Neonatal Screening for Phenylketonuria

MICHAEL L. NETZLOFF, M.D.*

Department of Pediatrics and Human Development,
Michigan State University, College of Human Medicine,
East Lansing, MI 48824

ABSTRACT

The case histories are reported of three patients with phenylketonuria (PKU) in whom the initial Guthrie screening for PKU was falsely negative. Possible explanations for this problem are reviewed, as well as the limited cost-effectiveness of general re-testing. Guidelines are suggested to improve the sensitivity of PKU screening procedures for newborns.

Introduction

Phenylketonuria (PKU) is an hereditary disorder of metabolism with an incidence of about one in 10,000 births. Without treatment, the metabolic insult on the intellect is devastating, frequently resulting in IQ’s less than 50, inability to ambulate or communicate, and behavioral abnormalities typified by hyperactivity, irritability, and hyperkinesis. Prior to the availability of effective therapy, PKU accounted for approximately one percent of mentally retarded persons in institutions. Early dietary restriction of phenylalanine and consequent lowering of the elevated blood levels of this amino acid have been highly effective in preventing the mental and physical retardation associated with untreated PKU. Since newborn PKU patients are entirely asymptomatic, biochemical screening is necessary to identify those patients requiring treatment. The screening method used in most state laboratories and general hospitals is the Guthrie technique. This test requires elevated blood phenylalanine levels, which may not be found during the first few days of life or until ingestion of protein-containing formula by the neonate with PKU. These observations may explain the eight percent incidence of false negative diagnoses reported for PKU patients during the first four days of life. The Division of Genetics, Department of Pediatrics, University of Florida is a referral center for diagnostic confirmation and treatment of PKU patients following screening by other state or private agencies. This report will summarize the experience over one year with three of the four new patients with PKU, which strongly suggests a false negative rate of diagnosis much higher than previously noted.

* Formerly Director, PKU Follow-Up Program, Department of Pediatrics, University of Florida, College of Medicine, Gainesville, FL.
Case Reports

Case 1

R.H. is a 13 month old white male who was the 2.98 kg product of a term pregnancy and normal labor and delivery. A Guthrie screening test was performed four days after birth and following three days of regular formula feedings and the results were negative. He was initially asymptomatic but was noted to be failing to thrive at ten weeks of age and was hospitalized in his community. Hyperchloremic acidosis suggested a renal tubular problem and he was started on a citrate buffer, resulting in some weight gain after discharge from the hospital.

A repeat Guthrie test was performed at six months of age and the results were positive, with a blood phenylalanine greater than 20 mg per dl. He was then admitted for the first time to the Shands Teaching Hospital at age seven months and had a length of 62 cm, a weight of 6.36 kg, and a head circumference of 41 cm; all parameters were below the third percentile. He showed little interest in his environment and had limited spontaneous movement. Pertinent laboratory findings included normal serum electrolytes, except a serum bicarbonate of 13 mEq per L, and a serum phenylalanine of 28 mg per dl (normal 2.28 ± 0.8 mg per dl), and a low serum tyrosine of 0.75 mg per dl (normal, 2.4 ± 1.0 mg per dl). Urine pH was 6. The buffer solution was discontinued, and the patient was discharged on a phenylalanine-restricted diet.

Upon readmission 20 days later, he had developed interest in his surroundings and had following movements with his eyes. He was able to move from prone to supine position but could not sit without assistance. His serum electrolytes, blood gases, and urinalysis were normal. Phenylalanine loading test results were consistent with classic PKU. Subsequent to discharge, levels of serum phenylalanine have been maintained within therapeutic range on a restricted diet and he is thriving. Development testing at approximately 9.5 months of age using the Bayley Scales of Infant Development, showed a mental function range of 5.7 to 5.8 months. Dihydropyridine reductase assay on the patient's skin fibroblasts showed normal activity, eliminating the diagnosis of the more severe reductase-enzyme-deficient variant of PKU.

Case 2

L.T. is a 28 month old white female who was first seen at 11 months of age and was the product of a full-term, uncomplicated pregnancy. The Guthrie screening test obtained at approximately 72 hours of age, and 48 hours after beginning protein-containing formula, was negative (<4 mg per dl). Her neonatal course was characterized by irritability, frequent crying, and delayed developmental milestones. At 10 months of age, a urine metabolic screen was positive and a subsequent serum phenylalanine was 38 mg per dl.

Upon admission to the Pediatric Service of the Shands Teaching Hospital at age 11 months, her length was 77 cm (97th percentile), her weight was 9.75 kg (50th percentile), and her head circumference was 43 cm (3rd percentile). The anterior fontanel was almost closed; her muscle tone was decreased. She demonstrated repetitive movements, made no attempt to roll over, and was unable to sit erect without assistance. A urine ferric chloride was positive for phenylketones, and serum phenylalanine determinations by fluorometer following a seven-hour fast and two-hours post prandial were both approximately 70 mg per dl. Serum tyrosine values were decreased at 1.3 and 0.56 mg/dl. She was discharged on a phenylalanine restricted diet to be followed in clinic where she has shown improvement in her neurologic status.

Developmental testing with the Bayley Infant Scales at a chronologic age of 17 months showed an eye-hand coordination of between four to six months, physical development between 9 to 11 months, personal-social between 11 to 13 months, and language development of 12 to 14 months. These same parameters at 19 months of age were as follows: eye-hand, 8 to 10; physical development, 14 to 16; personal-social, 11 to 13; and language, 12 to 14 months. More recent testing at 25 months continues to show a delay of approximately seven months in L.T.'s mental and physical development. Fibroblast enzyme assay for PKU variant disease was negative.

Case 3

S.T. is a white male aged four years and nine months whose diagnosis of PKU was discovered at four years and three months. He was the 2.64 kg product of a full-term pregnancy and had an Apgar score of 9. The Guthrie test result at 48 hours of age was less than 2 mg per dl. His developmental milestones were markedly delayed: he sat up at 18 months and walked at 30 months; he has never been toilet trained nor has he developed any language. He had reportedly worn clothes of the same size for the previous two years.

During an admission to the Shands Teaching Hospital at four years and three months to investigate his severe retardation, the patient had a length of 89 cm (4.75 standard deviations below the mean for his age), a weight of 13 kg (< 3rd percentile) and a head circumference of 50.5 cm (< 50th percentile). His height age was that of a child 2.5 years old and radiographs for bone age were consistent with two years and eight months. He was noted to be very hyperactive and had an unusual musty odor. On the ward, a urine screen with ferric chloride was found to be positive for phenylketones, and a subsequent serum phenylalanine was 29.4 mg per dl with a normal tyrosine of 1.4 mg per dl. A low phenylalanine diet using Lofenalac was begun, and the patient has had modest improvement in his hyperactivity without effect on his profound mental retardation. Fibroblast enzyme assays eliminated the possibility of PKU variant disease owing to dihydropteridine reductase deficiency.
Discussion

Holtzman, et al. reporting the results of a survey of eight states, found 23 infants whose PKU test results were initially negative, but who were subsequently shown to have blood phenylalanine values above 20 mg per dl. At the same time, screening in these states had detected 253 patients with PKU. Thus, approximately eight percent of infants were missed by screening. This false negative rate is a minimum estimate, since ascertainment and reporting of these cases is almost certainly incomplete. Sixty-five percent of the false negatives were screened on or before the third day, while only 44 percent of all infants were screened by that age. This suggests that the probability of missing a case of PKU increases as the age of the neonate decreases at the time of screening. Furthermore, this premise is evidenced by the finding of a 1.6-fold higher incidence of PKU among infants screened after the fourth day of life compared to those screened earlier (again indicating false negatives in the latter group).

Screening methods for detection of PKU in affected infants depend on the determination of the blood phenylalanine level, which is normal at birth probably because of maternal enzyme. The PKU infants' phenylalanine values rise to abnormal levels only after they have received protein-containing feedings. This gradual elevation of phenylalanine concentrations in infants with PKU has been shown to be directly proportional to the age at the time of screening and probably reflects the duration of exposure to oral protein. In the United States, as more neonates are discharged from the newborn nursery at younger ages, the blood phenylalanine levels at that time are less likely to be diagnostic in PKU patients. One of the three cases reported by us was screened on day two, and the others on days three and four of life. At the present time, discharge from the nursery at 24 hours and even earlier is not unusual. Thus, the incidence of screened PKU cases which are initially missed may greatly exceed the eight percent estimate. This higher incidence of false negatives may be indicated by our finding in one year that three of the four new patients with PKU from our region of Florida had initial false negative Guthrie tests. The fourth patient was discovered more easily than the other newly diagnosed patients, since she is the sibling of a known PKU patient.

As a consequence, a serum fluorometric determination, rather than the Guthrie test for phenylalanine, was done at three days of age, resulting in a value of 8.4 mg per dl. A value this high would presumably have resulted in a positive Guthrie screen.

What screening test for PKU should be used? Originally, the only screening test available was the urine ferric chloride reaction with phenylketones, which must be significantly elevated. This, in turn, is dependent on very high blood phenylalanine levels (>16 mg per dl). Furthermore, the enzyme which forms the diagnostic phenylketones may be delayed in maturation in the infant, thus preventing or delaying diagnosis for up to a month. For these reasons, the urine ferric chloride test should not be used to screen for or confirm neonatal PKU.

Early diagnosis can be achieved only by measuring blood phenylalanine levels, but when is the optimal time for screening? Previous data indicate that beginning dietary restriction before one month of age results in higher intellectual performance. More recent studies suggest that children with PKU treated within the first ten days of life obtained higher IQ scores than children treated later.

Thus, the advantage of early screening on the eventual intellectual function of children with PKU must be compared with the possibility of initially missing the diagnosis by screening too early. A repeat screening test at a later age (five days is the current recommendation by the Guide-
NEONATAL SCREENING FOR PHENYLKETONURIA

lines for the Florida Infant Screening Program)\(^{11}\) may prevent false negative diagnosis of PKU. However, a report by Sepe et al\(^{12}\) suggests few patients are identified by a repeat test on all patients previously tested and such procedures substantially decrease the cost-effectiveness of initial screening.

An hypothesis concerning failures of screening has been proposed by Berman\(^1\): that fetal phenylalanine hydroxylase isozyme can persist into neonatal life. Persistence of the fetal enzyme in a newborn with “classical” PKU could result in initially normal blood levels of phenylalanine, which would become diagnostic only when the fetal enzyme disappears. Two prospective studies with small numbers of patients were able to demonstrate phenylalanine levels above the lower limit for suspecting PKU (4 mg per dl) at 48 and 24 hours, respectively.\(^3\)\(^-\)\(^9\) Breast feeding did not affect detection. Centralized laboratories with quality controlled, quantitative determinations might detect all screened cases of PKU by a single test. However, up to 16 percent of PKU infants under 24 hours of age, and approximately four percent between 24 and 72 hours old, will be missed by current methods of Guthrie testing.\(^{12}\) In addition, the greater problem may be in failing to screen all neonates for PKU in the first place.\(^5\)\(^-\)\(^{12}\)

In those states in which repeat screening for PKU is not done routinely, physicians caring for neonates will have to maintain a high index of suspicion. Infants with signs or symptoms of untreated PKU and who have received earlier screening should be retested for PKU regardless of previous negative results. By limiting the retesting to those patients who are tested initially at an age less than 48 hours or who have been on protein feedings less than 24 hours, the cost-effectiveness of identifying previously missed patients may be substantially increased.

References