Archives of Razi Institute, Vol. 78, No. 2 (2023) 667-673

DOI: 10.22092/ARI.2022.359480.2431

Original Article

Neurotransmitters Disorders with Mild Hyperphenylalaninemia: The Ones That Should Not Be Missed

Thoalnoon, O. A[1], Kareem, A. A[2], Hammoodi, H. Z[2]*

1. Pediatric Neurologist, Children Welfare Teaching Hospital, Baghdad, Iraq
2. Consultant Pediatric Neurology, Children Welfare Teaching Hospital, Baghdad, Iraq

Received 23 July 2022; Accepted 13 August 2022
Corresponding Author: heshamdr1012@yahoo.com

Abstract

Phenylalanine (PHE) is an essential amino acid. Dietary PHE converts to tyrosine by phenylalanine hydroxylase (PAH) activity. Phenylketonuria (PKU) is an autosomal-recessive disorder resulting from PAH enzyme deficiency. Elevations of PHE in plasma are classified based on the degree of enzyme deficiency into classic PKU (PHE≥1200 µmol/l), mild PKU (PHE>600 µmol/l and <1200 µmol/l), and non-PKU-hyperphenylalaninemia (HPA) or mild hyperphenylalaninemia (MHP) (PHE≤600 µmol/l). This is a single-center study of consecutive patients managed at the Pediatric Neurology Department and the outpatient clinic at Children's Welfare Teaching Hospital, Medical City, Baghdad, Iraq, from the 1st of October 2019 to the 1st of October 2020. Five patients were selected who were proven to have non-PKU-HPA (PHE<600 µmol/L) confirmed by the high-performance liquid chromatography analysis and assured to have sapropterin response by the sapropterin loading test which showed >30% decrease in PHE level. All patients presented with a neurological complaint, they were between three months and 15 years, and they were treated with sapropterin, Levodopa (L-Dopa), and 5-hydroxytryptamine (5-HT). The study included the demographic and clinical profile, biochemical response to sapropterin, and clinical response to treatment according to the development quotient. The five patients enrolled in this study had a gross motor developmental delay as their main symptom. One case also had a seizure and dystonia, another had a fluctuation of symptoms, four had a consanguineous marriage, and two had a family history of the same condition. Moreover, all cases had a higher than 30% decrease in PHE level by the tetrahydrobiopterin (BH4) loading test, and all of them showed significant clinical improvements after treatment except for one that showed only a moderate improvement. The BH4 therapy significantly enhanced dietary PHE tolerance and permitted a PHE-free medical formula to be discontinued in all patients with PHE within an achieved therapeutic target (120-300 µM]. MHP is not a mild disease as it may be related to neurotransmitter disorders. Sapropterin, L-DOPA, and 5-HT are always used for patients suspected of having neurotransmitter diseases, particularly those with MHP.

Keywords: BH4 loading test, Mild hyperphenylalaninemia, Neurotransmitters Tetrahydrobiopterin

1. Introduction

Phenylalanine (PHE) is an essential amino acid. Dietary PHE is not utilized for protein synthesis; it converts to tyrosine by phenylalanine hydroxylase (PAH) activity. Phenylketonuria (PKU) is an autosomal-recessive disorder caused by PAH enzyme deficiency. Tetrahydrobiopterin (BH4) is a necessary cofactor in the PAH reaction, and elevated PHE levels may rarely be caused by inherited disorders of BH4 synthesis. These result in the accumulation of PHE in body fluids and the brain (1).

Elevations of PHE in plasma are categorized as follows, depending on the degree of enzyme deficiency:

1. Classic PKU (PHE≥1200 µmol/l, less than 1% residual PAH activity)

2. Hyperphenylalaninemia (HPA), mild PKU (PHE>600 μmol/l and <1200 μmol/l, 1-5% residual PAH activity)

3. Non-PKU-HPA or mild hyperphenylalaninemia (MHP) (PHE≤600 μmol/l, >5% residual PAH activity (2)

The brain is the main organ damaged by HPA PKU, but the exact mechanism of injury remains elusive. On the other hand, lower concentrations of PHE in plasma and brain tissue are associated with improved neurobehavioral outcomes and support the view that toxic levels of PHE are critical to the mechanisms of the disease (1). Patients with MHP whose PHE levels are virtually less than 600 μmol/l (even when metabolically stressed) are on unrestricted diets (3).

These cases are more likely to benefit from BH4 as previous studies showed that the response was inversely related to the PHE level, and those with MHP showed a complete response (100% of the respondents) (3-6). In 1-3% of infants with HPA, the defect resides in one of the enzymes necessary for producing or recycling the cofactor BH4. If these infants are misdiagnosed as PKU patients, they may deteriorate neurologically despite adequate control of plasma PHE (1).

Tetrahydrobiopterin (BH4) is considered the natural cofactor of the PAH enzyme, and different BH4 deficient syndromes may cause significant neurologic features. The deficiency of BH4 leads to PAH dysfunction, which in turn results in HPA, plus cerebral tyrosine and tryptophan hydroxylases dysfunction, subsequently leading to neurotransmitter L-DOPA and 5-hydroxytryptophan (5-HT) deficiencies (7). Kure, Hou (8) showed that blood PHE levels may well reduce in some patients with mutations in the PAH gene who received doses of BH4 (20 mg/kg). Therefore, HPA in these patients has a defect in one of the four enzymes responsible for maintaining tetrahydrobiopterin levels (9). Because tyrosine and tryptophan hydroxylases also require tetrahydrobiopterin for proper functioning, these disorders also result in deficiencies of L-DOPA and 5-TH neurotransmitters (9).

Despite the low incidence of BH4 synthesis defects, all newborns with HPA detected through newborn screening must be screened for BH4 synthesis defects. The therapy goals are to normalize plasma PHE levels and correct neurotransmitter deficiencies with exogenous supplementation.

The likelihood of adverse side effects after extended therapy, safety, and the benefits of BH4 treatment in young children or women during pregnancy and breastfeeding are still important issues to be assessed (10).

The neurological function may improve with therapy, but the overall prognosis for these disorders is variable. Generally, if treatment starts in the neonatal period, patients are more frequently asymptomatic or have a milder clinical course, including less severe cognitive impairment, movement disorders, and seizures (9).

2. Materials and Methods

This is a single-center study of consecutive patients managed in the Pediatric Neurology Department and the outpatient clinic at Children's Welfare Teaching Hospital, Medical City, Baghdad, Iraq, from the 1st of October 2019 to the 1st of October 2020. Five patients were confirmed as non-PKU-HPA (>120 μM and 600 μM/L) by MS/MS (Tandem mass spectrometry) and high-performance liquid chromatography (HPLC). We relied primarily on patients with a high index of suspiciously unexplained psychomotor delay or those previously diagnosed as MHP and treated with nutritional therapy alone with clinical deterioration despite a biochemical response. The confirmation response for BH4 was performed for them by means of a BH4 loading test. All patients were confirmed for their neurological service by their developmental delay, abnormal movement, or seizure or were suggested by a metabolic screen (not responsive to diet).

2.1. Inclusion Criteria

1. Patients with a neurological complaint due to HPA proven by HPLC and the level above the reference range but below or equal to 600 μmol/l

2. Patients three months to 15 years

3. Use of BH4 with or without L-DOPA and 5-HT

2.2. Exclusion Criteria

1. PHE level in the PKU range (>600 µmol/l)
2. Loss of follow-up

Patients recruited in this study were from all regions of Iraq, five cases ranging in age from three months to 15 years, who were diagnosed with MHP and seeking consultation from the Pediatric Neurology Department and the outpatient neuro-metabolic clinic at the Welfare Teaching Hospital, Medical City complex, Baghdad, Iraq. All patients were comprehensively interviewed, clinically examined, and investigated according to the approved medical and laboratory standards. Relationship status, family history, and development history focusing on significant areas affected before and after the treatment, their developmental quotient, seizure history, and age at treatment initiation were recorded. A general and neurological examination was performed for all patients focusing on the motor system, abnormal movement, and observation of rash if present.

2.3. Basic Investigations

Upon suspicion of an inborn error of metabolism, baseline metabolic investigations were performed using routine methods of measuring blood glucose, testing liver function, electrolytes, arterial blood gases, ammonia, lactate, and MS/MS (adjusted to patient need).

2.4. Specific Investigations

The protocol was followed to assess the response to BH4 therapy by giving patients 20 mg/kg of BH4 analog (Kuvan®) for two days. All patients were on a regular diet for at least two days before and during the test. The BH4 loading test was performed using BH4 at a 20 mg/kg dose, taken orally and directly after a baseline blood sample. The second blood sample was taken two hours after the second dose of BH4. Blood samples were collected and analyzed by HPLC (5). BH4 was determined by a higher than 30% decrement in PHE levels in plasma (11).

3. Results

It is evident in this study that consanguinity is a significant risk factor, and most patients denied any family history of the same condition. The main presentation of all cases was a gross motor delay, and one had a seizure and dystonia (Table 1).

Table 2 shows a decrease in PHE level (>30% decrement) after the BH4 loading test.

The clinical response after starting BH4, L-DOPA, and 5-HT (the dose tailored individually) was recorded according to the gross motor development question applied to all cases. The fifth case was 11 years old, and because he had severe developmental delay since his early days but was diagnosed too late, he showed significant improvement in motor skills and could walk with mild limitation. One case (the first) showed a complete response, two cases (the second and fourth) showed a significant response and reached the development state (near normal), and the third case showed a moderate response (Table 3).

The plasma pterins level was performed only for one patient. Elevated biopterin and a normal neopterin level are usually seen in dihydropyridine reductase (DHPR) deficiency.

Table 1. Demographic and clinical profile

Patient no	Age of start treatment	Gender	Consanguinity	Family HX	Main presentation	Others (Seizure, movement disorder, skin manifestation)
1	6 months	Female	-Ve	-Ve	Gross motor delay	
2	3 months	Female	1st cousin	+Ve	Gross motor delay	
3	6 months	Male	1st cousin	-Ve	Gross motor delay	
4	1 year	Female	1st cousin	+Ve	Gross motor delay	Seizure and dystonia
5	11 year	Male	1st cousin	-Ve	Gross motor delay	Fluctuation in symptoms

Table 2. Biochemical response to sapropterin

Patient no	Phe level before kuvan (µmol /l)	Phe level after kuvan (µmol /l)	% of decrement	Response
1	375.16	141.14	62.32	+ve
2	441.55	154.66	64.9	+ve
3	507.27	127.26	74.9	+ve
4	264	138	47.7	+ve
5	592.50	139.98	76.3	+ve

Table 3. Clinical description of patient's response (main complaint) after treatment according to development quotient

Patient no	The main development domain complained	Development question before treatment	Development question after treatment
1	Gross motor delay	50%	93%
2	Gross motor delay	50%	85%
3	Gross motor delay	42%	54%
4	Gross motor delay	60%	80%
5	Gross motor delay	25%	Walk with mild limitation *

*as long as his treatment started late

4. Discussion

The category referred to as MHP is the most clinically and biochemically benign of PKU cases (12). This is considered when the defect was on the enzyme (PHA) itself; therefore, these patients had residual activity and mild symptoms.

The BH4 loading test was initially used for differentiating between PKU and BH4 deficiency, and it was a valuable tool for identifying BH4 response cases from PKU since Kure, Hou (8) showed that four of five patients with MHP responded to oral sapropterin therapy since their PHA levels decreased (8, 13, 14).

In the current study, consanguinity was focused on as it is considered a real challenge in developing countries with high consanguinity marriage prevalence and was found a significant risk factor for MHP and other types of HPA. Other studies have demonstrated the same results as in Oman, 9 out of 11 cases had positive consanguinity (15), and 8 out of 9 cases in Kuwait had consanguineous parents (16). Another study in Iraq (17) indicated that all 7 cases had consanguineous parents, and 4 out of 7 had a family history. In the present study, only two cases had a positive family history.

Clinically, the main presentation of all cases reported in this study was a gross motor delay, and one of them also had epilepsy. Another study showed that approximately 50-75% of patients with any of these disorders have a gross motor delay, often combined with poor head control and peripheral hypertonia, mainly of the extremities (18). Nearly the same results were obtained in a study in Egypt (19). Another study showed that seizure occurs in 20-40% of cases (20).

The present study showed a significant biochemical response after therapy with BH4, as PHE levels dropped in all cases by more than 30%. Therefore, the response was shown in 100% of the cases, and the results agreed with the findings of several previous studies (3-6). However, other studies reported response in nearly 66% of cases in the USA (21), >80% of cases in Spain (22), and nearly 77% in Italy (23). This discrepancy might be due to the small sample of the present study.

Response to BH4 in different types of HPA varies in different studies from 20% to 62% (24). In addition to the interplay among the PAH enzyme, the coenzyme (BH4), and the substrate (PHE), the difference in the

BH4 responsive rate in patients is also related to the protocols, which varies in duration (24 h, 48 h, 1 week, or 4 weeks) and the testing dose of BH4 (10 mg/kg vs. 20 mg/kg of body weight) (25).

In principle, BH4 deficiency disorders are treatable, and the treatment requires the normalization of BH4 level and blood PHE concentration, as well as the restoration of the BH4-dependent hydroxylation of tyrosine and tryptophan. This is achieved by supplements of BH4, along with neurotransmitter precursors, as well as supplements of folinic acid and dietary modification in some DHPR deficiencies (2, 11).

This study reported the developmental responses after sapropterin, L-DOPA, and 5-HT therapy as complete improvement in one case, near complete improvement in two cases, and moderate in one case. A study in the USA (26) hypothesized that BH4 treatment in the PKU group would be associated with improved working memory and related neural activity. Moreover, the significant variability of BH4 responsiveness is unexplained, and subpopulations with a unique response to this medication have not been well characterized. Causes of variability are multifactorial and likely include individual patient and genotype differences, drug dose, and the age of starting treatment. When treatment begins in the neonatal period, patients are frequently asymptomatic or show less retardation, movement disorders, and convulsions (20, 27).

The fifth case was a known state of psychomotor delay since his early days, and his parent complained that their child was still not sitting, not standing, and had no active movement.

Nevertheless, sometimes he could achieve beneficial action in the early morning, which lasted only minutes or less (fluctuation), and his speech had a delay by few words, which worried his parents. His vocabulary was better in the early morning but deteriorated as he got tired.

The plasma pterin level was thus performed only for him, and the result showed an increase in biopterin with an average level of neopterin, which indicated DHPR deficiency. The same result in terms of biopterin and neopterin value was obtained in a study in Italy (28) and 91.4% in an Iranian study (29).

Monoaminergic neurotransmitters with HPA disorders are challenging and need a high index of suspicion.

MHP is not a mild disease as it may be related to neurotransmitter disorders.

Sapropterin, L-DOPA, and 5-HT are always used in suspected neurotransmitter disease patients, particularly those with MHP.

Authors' Contribution

Study concept and design: H. Z. H.
Acquisition of data: O. A. T.
Analysis and interpretation of data: A. A. K.
Drafting of the manuscript: H. Z. H.
Critical revision of the manuscript for important intellectual content: H. Z. H.
Statistical analysis: O. A. T.
Administrative, technical, and material support: O. A. T.

Ethics

The human study was approved by the ethics committee of the Children Welfare Teaching Hospital, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Oleg AS, Charles PV. Nelson textbook of pediatric. 2020.
2. Burgard P, Lachmann RH, Walter J. Hyperphenylalaninaemia. In: Saudubray J-M, Baumgartner MR, Walter J, editors. Inborn Metabolic Diseases:

Diagnosis and Treatment. Berlin, Heidelberg: Springer Berlin Heidelberg; 2016. p. 251-63.
3. Perez-Duenas B, Vilaseca MA, Mas A, Lambruschini N, Artuch R, Gomez L, et al. Tetrahydrobiopterin responsiveness in patients with phenylketonuria. Clin Biochem. 2004;37(12):1083-90.
4. Belanger-Quintana A, Garcia MJ, Castro M, Desviat LR, Perez B, Mejia B, et al. Spanish BH4-responsive phenylalanine hydroxylase-deficient patients: evolution of seven patients on long-term treatment with tetrahydrobiopterin. Mol Genet Metab. 2005;86(1):61-6.
5. Kareem AA, Al-Auqbi TFR, Hammoodi HZ, Shreef HR, Sallih MM. Significance of tetrahydrobiopterin in management of hyperphenylalaninemia. Iraqi J Community Med. 2020;33(1):1.
6. Matalon R, Koch R, Michals-Matalon K, Moseley K, Surendran S, Tyring S, et al. Biopterin responsive phenylalanine hydroxylase deficiency. Genet Med. 2004;6(1):27-32.
7. Highland K. PKU and BH4. Advances in Phenylketonuria and Tetrahydrobiopterin. In: Blau N, editor. Advances in Phenylketonuria and Tetrahydrobiopterin: SPS Publications; 2006.
8. Kure S, Hou DC, Ohura T, Iwamoto H, Suzuki S, Sugiyama N, et al. Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. J Pediatr. 1999;135(3):375-8.
9. Gallagher RC, Enns GM, Cowan TM, Mendelsohn B, Packman S. Aminoacidemias and Organic Acidemias. 6th ed: Swaiman's Pediatric Neurology Principles and Practice; 2018.
10. Trefz FK, Belanger-Quintana A. Sapropterin dihydrochloride: a new drug and a new concept in the management of phenylketonuria. Drugs Today (Barc). 2010;46(8):589-600.
11. Regier D, Greene C. Phenylalanine Hydroxylase Deficiency. In: Adam M, Ardinger H, Pagon R, editors. GeneReviews. University of Washington, Seattle: Seattle (WA); 2000.
12. Campistol J, Gassio R, Artuch R, Vilaseca MA, Unit PKUF-u. Neurocognitive function in mild hyperphenylalaninemia. Dev Med Child Neurol. 2011;53(5):405-8.
13. Leuret O, Barth M, Kuster A, Eyer D, de Parscau L, Odent S, et al. Efficacy and safety of BH4 before the age of 4 years in patients with mild phenylketonuria. J Inherit Metab Dis. 2012;35(6):975-81.
14. Muntau AC, Roschinger W, Habich M, Demmelmair H, Hoffmann B, Sommerhoff CP, et al. Tetrahydrobiopterin as an alternative treatment for mild phenylketonuria. N Engl J Med. 2002;347(26):2122-32.
15. Al Riyami S, Al Maney M, Joshi SN, Bayoumi R. Detection of Inborn Errors of Metabolism using Tandem Mass Spectrometry among High-risk Omani Patients. Oman Med J. 2012;27(6):482-5.
16. Yadav GC, Reavey PC. Aminoacidopathies: a review of 3 years experience of investigations in a Kuwait hospital. J Inherit Metab Dis. 1988;11(3):277-84.
17. Thijeel RF. The Prevalence of Phenylketonuria and other metabolic diseases in sick Iraqi children; the importance of the newborn screening program. J Fac Med Baghdad. 2013;55(3):214-8.
18. Opladen T, Lopez-Laso E, Cortes-Saladelafont E, Pearson TS, Sivri HS, Yildiz Y, et al. Correction to: Consensus guideline for the diagnosis and treatment of tetrahydrobiopterin (BH4) deficiencies. Orphanet J Rare Dis. 2020;15(1):202.
19. Sadek AA, Hassan MH, Mohammed NA. Clinical and neuropsychological outcomes for children with phenylketonuria in Upper Egypt; a single-center study over 5 years. Neuropsychiatr Dis Treat. 2018;14:2551-61.
20. Opladen T, Hoffmann GF, Blau N. An international survey of patients with tetrahydrobiopterin deficiencies presenting with hyperphenylalaninaemia. J Inherit Metab Dis. 2012;35(6):963-73.
21. Michals-Matalon K, Bhatia G, Guttler F, Tyring SK, Matalon R. Response of phenylketonuria to tetrahydrobiopterin. J Nutr. 2007;137(6):1564-7.
22. Zurfluh MR, Zschocke J, Lindner M, Feillet F, Chery C, Burlina A, et al. Molecular genetics of tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. Hum Mutat. 2008;29(1):167-75.
23. Swoboda KJ, Walker MA. Neurotransmitter-Related disorders: Swaiman's Pediatric Neurology Principles and Practice; 2018.
24. Vernon HJ, Koerner CB, Johnson MR, Bergner A, Hamosh A. Introduction of sapropterin dihydrochloride as standard of care in patients with phenylketonuria. Mol Genet Metab. 2010;100(3):229-33.
25. Cerone R, Andria G, Giovannini M, Leuzzi V, Riva E, Burlina A. Testing for tetrahydrobiopterin responsiveness in patients with hyperphenylalaninemia due to phenylalanine hydroxylase deficiency. Adv Ther. 2013;30(3):212-28.

26. Christ SE, Moffitt AJ, Peck D, White DA. The effects of tetrahydrobiopterin (BH4) treatment on brain function in individuals with phenylketonuria. Neuroimage Clin. 2013;3:539-47.
27. Lindegren ML, Krishnaswami S, Reimschisel T, Fonnesbeck C, Sathe NA, McPheeters ML. A Systematic Review of BH4 (Sapropterin) for the Adjuvant Treatment of Phenylketonuria. JIMD Rep. 2013;8:109-19.
28. Ponzone A, Guardamagna O, Spada M, Ponzone R, Sartore M, Kierat L, et al. Hyperphenylalaninemia and pterin metabolism in serum and erythrocytes. Clin Chim Acta. 1993;216(1-2):63-71.
29. Khatami S, Dehnabeh SR, Zeinali S, Thony B, Alaei M, Salehpour S, et al. Four Years of Diagnostic Challenges with Tetrahydrobiopterin Deficiencies in Iranian Patients. JIMD Rep. 2017;32:7-14.