PHENYLKETONURIA DETECTED BY THE NEONATAL SCREENING PROGRAM IN THAILAND

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Abstract. Neonatal screening for phenylketonuria (PKU) was introduced as a pilot project in Thailand from 1992-1995, and mass screening was started in 1996 by the Department of Medical Sciences, Ministry of Public Health. Blood samples were collected by heelprick on filter paper either at 48 hours of life or before discharge from the hospital. Elevated blood phenylalanine was identified by screening with the Guthrie method, then followed by the fluorometric method. All infants with a phenylalanine level equal to or greater than 4 mg/dl were recalled and retested using the fluorometric method and confirmed by plasma amino acid analysis and urinary pterins for tetrahydrobiopterin deficiency. A total of 1,062,676 newborns were screened from October 1992 – March 2001, with 5 cases confirmed with PKU. The incidence was 1 in 212,535. All patients have been treated with low phenylalanine diet. The results of this study confirm the benefit of early detection and treatment of PKU through the screening program.

INTRODUCTION

Phenylketonuria (PKU) is an autosomal recessive disorder caused by a deficiency of hepatic phenylalanine hydroxylase activity. The phenylalanine hydroxylase gene was mapped to chromosome 12q22-24.1 (Scriver *et al*, 1995). The enzyme deficiency leads to elevated levels of phenylalanine and its metabolites in the blood. In untreated cases, patients gradually develop irreversible severe mental retardation, seizure, microcephaly, hypopigmented hair and skin, and behavior abnormalities (NIH, 2001; Irons, 1993; Smith and Brenton, 1996). PKU was first described in 1934 by Folling (Erbe and Levy, 1997). In 1947, Jervis identified that this disease was caused by phenylalanine hydroxylase deficiency (Matalon and Michals, 1991). In 1953, treatment with low phenylalanine diet was started (Bickel *et al*, 1953). In 1963, Robert Guthrie developed a simple neonatal screening method for PKU, known as the Guthrie test (Guthrie, 1963). Diagnosis of PKU by clinical criteria is usually made later than 6 months of age after the patient develops irreversible brain damage (Matalon and Michals, 1991). If PKU is detected early, it can be treated with a low phenylalanine diet thus preventing mental retardation (Smith *et al*, 1991; Seashore, 1990; Levy, 1986; Anonymous, 1993). Neonatal screening for PKU allows early detection and treatment of affected infants. Screening for PKU was introduced as a pilot project in Thailand from 1992–1995 and the Department of Medical Services, Ministry of Public Health, started mass screening in 1996.

MATERIALS AND METHODS

Blood samples for screening were obtained by heelprick on filter paper and collected from several provinces all over the country during October 1992 – March 2001. Samples were taken from infants at either 48 hours of age or before they were discharged from the hospital. Dried blood spots were screened for phenylalanine levels using the Guthrie method. Samples screened by the Guthrie method having a phenylalanine level equal to or greater than 4 mg/dl and those on antibiotic treatment were then tested by the fluorometric method. Positive cases were recalled and retested. If results remained elevated, confirmatory testing was done by plasma amino acid analysis using high performance liquid chromatography. Urinary pterins were determined for tetrahydropterine (BH4) deficiency. The diagnosis of PKU was assigned when the blood phenylalanine level was equal to or greater than 20 mg/dl and urinary pterins were normal.

RESULTS

A total of 1,062,676 newborns were screened during October 1992 – March 2001. Five cases were confirmed to have PKU. The incidence of PKU was 1: 221,535.

Case 1: A 2-month old female infant was referred from Saraburi province. She was born full term and

weighed 3,450 grams after an uncomplicated pregnancy, labor and delivery. Parents were healthy and unrelated. Family history was unremarkable. Newborn screening was done on the third day of life. Her first blood sample test showed a phenylalanine level of 5.89 mg/dL. The second blood sample showed a phenylalanine level of 32.2 mg/dL. She has been treated with a low phenylalanine diet since then. Currently, she is 3 years old with normal growth and development, as assessed by Denver Developmental Screening Test II (DDST II).

Case 2: A 3-month old male infant was referred from Udonthani province. He was born weighing 3,500 gm, a product of a term pregnancy, delivered by spontaneous vaginal delivery. Parents were healthy and unrelated. His initial blood sample test showed a phenylalanine level of 6.4 mg/dl. The retested blood sample revealed phenylalanine level of 27.7 mg/dl. The patient was recalled and started on a restricted phenylalanine diet. His parents were separated and he had been in the care of his grandmother since birth. Currently, he is 2 years and 10 months old with a developmental quotient (DQ) of 100.

Case 3: A 1-month old male infant was referred from Rathchaburi province. He was born weighing 3,500 gm after an uneventful pregnancy, labor and delivery. He developed neonatal jaundice on day 3 and was treated by phototherapy for 2 days. His parents were healthy and unrelated. He had a 4-year old mentally retarded brother whom we later proved to have PKU. His phenylalanine level on newborn screening was 5.92 mg/dl. The repeat sample showed a phenylalanine level of 30.68 mg/dl. He was treated with a low phenylalanine diet. At present, he is 4 months old with normal growth and developmental milestones.

Case 4: A 3-month old male infant was referred from Lumpoon province. He was born weighing 3,590 gm after an uncomplicated pregnancy, labor and delivery. His parents were healthy and unrelated. He had a 12-year old severely retarded sister. Subsequently, she was confirmed to have PKU. His initial blood test for phenylalanine level was 8 mg/dl. A confirmatory test sample showed a phenylalanine level of 22.42 mg/dl. He was recalled and started on a low phenylalanine diet. At present, he is 6 months old with normal growth and developmental milestones.

Case 5: A 2-month old male infant from Nan province was the product of a full term uncomplicated pregnancy, labor and delivery. His parents were normal and a history of consanguinity was denied. Family history was unremarkable. Phenylalanine level from the newborn screening test was 5.10 mg/dl. His second blood sample for phenylalanine level was 30.03 mg/dl. He was treated with a low phenylalanine diet only until 7 years of age due to noncompliance. He is currently 9 years and 4 months old with an intelligence quotient (IQ) of 65 due to poor diet control and is now studying in a regular second grade class.

DISCUSSION

Our study has identified 5 cases of PKU. All patients detected by neonatal screening came for treatment after 1 month of age, which was rather late. This delay was caused by many factors including, delay in retesting and/or confirmation of the initial results, disinterested parents, lack of concern of health care providers towards screening, difficulty in tracing patients because of inaccurate address or lack of a telephone. All cases received low phenylalanine dietary treatment controlling the phenylalanine level between 2-6 mg/dl. After dietary treatment, the patients showed normal growth and development except for 1, who had mild mental retardation due to poor diet control. Although lifelong dietary control was recommended, poor dietary compliance by our patients started during childhood. One reason was the bad taste and smell of low phenylalanine food. The other reason was parental relaxation in diet control because the children were apparently normal.

In our study, 2 families had more than one child affected with PKU. Within each family, the untreated affected sibling was mentally retarded because of delay in diagnosis and treatment. The PKU infants who were not detected by neonatal screening were easily missed clinically especially in the early infancy period. This study emphasizes that early detection and treatment of PKU through newborn screening is very important to prevent mental retardation.

The incidence of PKU thus far appears to be 1: 212,535. The incidence of PKU in other countries ranges from 1:10,000 to 1:85,000 (American Academy of Pediatrics, 1996; Naruse, 1980). The incidence of PKU in Thailand is lower than the worldwide incidence. PKU appears to be a rare disease in Thailand and, considering the incidence rate, the cost-benefit analysis of the program should be re-evaluated periodically. Nonetheless, a bigger sample size is needed to verify the incidence of PKU in Thailand.

CONCLUSION

The neonatal screening program is beneficial for patients for early detection and treatment of PKU, especially for preventing mental retardation. At present, a nationwide program has been established with the goal of

screening every infant born in Thailand in the near future.

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