Original Article

Significance of Tetrahydrobiopterin in Management of Hyperphenylalaninemia

Adel A. Kareem, Tawfeeq Fakhir R. Al-Auqbi[1], Husham Z. Hammoodi, Hula R. Shreef, Maher M. Sallih[2]

Children Welfare Teaching Hospital, Department of Neurology, [1]National Diabetes Center, Al-Mustansiriyah University, [2]Children Welfare Teaching Hospital, Department of Neurology, Baghdad Medical City Teaching Complex, Baghdad, Iraq

Abstract

Background: The Sapropterin dihydrochloride enzyme cofactor, a synthetic analog form of 6R-BH4, was offered as an oral treatment for hyperphenylalaninemia. The sapropterin dihydrochloride role in patients with phenylketonuria (PKU) is to activate the endogenous phenylalanine hydroxylase (PAH) and to restore partially the oxidative metabolism of phenylalanine (Phe), when patients have insufficiency or lack of tetrahydrobiopterin. The sapropterin dihydrochloride anticipated to restore PAH activity through providing an exogenous source of the lost or deficient cofactor. **Aim of Study:** This study aims to evaluate the effects and clinical significance of tetrahydrobiopterin supplementation in hyperphenylalaninemia. **Patients and Methods:** This study describes the use of tetrahydrobiopterin in 58 patients with hyperphenylalaninemia treated before the age of 15 years with the use of tetrahydrobiopterin loading test. More than 30% decrement in Phe consider positive. **Results:** Fifty-eight patient been enrolled in this prospective study as follows; seven patient non PKU hyperphenylalaninemia (Phe level <600 μM), 13 patients mild to moderate PKU (Phe level between 600 and 1200 μM) and 38 with classic PKU (Phe level equal or above 1200 μM). The mean of Phe at the diagnosis was 377.00 ± 150.240 μM, 843.00 ± 133.899 μM and 1513.736 ± 274.372 μM sequentially. The response to treatment with tetrahydrobiopterin was inversely related to the level of Phe where its 100% in non PKU hyperphenylalaninemia, 70% in mild-to-moderate PKU and 16% in classic PKU. Tetrahydrobiopterin therapy significantly enhanced dietary Phe tolerance and permitted a Phe-free medical formula to be discontinued in a significant number of patients in whom phenylalaninemia within therapeutic target (120–300 μM) were achieved. Tetrahydrobiopterin displays the safety and usefulness of this treatment for patients mild PKU. **Conclusions:** Tetrahydrobiopterin therapy should be tried in all patients with hyperphenylalaninemia and/or PKU as it may significantly decrease the Phe level and improvement his milestone moreover safe in mild PKU.

Keywords: BH4, hyperphenylalaninemia, kuvan, phenylketonuria, sapropterin dihydrochloride

Introduction

The recognition of phenylketonuria (PKU) by Dr. Asbjørn Følling was avitall and mark in the, field of metabolic disorders, medicine. The PKU model was used to explain how the metabolic abnormalities could have neurological effects, and how the management may extremely affect the clinical feature of the disorder. The establishment of Guthrie's screening test and dietary management make the avoidance of cerebral damage possible, in affected kids all over the world. Furthermore, the PKU model, since its first use, was applied for research of more than 200 other inborn errors of metabolism.[1,2]

The PKU and its milder variant hyperphenylalaninemia (HPA) are inherited conditions characterized by a deficiency of phenylalanine hydroxylase (PAH), an essential enzyme to metabolize the phenylalanine (Phe) to Tyrosine (Tyr). When the blood level of Phe is higher than the upper reference limit, the PAH deficiency classified into classic PKU (Phe >1200 μmol/L), mild PKU (Phe 600–1200 μmol/L), and mild HPA (Phe <600 μmol/L).[3]

Tetrahydrobiopterin (BH4) is considered as the natural cofactor of the PAH enzyme. In the different BH4 deficient syndromes that may cause significant neurologic features

Access this article online

Quick Response Code:

Website: www.journalijcm.org

DOI: 10.4103/IRJCM.IRJCM_4_20

Address for correspondence: Dr. Tawfeeq Fakhir R. Al-Auqbi, National Diabetes Center, Al-Mustansiriyah University, Baghdad, Iraq.
E-mail: tawfeeqauqbi@yahoo.co.uk

Submitted: 08-Sep-2019 **Revised:** 01-Oct-2019
Accepted: 30-Dec-2019 **Published:** 29-Jun-2021

How to cite this article: Kareem AA, Al-Auqbi TR, Hammoodi HZ, Shreef HR, Sallih MM. Significance of tetrahydrobiopterin in management of hyperphenylalaninemia. IRAQI J COMMUNITY MED 2020;33:1-5.

the deficiency of BH4 lead to PAH dysfunction, which in turn result in hyperphenylalaninemia; plus cerebral Tyr and tryptophan hydroxylases dysfunction, subsequently lead to neurotransmitter deficiencies.[4] Kure *et al*. in 1999 was first shown that some patients with mutations in the PAH gene who received doses of BH4 (20 mg/kg) may well reduce blood Phe levels;[5] subsequently, 10%–60% of patients with enzyme deficiency of the PAH have been established to be BH4 responsive, depends on the mutations dominant in the different inhabitants.[6,7] In the beginning, few centers had the right to use the noncommercial form of BH4 (Shircks Laboratories, Jona, Switzerland), but later on the sapropterin dihydrochloride was industrialized and commercially launched after getting approval in the USA in 2007, and worldwide since 2008 in Europe and other countries.

In general, not all the PAH deficient PKU patients were responding to BH4; there is a wide range in the degree of response was noticed among patient who considered as responders. So that, a challenging test is performed to evaluate whether a PAH deficiency PKU patient is responsive to BH4 or not. In general, European centers prefer the shorter duration challenging tests, like that suggested by Blau *et al*.,[8] Often, 48-h tests identify the most BH4responders, if frequent blood Phe measurements are done. Short-term challenging test helps in decision about the treatment strategy to be prepared sooner; moreover, it was lower in cost with less intervention of the potential dietary regimen. Yet, the longer challenges are less probable missing potential late responders,[9] and possibly to assess other advantages of the treatment like special effects upon neurocognitive processing.

Once the response to BH4 was proved, it is necessary to gradually modify the diet till the maximum Phe tolerance is established. The early months of treatment require a very close nutritional follow up, for explaining the essential nutritional modifications to the family, be sure about family compliance and identify any opinion about food phobia and/or misinterpretations which may lead to dietary insufficiencies on the long-term. The typical approach to determine the patient's Phe tolerance is to provide a little amount of extra Phe weekly with a close monitoring of blood Phe levels; the medical Phe-free formulas should be decreased consequently to the recent protein requirement.[10] There are some suggested guiding principles, how to reach the new dietary regimen; many published papers showed that, patients with very mild phenotypes and a significant declining in their Phe levels throughout the challenge testing period could be reach feeding with normal diet. While, marked and severe phenotypes patients show a little reduction in their Phe levels or a delay in treatment response, who receiving BH4 could tolerate an increase in dietary Phe intake, but not entirely relieve and they need the dietary restriction in the long-term.[11,12] Pharmacologically treated patients with positive results shows a rise in nutritional Phe tolerance; however, If Phe tolerance does not obtain, its maybe due to a false positive result of the challenging test and long-term treatment must be re-evaluated.

Regarding the optimal method for assessing BH4 responding, many questions should be answered; on the long-term, BH4 therapy enhances the improvement of neurological function, dietary state and the psychosocial comfort of patients. The likelihood of adverse side effect after extended therapy, safety and the benefits of BH4 treatment in very young children or women during pregnancy and breastfeeding are still being important issues to be assessed.[13] Presentlysapropterin is becoming accessible worldwide, and involved in different ongoing register office is crucial in order to collectenough data to getsolid conclusions.

Aim of study

To evaluate the effects and clinical significance of tetrahydrobiopterin supplementation in hyperphenylalaninaemia.

Patients and Methods

Study design

A longitudinal follow-up designed study was carried on 58 patients, who prove to have hyperphenylalaninemia during their work up for psychomotor delay. The study was conducted from August 1, 2016 to January 31, 2019; at the pediatric neurology department of the Welfare Teaching Hospital, Medical City Complex, a tertiary health care government sponsored teaching hospital in Baghdad.

Patients

Patients recruited in this study were from all the regions of Iraq, a total of 58 cases aged from 1 month to 15-year-old, who diagnosed as PKU or hyperphenylalaninemia and seeking consultation from the pediatric neurology and neurometabolic clinic at the Welfare Teaching Hospital.

Patients were classified into three groups according to their blood Phe level, the classic PKU group (38 patient, Phe >1200 μmol/L), mild to moderate PKU group (13 patients, Phe 600–1200 μmol/L) and mild HPA group (7 patients, blood Phe is higher than the upper reference limit, but <600 μmol/L).[3]

Inclusion criteria

1. Newly diagnosed children, HPA proved by high performance liquid chromatography (HPLC)
2. An age between 1 month and 15 years.

BH4(kuvan) loading test

To assess the response to BH4 therapy; the protocol was followed by giving patients 20 mg/kg of BH4 analogue (Kuvan®) for 2 days.

All patients were on a normal diet at least 2 days prior and during the test. The BH4 loading test (kuvan ®test) was performed using 20 mg/kg BH4, ordered orally and directly after taking a baseline venous blood sample. The second blood samples were taken 24 h after the second dose of BH4. Blood samples were collected and analyzed by HPLC.

Ethical approval

This study was performed according to the Helsinki II Declaration and approved by the ethics committee and the scientific research committee by the pediatric neurology department of the Welfare Teaching Hospital and the National Diabetes Center. All patients were informed about the aim and the suspected benefit of the study before obtaining their agreements for participation; a written consent was obtained from all enrolled participants and their families. All the medical research ethics rules and instructions regarding patient's privacy, humanity and security; as well as the medical research, laboratory data and investigation results were strictly considered throughout all the steps of the study.

Statistical analyses

Statistical analysis and reporting of obtaining data were carried out by the Statistical Package for the Social Sciences (SPSS) (version 20.0) program (IBM SPSS Statistics, SPSS Inc., Chicago, Illinois, USA).

The Anderson–Darling test was performed to test the adherence of continuous, parametric, variables to the normal distribution. Data were reported and presented as mean ± standard deviation and/or 95% confidence interval for the normally distributed continuous parametric variables. The discrete, nonparametric variables presented as number and percentages.

Independent 2 samples student *t*-test was used to analyze the statistical significance of differences between the mean of two parametric variables. While, the Chi-square test was used for analysis of the statistical significance of discrete nonparametric variables.

Statistical tests were considered statistically significant, when the $P \leq 0.05$ and $P \leq 0.001$ considered of high statistical significance.

Results

It is clear in this study that the female was the more of both groups [Table 1].

Regarding age at diagnosis, although the group of respondents was earlier, without statistically significant difference, $P \geq 0.05$.

After kuvan loading test, for the no response group, the level of Phe tends to increase, while the good response group shows statistically significant decrease of Phe. While the Tyr shows insignificant statistical difference between the two groups Table 2;

Our patients were classified in a three group of Phe, Table 3;

The clinically abnormal movement represents the main presentation of the respondent and then psychological delay. While in the no-respondent group, the psychomotor delay was the main presentation and shows statistical significant difference from the response group [Table 4].

The consanguinity Prominent in both groups and that is why there is no constant significance [Table 4].

Patients with non-PKU hyperphenylalaninemia show 100% response to Kuvan [Table 5].

Discussion

Newborn screening for PKU and HPA was established in Iraq since 2010, but it is limited just for two provinces, i.e., is why it is not uncommon to see PKU and/or HPA patients in pediatric neurology clinic.

The study announces, for many reasons, to accept treatment of hyperphenylalaninemia by medication better than dietary modification, special diet, First, the elevation of Phe concentration in the circulating blood, as a result of PKU and HPA, has a neurotoxic effect on the brain,[13,14] secondly; there is no test for diagnosis of nonclassical type of PKU at the moment to monitor and follow up patients, thirdly: Even those patients who diagnosed by neonatal screening facing difficulty to keep their Phe level within an acceptable range for several reasons like shortage of dietitian specialized in PKU management, feeding with special formula diet was not always accessible, poor compliance of patients and families, and long-term low protein diet apply financial burden for parents. In the last few years, all metabolic units that treat PKU patients have conducted a BH4 load test in an attempt to identify patients who can get benefit from the kuvan; however, the mild HPA cases are more likely to benefit from BH4.[6,15,16]

The description of the BH4 reception was studied for the first time in the neurometabolic disorders unit in Iraq. A total of 58 patients with HPA and PKU underwent a BH4 load test; generally, the response was inversely related to the Phe level and those with mild HPA shows a full response, 100% of respondents, our results was corresponds to many studies in the world.[17-19]

Clinically, patients with BH4 deficiency show malignant forms of PKU although they had mild hyperphenylalaninemia, because it is a cofactor for PAH, Tyr hydroxylase and tryptophan hydroxylase.[20] On the other hand, patients with enzyme deficiency may get a benefit because it increases residual activity of PAH mostly by a pharmacological chaperone action, even at an early age. However, the BH4 loading test was initially used for differentiating between PKU

Table 1: Sex distribution of study groups according to the response to kuvan

Sex	All (n=58), n (%)	No response (n=36), n (%)	Good response (n=22), n (%)	P
Males	23 (39.66)	14 (38.89)	9 (40.90)	0.0233 (0.879)
Females	35 (60.34)	22 (61.11)	13 (59.10)	

Table 2: Description the study participants according to responding to kuvan

Variables	Mean±SD (95% CI)			*P*
	All (*n*=58)	No response (*n*=36)	Good response (*n*=22)	
Age at diagnosis (months)	7.413±6.282 (7.362-7.465)	8.222±6.542 (8.153-8.290)	6.090±5.731 (6.014-6.167)	0.1992
Phe befor kuvan (nmol/mL)	1226.206±481.857 (1222.23-1230.17)	1460.305±292.303 (1457.250-1463.360)	843.136±490.042 (836.584-849.687)	0.00001
Phe after kuvan (nmol/mL)	933.668±562.932 (929.033-938.304)	1284.416±311.086 (1281.165-1287.667)	359.718±377.944 (354.665-364.770)	0.00001
Phe reduction (nmol/mL)	292.537±250.662 (290.474-294.601)	175.888±180.667 (174.00-177.777)	483.418±233.761 (480.292-486.543)	0.00001
Phe reduction (%)	31.130±29.8193 (30.884-31.375)	11.853±13.307 (11.714-11.993)	62.672±20.953 (62.392-62.952)	0.00001

SD: Standard deviation, CI: Confidence interval

Table 3: Description of the study participants according to the type of phenylketonuria

Variables	Mean±SD (95% CI)			*P*
	Hyperphenylalaninaemia (*n*=7)	Mild-moderate pku (*n*=13)	Classic pku (*n*=38)	
Age at diagnosis (months)	3.7142±3.251 (3.637-3.791)	8.307±6.498 (8.194-8.420)	7.789±6.510 (7.723-7.855)	0.25
Phe befor kuvan (nmol/mL)	377.00±150.240 (373.439-380.560)	843.00±133.899 (840.671-845.328)	1513.736±274.372 (1510.945-1516.527)	0.0001
Phe after kuvan (nmol/mL)	122.114±87.918 (120.030-124.198)	456.846±402.661 (449.843-463.849)	1246.289±359.843 (1242.629-1249.949)	0.0001
Phe reduction (nmol/mL)	254.885±143.949 (251.473-258.297)	386.153±318.985 (380.606-391.701)	267.447±237.570 (265.030-269.864)	0.003
Phe reduction (%)	66.880±19.085 (66.428-67.333)	49.413±39.186 (48.731-50.094)	18.289±16.856 (18.118-18.461)	0.0001

SD: Standard deviation, CI: Confidence interval

Table 4: Clinical description of study participants according to kuvan response

Clinical variables	All (*n*=58), *n* (%)	No response (*n*=36), *n* (%)	Good response (*n*=22), *n* (%)	*P*
Skin lesion				
Negative	33 (56.89)	17 (47.22)	14 (63.63)	0.030
Positive	25 (43.10)	19 (52.78)	8 (36.37)	
Seizure				
Negative	34 (57.89)	17 (47.22)	15 (68.18)	0.078
Positive	24 (41.37)	19 (52.78)	7 (31.82)	
Abnormal movement				
Negative	28 (48.27)	19 (52.78)	9 (40.9)	0.380
Positive	30 (51.72)	17 (47.22)	13 (59.01)	
Psychomotor delay				
Negative	21 (36.20)	5 (13.89)	12 (54.54)	0.001
Positive	37 (63.79)	31 (86.11)	10 (14.46)	
Consanguinity				
1st	55 (94.82)	35 (97.22)	20 (90.9)	0.139
2nd	1 (1.72)	1 (2.78)	0	
Negative	2 (3.44)	0	2 (9.1)	

Table 5: Clinical description of patient's response, according to the type of phenylurea

Sex	Hyperphenylalaninaemia (*n*=7), *n* (%)	Mild-moderate pku (*n*=13), *n* (%)	Classic pku (*n*=38), *n* (%)	χ^2 (*P*)
Respond patients	7 (100.00)	9 (69.23)	6 (15.79)	24.8 (0.0001)†
Not respond patients	0	4 (30.77)	32 (84.21)	

†Difference of very high statistical significance

and BH4 deficiency, but it was a valuable tool for selecting BH4 response cases from PKU since Kure *et al*. showed that four of five patients with HPA responded to oral BH4 therapy by lowering their PHA levels.[5,21]

The BH4 loading test was carried for all participants, to evaluate responsiveness to BH4, including 16% of classic PKU subjects; to confirm the previous reports of the BH4 response in the classic PKU.[22,23] A significant response was observed, 70% of patients enrolled with mild to moderate PKU responded to BH4, confirming that phenotype is not the only indicator of response to BH4, but other aspects must be considered.

In the meantime, responsiveness to BH4 cannot be predicted depending on the basis of pretreatment Phe concentrations,

therefore we classify the patients to: Tetrahydrobiopterin-unresponsive HPA and tetrahydrobiopterin-responsive HPA, the responsive patients include tetrahydrobiopterin-responsive PAH deficiency and defects in the synthesis of tetrahydrobiopterin. Our short protocol BH4 regimen does not exclude the possibility of subtle effects that may become noticeable only after a long protocol even in some patients with classic PKU.

Many difficulties limit the tetrahydropterin therapy to its routine use, especially for detection later responsive, the first: This medication is costly, Second: Dose-finding studies and scientific trials are necessary to determine the bioavailability and long-term effects of tetrahydrobiopterin therapy in patients with PAH deficiency.

Conclusions

The pharmacological doses of tetrahydrobiopterin BH4 correct impaired Phe oxidation in the vast majority of patients with mild HPA phenotypes.

The success output and results have a consequence on diagnostic work-up and clinical classification of HPA and PKU for therapeutic interferences. Therefore, a significant number of patients with HPA, BH4 treatment may preclude the need for the most troublesome dietary special regime.

Recommendations

Expanding and extending of neonatal screening to include all Iraq.

Acknowledgments

The authors would like to thank all the patients with PKU and their families for their participation and cooperation in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Applegarth DA, Toone JR, Lowry RB. Incidence of inborn errors of metabolism in British Columbia. Pediatrics 2000;105:e10.
2. Raghuveer TS, Garg U, Graf WD. Inborn errors of metabolism in infancy and early childhood: An update. Am Fam Physician 2006;73:1981-90.
3. Hanley WB. Adult phenylketonuria. Am J Med 2004;117:590-5.
4. Hyghland K. Tetrahydrobiopterin deficiencies with hyperphenylalaninemia. In: Blau N, editor. PKU and BH4. Advances in Phenylketonuria and Tetrahydrobiopterin. india: SPS Publications; 2006.
5. Kure S, Hou DC, Ohura T, Iwamoto H, Suzuki S, Sugiyama N, *et al*. Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. J Pediatr. 1999 Sep;135(3):375-8. doi: 10.1016/s0022-3476(99)70138-1. PMID: 10484807.
6. Bernegger C, Blau N. High frequency of tetrahydrobiopterin-responsiveness among hyperphenylalaninemias: A study of 1,919 patients observed from 1988 to 2002. Mol Genet Metab 2002;77:304-13.
7. Karacić I, Meili D, Sarnavka V, Heintz C, Thöny B, Ramadza DP, *et al.* Genotype-predicted tetrahydrobiopterin (BH4)-responsiveness and molecular genetics in Croatian patients with phenylalanine hydroxylase (PAH) deficiency. Mol Genet Metab 2009;97:165-171.
8. Blau N, Bélanger-Quintana A, Demirkol M, Feillet F, Giovannini M, MacDonald A, *et al*. Optimizing the use of sapropterin (BH(4)) in the management of phenylketonuria. Mol Genet Metab 2009;96:158-63.
9. Levy H, Burton B, Cederbaum S, Scriver C. Recommendations for evaluation of responsiveness to tetrahydrobiopterin (BH4)) in phenylketonuria and its use in treatment. Mol Genet Metab 2007;92:287-91.
10. MacDonald A, Ahring K, Dokoupil K, Gokmen-Ozel H, Lammardo AM, Motzfeldt K, *et al*. Adjusting diet with sapropterin in phenylketonuria: What factors should be considered? Br J Nutr 2011;106:175-82.
11. Singh RH, Jurecki E, Rohr F. Recommendations for personalized diet adjustments based on patients response to tetrahydrobiopterin (BH4) in phenylketonuria. Top Clin Nutr 2008;23:149-57.
12. Quintana BA, García MJ, Castro M, Desviat LR, Pérez B, Mejía B, *et al.* Spanish BH4-responsive phenylalanine hydroxylasedeficient patients: Evolution of seven patients on long-term treatment with tetrahydrobiopterin. Mol Genet Metab 2005;86:S61-6.
13. Trefz FK, Belanger-Quintana A. Sapropterin dihydrochloride: A new drug and a new concept in the management of phenylketonuria. Drugs Today (Barc) 2010;46:589-600.
14. Feillet F, van Spronsen FJ, MacDonald A, Trefz FK, Demirkol M, Giovannini M, *et al.* Challenges and pitfalls in the management of phenylketonuria. Pediatrics 2010;126:333-41.
15. Weglage J, Grenzebach M, von TeeVelen-HeithoV A, Marquardt T, Feldmann R, Denecke J, *et al*. Tetrahydrobiopterin responsiveness in a large series of phenylketonuria patients. J Inherit Metab Dis 2002;25:321-2.
16. Desviat LR, Perez B, Quintana AB, Castro M, Aguado C, Sanchez A, *et al.* Tetrahydrobiopterin responsiveness: Results of the BH4 loading test in 31 Spanish PKU patients and correlation with their genotype. Mol Genet Metab 2004;83:157-62.
17. Duenas BP, Vilaseca MA, Mas A, Lambruschini N, Artuch R, Gomez L, *et al*. Tetrahydrobiopterin responsiveness in patients with phenylketonuria. Clin Biochem2004;37:1083-90.
18. Matalon R, Koch R, Matalon KM, Moseley K, Surendran S, Tyring S, *et al*. Biopterin responsive phenylalanine hydroxylase deWciency. Genet Med 2004;6:27-32.
19. Quintana AB, García MJ, Castrob M, Lourdes R, Pérezb DB, Mejía 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:S61-6.
20. Pin ˜a-Garza JE, James KC. Psychomotor retardation and regression. Fenichel's Clinical Pediatric Neurology. 8th ed. eBook ISBN: 9780323496858: Elsevier CH.5 2019:127.
21. Leuret O, Barth M, Kuster A, Eyer D, deParscau 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;35:975-81.
22. Hennermann JB, Bührer C, Blau N, Vetter B, Mönch E. Long-term treatment with tetrahydrobiopterin increases phenylalanine tolerance in children with severe phenotype of phenylketonuria. Mol Genet Metab 2005;86 Suppl 1:S86-90.
23. Ponzone A, Spada M, Roasio L, Porta F, Mussa A, Ferraris S. Impact of neonatal protein metabolism and nutrition on screening for phenylketonuria. J Pediatr Gastroenterol Nutr 2008;46:561-9.