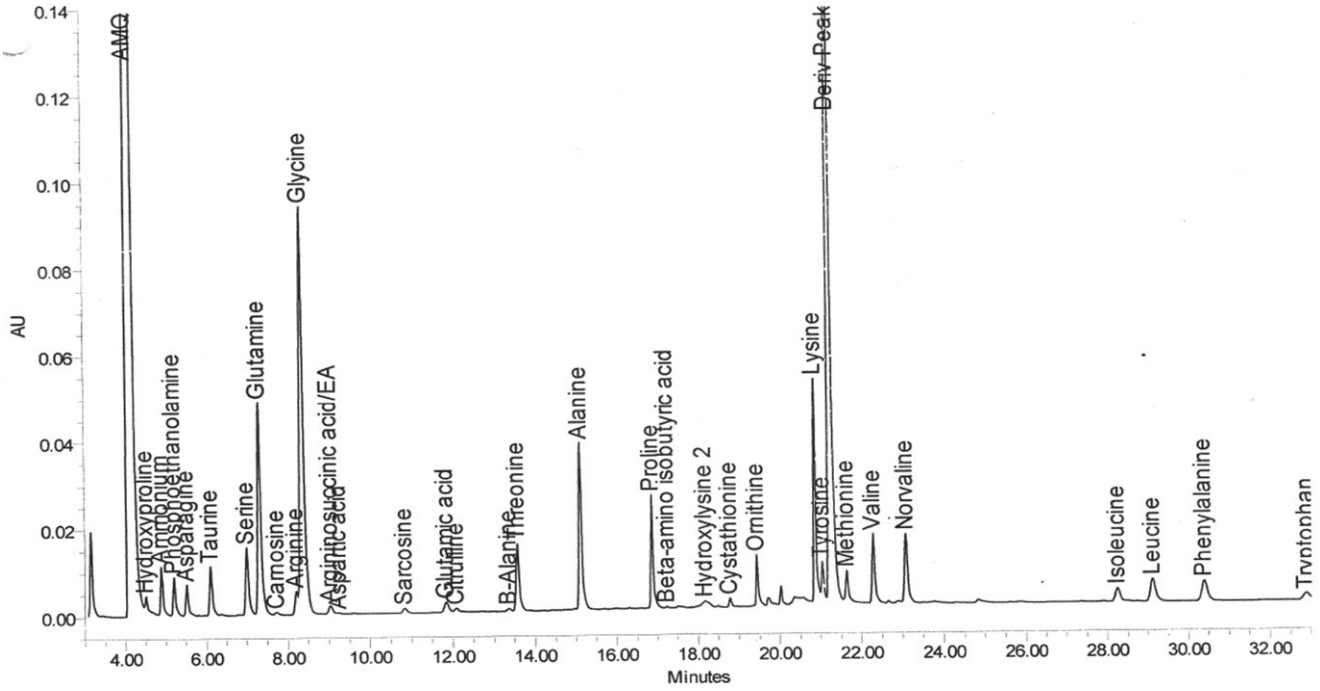

Figure 2. Plasma amino acid analysis chromatogram showing grossly increased glycine.

Table 1. Quantitative values of plasma amino acids showing markedly elevated glycine (Reference values based on Princess Margaret Hospital Perth, Western Australia). Some amino acids were slightly increased which could be attributed to other factors such as patient's parenteral nutrition intake

Amino Acids	Reference Intervals	Amount (μmol/L)	Remarks
Hydroxyproline	<92	31	Normal
Histidine	30-138	BELOW ASSAY RANGE	Outside Normal Values
Taurine	46-492	173	Normal
Serine	99-395	266	Normal
Glutamine	340-910	931	Outside Normal Values
Arginine	6-140	89	Normal
Glycine	150-560	2116	Outside Normal Values
Sarcosine	<18	30	Outside Normal Values
Citrulline	10-45	19	Normal
Threonine	90-330	252	Normal
Alanine	130-460	561	Outside Normal Values
Proline	110-417	357	Normal
Ornithine	48-211	206	Normal
Cystine	17-98	BELOW ASSAY RANGE	Outside Normal Values
Lysine	92-325	373	Outside Normal Values
Tyrosine	55-147	117	Normal
Methionine	10-60	94	Outside Normal Values
Valine	86-190	222	Outside Normal Values
Isoleucine	26-91	79	Normal
allo-Isoleucine	<5	NOT DETECTED	Normal
Leucine	48-160	145	Normal
Phenylalanine	38-137	143	Outside Normal Values

Table 2. Quantitative values of cerebrospinal fluid amino acids showing marked and disproportionately increased glycine (Reference values based on Princess Margaret Hospital Perth, Western Australia). All other amino acids were slightly increased probably due to other factors such as some degree of blood contamination, either a traumatic tap or previous bleed into CSF.

Amino Acids	Reference Intervals	Amount (μmol/L)	Remarks
Hydroxyproline	No Reference Available	4.4	~
Histidine	10.5-30.1	71.4	Outside Normal Values
Taurine	5.3-13.7	81.8	Outside Normal Values
Serine	29.6-85.9	107.6	Outside Normal Values
Glutamine	387.6-823.6	1946.1	Outside Normal Values
Arginine	No Reference Available	15.5	~
Glycine	3.8-8.0	834.6	Outside Normal Values
Sarcosine	No Reference Available	6.7	~
Citrulline	1.3-7.8	8.7	Outside Normal Values
Threonine	15.1-130.2	149.8	Outside Normal Values
Alanine	16.5-41.0	71.3	Outside Normal Values
Proline	No Reference Available	9.7	~
Ornithine	2.8-19.2	BELOW ASSAY RANGE	Outside Normal Values
Cystine	No Reference Available	BELOW ASSAY RANGE	~
Lysine	11.6-36.5	65.6	Outside Normal Values
Tyrosine	6.9-24.7	57.9	Outside Normal Values
Methionine	1.6-7.4	37.5	Outside Normal Values
Valine	11.9-29.4	82.8	Outside Normal Values
Isoleucine	4.7-11.7	17.8	Outside Normal Values
allo-Isoleucine	No Reference Available	NOT DETECTED	~
Leucine	12.4-19.6	47.3	Outside Normal Values
Phenylalanine	5.2-22.5	68.5	Outside Normal Values

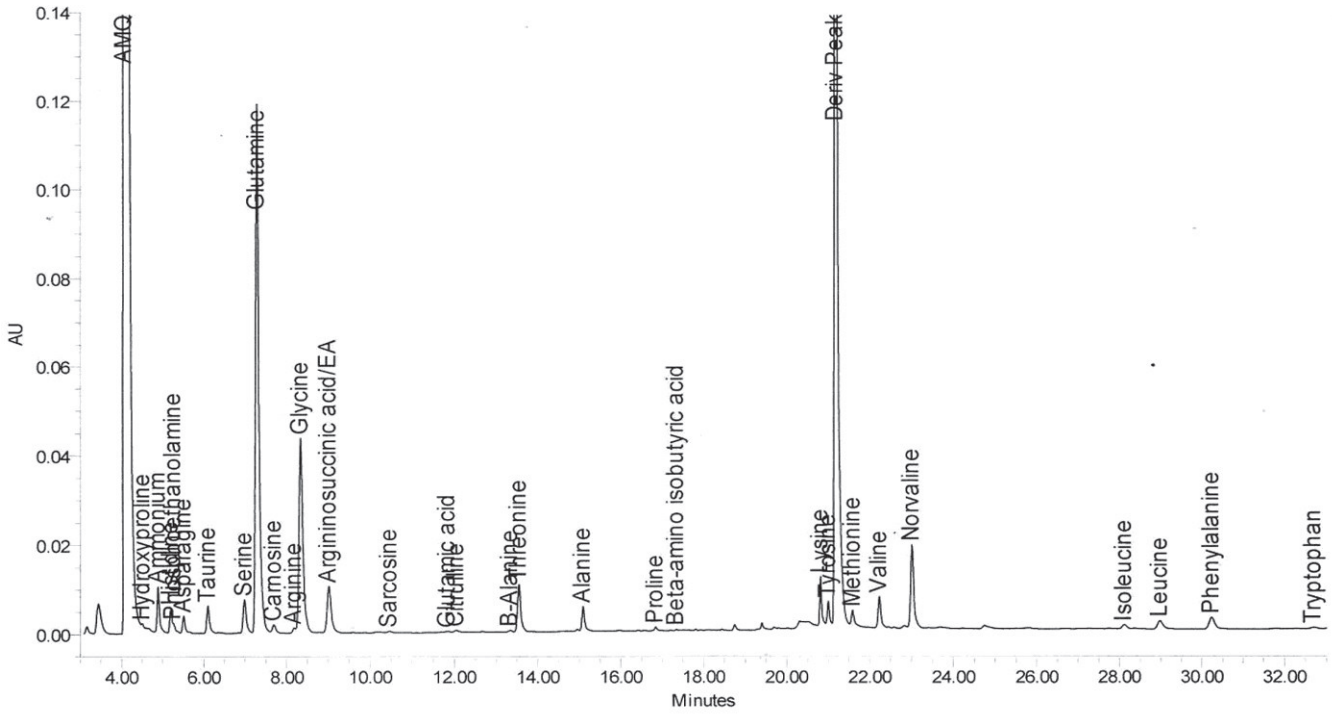

Figure 3. Cerebrospinal fluid amino acid analysis chromatogram showing grossly increased glycine.

Discussion

Several forms of NKH are known. The *neonatal* or *classical* form presents with lethargy, hypotonia, myoclonic seizures, and apnea occurring suddenly within one or two days after birth. It is the form with the most severe phenotype which rapidly progresses to unresponsiveness and often death. The *transient* form is characterized by clinical and biochemical findings similar to those found in classic NKH; however, a number of enzymes are transiently immature during the neonatal period causing a delay in the maturation of the glycine cleavage system, hence the clinical presentation. [10]

Some other atypical and mild forms have been reported. Patients diagnosed in this category had rather disparate manifestations such as mild mental retardation, spinocerebellar degeneration, and progressive neurodegeneration. [11]

It is therefore important to distinguish the transient type from the classical type to predict the outcome of the patient, as well as from the phenotypic variabilities of atypical forms to allow accurate assessment of treatment efficacy. [10,11]

The laboratory diagnosis of NKH begins with measurement of plasma and cerebrospinal fluid glycine wherein samples should be taken as closely as possible at the same time.[7] CSF/plasma ratios are an estimation of the sum of all of the transport mechanisms (influx and efflux) in the brain-blood barrier (a unique dynamic regulatory interface separating bloodstream from the remaining extracellular space of the body), blood-CSF barrier, and between extracellular fluid and CSF. In metabolic diseases which affect CNS and peripheral organs, amino acid CSF/plasma ratios change because of the degree of metabolic disturbance.[12] Glycine acts through inhibitory receptor distributed in the brain and spinal cord (responsible for respiratory depression and lethargy) and excitatory receptor in the brain (responsible for the hyperirritability and impairment of the voluntary movements).[10] The advantage of calculating the ratio of CSF/plasma glycine levels taken simultaneously is that the CSF glycine is likely to be increased only in the same proportion relative to normal as is the plasma glycine in non-NKH patients[7].

In classical neonatal NKH which is most probably the type that our patient has, the glycine CSF/plasma ratio of >0.08 is usually considered diagnostic. CSF glycine can be more than 30X the upper limit of normal while plasma glycine can be very high in untreated patients, however can be occasionally normal. More mildly affected patients are reported with CSF/plasma glycine ratios of 0.04-0.1.[7] Our patient's plasma and CSF glycine levels, and CSF/plasma glycine ratio were all markedly elevated confirming the diagnosis.

Caution is specified on borderline elevations which could be caused by valproate treatment. In addition, differential diagnosis of hyperglycinemia (with ketosis) includes organic acidurias wherein glycine cleavage system is inhibited by pathological metabolites; and prolonged fasting. [9] Our patient was not administered with valproic

acid, and glycine levels of both plasma and CSF were markedly elevated. Likewise, ketones were not observed as what can usually be seen in organic acidurias.

Further tests to complement NKH diagnosis include a liver biopsy for glycine cleavage enzyme (GCE) complex assays, and molecular diagnosis of the four proteins of the GCE. Because of the difficulty in obtaining sample for the enzyme assays, this test is not done in most patients, while even fewer patients are tested for their specific protein defect.[7]

For our patient, the diagnosis of NKH was made through clinical history as well as the disproportionately high plasma and CSF glycine with a corresponding high ratio. Without the benefit of enzyme assay and molecular analysis, the value to biochemical testing is emphasized in this paper for the diagnosis of such types of disorders.

Conclusion

The prognosis of classical nonketotic hyperglycinemia is generally poor.[9] However, coming up with a definite diagnosis confirms to be of value to the patient's family for therapeutic decisions and genetic counseling issues. (i.e. NKH being an autosomal recessive disease). Finally, this paper highlights the benefits of locally available biochemical genetic services for the assessment and management of inborn errors of metabolism.

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