Clinical Profiles of Hyperphenylalaninemia Patients Diagnosed by Newborn Screening

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ABSTRACT

Hyperphenylalaninemia is due to problems in phenylalanine metabolism caused by defects in phenylalanine hydroxylase enzyme and its co-factor, tetrahydrobiopterin (BH4). This paper presents a review of patients with hyperphenylalaninemia (HPA) diagnosed by Newborn Screening Center-National Institutes of Health from 1996 to 2009. Thirteen cases were diagnosed: five classical phenylketonuria (PKU), one mild PKU, three 6-pyruvoyl tetrahydrobiopterin synthase (6-PTPS) deficiency, and four mild hyperphenylalaninemia (HPA). The clinical profile of the patients highlights the importance of early diagnosis and dietary treatment, good metabolic control and regular monitoring, for better outcome.

Key Words: phenylketonuria, hyperphenylalaninemia, phenylalanine hydroxylase enzyme deficiency, BH4 deficiency, 6-Pyruvoyl tetrahydrobiopterin synthase (PTPS) Deficiency

Introduction

Hyperphenylalaninemia (HPA) is a group of autosomal recessive inborn errors of phenylalanine metabolism caused by a deficiency or a decreased activity of phenylalanine hydroxylase (PAH) enzyme or a deficiency of its cofactor BH4.[1,2] The overall incidence of HPA is highest among Caucasians, occurring in approximately 1 in 10,000 births with about 98-99% of HPA due to phenylalanine hydroxylase deficiency. BH4 deficiency, which only accounts for 1-2% of HPA in Caucasians, occur more commonly in Asian populations.[1,3] Dietary intake of phenylalanine (Phe) along with endogenous recycling of amino acid stores are the major sources of Phe, whereas, utilization of Phe occurs via integration into proteins, oxidation to Tyr, or conversion to other metabolites. The conversion of Phe to Tyr occurs by a hydroxylating system consists of PAH, its cofactor BH4 , and enzymes which serve to regenerate BH4 (Figure 1).[1] On the basis of blood Phe concentrations, PAH deficiency can be classified into classic phenylketonuria (PKU) (Phe>1200 umol/L in the untreated state), mild PKU (Phe = 600-1200 umol/L), and mild HPA, where blood Phe is elevated above upper reference limit, but <600 umol/L[1] Untreated children with persistent severe HPA show impaired brain development which is manifested as microcephaly, severe mental retardation, epilepsy, and behavioral problems.[4]

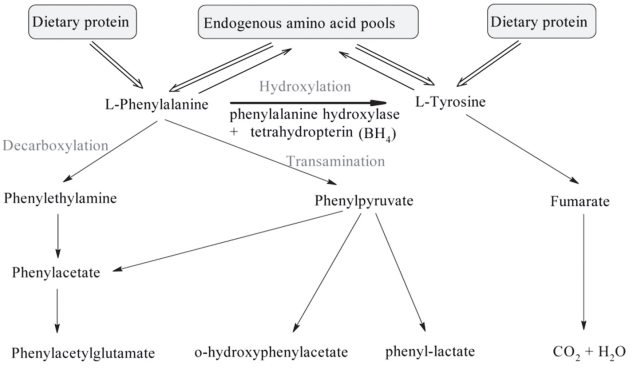

Figure 1. Phe metabolism. Intake of L-Phe is via the diet and it is recycled through amino acid pools. Hydroxylation by PAH with its cofactor BH4 produces L-Tyr. Alternative metabolism of L-Phe by decarboxylation or transamination produces various metabolites which are excreted in urine.[1]

This paper reviews the clinical profiles of 13 Filipino patients with HPA diagnosed by newborn screening and who are currently seen at the metabolic clinic of the University of the Philippines-Philippine General Hospital.

Description of Cases

Of 1,487,396 samples received by the newborn screening laboratory from 1996 to 2009, 13 patients were noted to have elevated Phe levels (above the reference limit of 200 umol/L; Newborn Screening Center, National Institutes of Health) which gives a local incidence of 1:114,000. The breakdown of cases include 5 classical PKU, 1 mild PKU, 3 6-PTPS deficiency, and 4 mild HPA. Table 1 shows their clinical characteristics.

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Table 1. Characteristics of Patients with Hyperphenylalaninemia

Patient	Diagnosis	Age/ Sex	Age at Diagnosis	Phenylalanine Level at Diagnosis (umol/L)	Metabolic Control	Developmental Outcome	Diet Compliance
1	PKU	5/F	At birth	240	Phe 63-1030 umol/L	Normal	Good
2	PKU	3/M	2 ½ yrs	2157	Phe 236-1157 umol/L	GDD	Good
3	PKU	1/F	At birth	3000	Phe 20 -1236 umol/L	Normal	Good
4	PKU	2 ½/F	At birth	400	Phe 479-1071 umol/L	Mild speech delay	Fair
5	PKU	10m/M	At birth	1808	Phe 60-1024 umol/L	Normal	Good
6	Mild PKU	1/F	At birth	357	Phe 25- 690 umol/L	Normal	Good
7	6-PTPS def	2/F	7 months	1400	Phe 39-120 umol/L Prolactin 602-963 uIU/ml	Mild GDD	Good
8	6-PTPS def	3/F	2 yrs	1500	Phe 60-100 umol/L Prolactin 800-1022 uIU/ml	GDD	Good
9	6-PTPS def	10mo/F	At birth	741	Phe 35-197 umol/L Prolactin 295-1316 uIU/ml	Normal	Good
10	Mild HyperPhe	1/F	At birth	345	Phe 303- 352 umol/L	Normal	No restriction
11	Mild HyperPhe	7mo/M	At birth	226	Phe 226-402 umol/L	Normal	No restriction
12	Mild HyperPhe	7mo/F	At birth	254	Phe 215-262 umol/L	Normal	No restriction
13	Mild HyperPhe	4mo/F	At birth	221	Phe 152-221 umol/L	Normal	No restriction

Classical Phenylketonuria

The 5 patients with classical PKU, 2 males and 3 females, had ages ranging from 6 months to 5 years. Four out of the 5 cases were diagnosed and treated shortly after birth. Two out of the 5 cases are siblings. All are developmentally normal except for two patients. A 2 ½-year-old female who was diagnosed and managed shortly after birth, had poor compliance with the diet, and has mild speech delay. Another 3-year-old male was diagnosed late at 2 ½ years old after his younger sister was diagnosed with PKU by newborn screening. This patient had global developmental delay at the time of ascertainment. Metabolic control in all the classical PKU patients leaves much room for improvement, since their plasma phenylalanine levels range from 400 to 1100 umol/L with only 1 patient having phenylalanine level below 600 umol/L.

Mild Phenylketonuria

This case of mild phenylketonuria is a 1-year-old female who was diagnosed at 15 days of age with a phenylalanine level of 690 umol/L. Upon diagnosis, she was placed on protein-restricted diet and phenylalanine-free milk. Her phenylalanine levels are well controlled at below 350 umol/L and she has normal growth and development.

6-Pyruvoyl tetrahydrobiopterin synthase (PTPS) Deficiency

Two of our PTPS deficient patients, both females, were diagnosed late. The first patient was diagnosed initially with classical PKU at 3 months of age after presenting with spasticity, seizures and with initial phenylalanine level of 1500 umol/L. The second patient was diagnosed at 19 days of life by newborn screening with classical PKU after an initial level of 1400 umol/L. Both were initially managed as such with succeeding phenylalanine levels ranging from 70-1500 umol/L. However, despite good compliance with prescribed protein-restricted diet and phenylalanine-free milk, both patients worsened neurologically. Patient 1 had persistent hypotonia, seizures and poor developmental progression. Patient 2 developed seizures at 4 months of age and subsequently presented with developmental regression, loss of head control, and hypotonia. Because of these symptoms, a pterin defect was suspected. DHPR assay (done at Children's Hospital at Westmead, Sydney, Australia) showed normal results while pterin analysis (done at Taipei Veterans General Hospital, Taiwan) revealed PTPS deficiency. Upon confirmation of PTPS deficiency for Patient 1 at 2 years of age and Patient 2 at 7 months of age, they were given Levodopa, 5-hydroxytryptophan (5-HT), and BH4. Seizures were controlled a few weeks after commencement of these medications. At present, Patient 1 has global developmental delay with better seizure-control. Patient 2 has mild speech delay but has improvement in motor skills, muscle tone and seizure control.

The third patient with PTPS deficiency is a 10-month-old male who was diagnosed at 3 weeks of age after BH4 loading test and pterin analysis. He was started on Levodopa/Carbidopa, 5-HT and BH4. He is developmentally doing well.

Mild hyperphenylalaninemia

The 4 patients with mild hyperphenylalaninemia include two males and two females with ages ranging from 4 months to 1 year and 3 months. They were all diagnosed shortly after birth. The patients are monitored for phenylalanine levels monthly. Phenylalanine levels range from 152 to 402 umol/L. They are not on restricted diet or on

any protein-free supplement. All are doing well in terms of growth and development.

Management of Cases

The patients are diagnosed and managed following diagnostic and treatment protocols (Figure 2). Urine pterins and Dihydropteridine reductase (*DHPR*) analysis are done to delineate BH4 deficiency. Classical and mild PKU patients are placed on a protein-restricted diet while mild hyperphenylalaninemia patients are placed on a normal diet with strict Phe monitoring. Phenylalanine levels are monitored monthly for the first year of life then every 3 months thereafter. More frequent monitoring is done when Phe levels rise above 350 in classical PKU, mild PKU, and mild hyperphenylalaninemia patients. For the PTPS-deficient patients, treatment response is monitored by determining prolactin levels every 3 months. The patients are seen regularly by the dietician for review of diet and nutritional assessment. Other co-managing specialists include the neurologists, developmental pediatricians and the rehabilitation specialists.

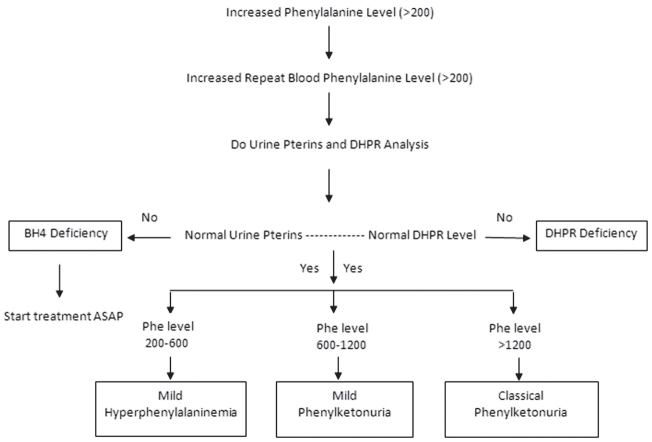

Figure 2. Hyperphenylalaninemia protocol
(Institute of Human Genetics, National Institutes of Health, University of the Philippines Manila)

Discussion

Hyperphenylalaninemia shows an extensive genetic and clinical variability. Untreated classical phenylketonuria results in profound and irreversible mental retardation, growth failure, microcephaly and seizures.[1] This was true for our 3-year-old classical PKU patient who was diagnosed late at 2 ½ years of age after presenting with global developmental delay. In contrast, the patients who were diagnosed and maintained on the protein restricted diet early are currently developmentally at par with age. BH4 deficiency, on the other hand, may not only produce the typical signs and symptoms of phenylketonuria but may also lead to other neurological signs and symptoms due to impaired dopamine and serotonin synthesis such as truncal hypotonia, increased limb tone, choreic or dystonic limb movements, gait abnormalities, hypersalivation and dysphagia.[5] The 2 PTPS deficient patients who were diagnosed late initially had hypotonia and poor developmental progression. After treatment, residual neurologic abnormalities are still seen such as spasticity, seizures, and developmental delay. In contrast, the PTPS deficient patient who was diagnosed early is doing well in terms of growth and neurologic development. In the milder forms of hyperphenylalaninemia, there is a much lower risk of cognitive impairment even in the absence of dietary restriction[6] however, it is important to monitor these patients closely in the early years as phenylalanine may occasionally rise to harmful levels.

The clinical effect of elevated phenylalanine levels is mainly of disturbance in brain development and cognitive function. Studies show that high cerebral phenylalanine concentrations may compete with cerebral tyrosine for hydroxylation, so that tyrosine is formed in the brain but its conversion to dopamine is blocked. Hyperphenylalaninemia also inhibits the transport of large neutral amino acids (LNAA) into the brain. Reduction of LNAA in the brain is thought to cause inhibition of protein synthesis and neurotransmitter synthesis, leading to deficient dopamine and serotonin levels.[6]

Implementing a phenylalanine-restricted diet early in life can significantly reduce mental deficiencies associated with phenylketonuria. Although there is no complete consensus on treatment protocols, it is most commonly agreed that babies with blood Phe levels >6mg/dL (>360 umol/L) should be started on treatment ideally by the time the neonate is 7 days old.[4] The degree of metabolic control is related to the development of cognitive skills and behavior. Those with poorer metabolic control show significantly lower scores on IQ, attention and reaction time.[6] A study done by Smith et. al. on the effect on intelligence of relaxing the low phenylalanine diet in phenylketonuria showed that for each 300 umol/l rise in average phenylalanine concentrations, the IQ fell by 7-10 points, and for those aged 5 to 8 years, IQ at 8 years fell by 4-6 points for a similar rise in phenylalanine concentration.[7] As observed in the classical PKU patients reported, those who were diagnosed and treated early in life had normal development except for one who despite early diagnosis, has mild speech delay. The latter could probably be attributed to poor compliance with the prescribed diet resulting to chronic exposure to high phenylalanine levels.

Possible causes of elevated phenylalanine levels in treated patients include catabolism due to infection, surgery or other illnesses, inadequate intake of energy or other amino acids, reduced requirement due to reduced growth rate and non-compliance with the diet.[8] It has been observed that the phenylalanine levels of the reported patients had a tendency to be high in the presence of intercurrent illnesses

such as respiratory tract infection, or when there is poor compliance with the diet wherein adequate calories and daily protein requirements are not met. In general, achieving the best metabolic control for our patients remains a challenge because of frequent occurrences of illnesses in these children, non palatable taste of the phenylalanine-free formula, occasional unavailability of the phenylalanine formula and poor compliance with the prescribed diet resulting from poor family dynamics.

It is important to exclude BH4 deficiency upon diagnosis of hyperphenylalaninemia. Unfortunately, a BH4 loading test was not done for the first 2 reported patients with 6-PTPS deficiency due to unavailability of BH4 tablets at that time. As seen in these 2 patients, lack of effective management led to severe neurologic complications.

There is an international consensus that patients with mild hyperphenylalaninemia with Phe levels <360 umol/L do not need Phe-lowering dietary treatment whereas patients with levels >600 umol/L do. A study done by Weglage et al. in 2001 showed no brain MRI changes, and normal intellectual and educational outcomes in mild hyperphenylalaninemia patients on unrestricted diet.[9] Nevertheless, because of a possible late-onset phenylketonuria, phenylalanine levels of untreated patients should be monitored carefully at least during the first year of life.[9] Our mild PKU patient is treated with a more relaxed restriction of phenylalanine in the diet compared to the classical ones. The 4 patients with mild hyperphenylalaninemia, on the other hand, are not placed on any dietary restrictions but are being monitored once every month with plans of commencement of treatment if levels become consistently high.

Conclusion

Early diagnosis and intervention of hyperphenylalaninemia prevent serious metabolic and neurological consequences. Lifelong adherence to prescribed diet and medications along with regular monitoring and family education are necessary for optimal results. The success of treatment greatly depends not only on the neonatal screening programs but also on the follow up care of patients that necessitate a multidisciplinary approach in the management of metabolic and neurologic problems.

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