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Original article

Diagnosis, treatment and follow-up of patients with tetrahydrobiopterin deficiency in Shandong province, China

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Abstract

Objectives: To summarize the clinical and biochemical data, mutation analysis, treatment, outcome and the follow-up data of patients with BH4 deficiency from 2004 to 2012 in Shandong province, China.

Methods: We analyzed the clinical, biochemical and treatment data of 40 patients with BH4 deficiency. Urinary neopterin and biopterin were analyzed. Further BH4 loading tests were performed in suspected patients with abnormal urinary pterin profiles. The patients with BH4 deficiency were treated with BH4 and neurotransmitter after diagnosis. Blood phenylalanine level, clinical symptoms and mental development were followed up.

Results: 40 cases with BH4 deficiency were identified and all classified as PTPS deficiency between 2004 and 2012 in Shandong province, China. They were diagnosed at the age of 20 d – 41 m and most patients received treatment with BH4, L-dopa and 5-HTP after diagnosis. Seven different mutations (P87S, K91R, T106M, D96N, N52S, S21R, and L127F) were detected in 11 patients. But outcome assessments were not always available. We obtained 19 records of DQ/IQ assessment. In 9 patients (7 early and 2 late diagnosed) no development delay is observed, while in 10 patients (8 early and 2 late diagnosed) development was delayed.

Conclusions: Our study emphasized that screening for BH4 deficiency should be carried out in all patients with HPA in order to minimize misdiagnosis. Although the outcomes of BH4 deficiency are highly variable, early diagnosis and treatment is essential for good outcomes.

Keywords: HPA; BH4 deficiency; PTPS; Treatment; Follow-up

Abbreviations: HPA, hyperphenylalaninemia; PAH, phenylalanine hydroxylase; PKU, phenylketonuria; BH4, tetrahydrobiopterin; GTPCH, guanosine 5′-triphosphate cyclohydrolase I; PTPS, 6-pyruvoyl-tetrahydropterin synthase; PCD, pterin-4-carbinolamine dehydratase; DHPR, dihydropteridine reductase; 5-HTP, 5-hydroxytryptophan; DQ, developmental quotient; IQ, intelligence quotient

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1. Introduction

Hyperphenylalaninemia (HPA), the most common inborn error of amino acid metabolism, is an autosomal recessive metabolic disease caused by a deficiency of phenylalanine hydroxylase (PAH) or by deficiency of enzymes involved in tetrahydrobiopterin (BH4) synthesis or recycling [1]. In most cases, HPA results from PAH deficiency, which converts phenylalanine to tyrosine. Classical therapy of PAH deficiency consists of phenylalanine (Phe) intake restriction. Dietary treatment has proved to be very effective in preventing the devastating consequences of PAH deficiency when started early in life [2].

A rare form of HPA can arise from the deficiency of an essential cofactor, BH4, which leads to decreased metabolism of Phe into tyrosine. In addition to being a cofactor for PAH, BH4 is also a significant cofactor for tyrosine hydroxylase and tryptophan hydroxylase, which involved in conversion of tyrosine and tryptophan into catecholamine and serotonin [3]. BH4 deficiency can be caused by a defect in one of four enzymes responsible for its synthesis and recycling, including guanosine 5′-triphosphate cyclohydrolase I (GTPCH), 6-pyruvoyl-tetrahydropterin synthase (PTPS), pterin-4-carbinolamine dehydratase (PCD), and dihydropteridine reductase (DHPR) [4]. The most common form is PTPS deficiency followed by DHPR deficiency. Due to involving the deficiency of neurotransmitters whose synthesis depends on the normal activity of BH4-dependent tyrosine and tryptophan hydroxylases, patients lacking BH4 show signs of progressive neurological impairment when treated with a Phe-restricted diet alone [5]. The predominant symptoms are muscular hypotonia, mental retardation, dystonia, convulsions and movement disorder [5]. BH4 deficiency is treatable disease. Consequently, these patients had to be rapidly recognized among hyperphenylalaninemic patients. Because BH4 does not penetrate the blood–brain barrier well, supplementation with the neurotransmitters precursors (5-HTP and L-dopa) is necessary to improve the turnover of dopamine and serotonin. The patients should receive combination treatment with BH4 and neurotransmitter precursors as soon as possible to reduce neurological deterioration [5]. DHPR-deficient patients need additional supplementation with folinic acid [6].

Neonatal screening for the detection of HPA has been established in numerous countries. In China, newborn screening was started in Shanghai in 1981 [7]. Since 1990 screening for BH4 deficiency among patients with HPA has been carried out in Shanghai [7]. The aim of this study was to summarize the clinical and biochemical data, mutation analysis, treatment, and the follow-up data of patients with BH4 deficiency from 2004 to 2012 in Shandong province, China.

2. Materials and methods

2.1. Patients

Total patients with HPA were diagnosed by newborn screening in Jinan maternal and Child Care Hospital from 2004 to 2012. Elevated phenylalanine concentrations were detected by newborn screening in dried blood spots. All tests were performed within routine clinical and biochemical investigation and in accordance with the ethical principles of the Declaration of Helsinki. Informed consent was obtained from guardians of all patients for being included in the study.

2.2. Urinary pterin analysis

Fresh urine samples were collected for the determination of pterin profile. Briefly, 100 mg ascorbic acid was added into 10 ml urine to keep BH4 in its reduced form. After centrifugation, 50 μl of the supernatant was diluted into 450 μl of H_2O. Then urine was added 50 μl of 0.1 mol/L HCl and 10 mg MnO_2 and incubated for 15 min at room temperature. The mixture was centrifuged at 13,000 g for 10 min. The remaining supernatant was used for pterin analysis by high-performance liquid chromatography. Two major peaks were detected: neopterin (N) and biopterin (B). Furthermore, B% (B/B + N%) was calculated.

2.3. BH4 loading test

When blood Phe concentration was >600 μmol/L, BH4 loading test would be performed by oral administration of BH4 at a dose of 20 mg/kg. Blood samples were collected at 2 h, 4 h, 6 h, 8 h, and 24 h after BH4 intake to measure the blood Phe concentration. When blood Phe concentration was <600 μmol/L, the combined phenylalanine and BH4 loading test was performed. Patients received BH4 (20 mg/kg) at 3 h after the Phe loading (100 mg/kg), then blood samples were collected at 2 h, 4 h, 6 h, 8 h, and 24 h after oral BH4 tablets.

2.4. DNA sequence analysis

Blood samples were collected from the BH4 deficient patients and genomic DNA was extracted by standard method. PCR primers were designed to amplify all six exons of *PTS* gene [8,9]. The primers used in this study are shown in Suppl. 1. The PCR products were purified by Gel Extraction kit and sequenced by using ABI Prism BigDye Terminator Cycle Sequencing kit on ABI Prism 3100 sequencer. Analyzed sequences were compared with genomic DNA sequences in Gen-Bank accession.