

Detection of Maple Syrup Urine Disease on Newborn Screening Second Tier Testing for Phenylketonuria

Sylvia Capistrano-Estrada^{1,2}, Charity M. Jomento¹

¹ Institute of Human Genetics, National Institutes of Health, University of the Philippines Manila

² Department of Pediatrics, College of Medicine and Philippine General Hospital, University of the Philippines Manila

ABSTRACT

Newborn screening for phenylketonuria (PKU) in the Philippines uses a bacterial inhibition assay to detect elevations in phenylalanine (PHE). The BIA sensitivity is affected by substances such as antimicrobials. Semiquantitative second-tier screening with thin layer chromatography (TLC) verifies inconclusive PKU screens. Maple syrup urine disease (MSUD) is a prevalent inborn error of metabolism in the Philippines that is currently not part of the Philippine newborn screening program. We report on the incidental detection of MSUD by second-tier TLC PKU screening in order to begin to establish the evidence necessary for its inclusion in the Philippine Newborn Screening Program.

We reviewed the PKU newborn screening database from September 1, 1996 to June 12, 2008 to document the number of cases of elevated LEU detected incidental to confirming PHE elevations by second-tier TLC. Elevated LEU findings were studied further to document the number of MSUD cases. From September 1, 1996 to June 12, 2008, 966,096 babies were screened for PKU and 28,248 (2.9%) required second-tier testing. Of these, 403 had elevated PHE and 9 were confirmed to have either classic PKU or hyperphenylalaninemia. Fifty-three of 28,248 babies had normal PHE concentrations but elevated LEU concentrations. These babies were recalled and a second dried blood spot was requested. Of these, 15 had elevated LEU and were subsequently confirmed to have MSUD. Two babies had concurrent elevations of PHE and LEU, but both were deceased at the time of recall.

Confirmation of 15 MSUD cases was almost twice as high as the number of PKU/HPA confirmed cases. Since MSUD patients were detected incidental to PKU screening and there was no initial MSUD screening, the incidence of MSUD is almost certainly much greater. The number of MSUD cases incidentally detected confirms that MSUD exists at a significantly higher prevalence in the Philippine newborn population than PKU, and its inclusion in the newborn screening panel should be considered as soon as feasible.

Key Words: Maple syrup urine disease, phenylketonuria, newborn screening

Introduction

Maple syrup urine disease (MSUD) appears to be the most common inborn error of metabolism in the Philippines.¹ MSUD is an autosomal recessive disorder caused by decreased activity of branched-chain α -ketoacid dehydrogenase, one of the enzymes involved in the degradation of leucine (LEU), isoleucine, and valine.^{2,3} If left untreated, the clinical course of classical MSUD is often progressively severe, including overwhelming neonatal illness and eventually death. MSUD is relatively rare with a newborn incidence ranging from 1:185,000 worldwide to 1:560,000 among the Japanese. However, it may be as common as 1:176 births in Mennonites in the United States.^{2,4} In the Philippines, at least 70 patients with MSUD are listed in the registry of the Biochemical Genetics Unit (Metabolic Clinic census) of the Institute of Human Genetics, National Institutes of Health (NIH).⁵

Phenylketonuria (PKU) is an autosomal recessive disorder of aromatic amino acid metabolism. In PKU, phenylalanine (PHE) is not converted to tyrosine because of a deficiency in the PHE-hydroxylase enzyme. Untreated PKU results in microcephaly, mental delay, chronic disability and/or seizures. The reported incidence of PKU ranges from about 1:13,500 to about 1:19,000.⁶ In the Philippines, 9 PKU/hyperphenylalanine (HPA) patients were detected by screening 966,096 newborns from September 1996 to June 2008.

It has been recognized that early diagnosis and intervention in disorders of inborn errors of metabolism like PKU and MSUD is advantageous because it markedly reduces morbidity, mortality and healthcare costs.³ Currently, MSUD is not included in the newborn screening program of the Philippines. Nonetheless, there has been a steady increase in the number of MSUD patients diagnosed in the recent years. A significant number have been brought to medical attention because of the elevated LEU detected incidentally during second tier testing for PKU. In order to more clearly assess the impact of these incidental case findings, a review of the PKU newborn screening database was undertaken.

Materials and Methods

From September 1, 1996 to June 12, 2008, 966,096 blood samples were collected by heel prick as part of routine

Corresponding author: Sylvia Capistrano-Estrada, MD
Institute of Human Genetics,
National Institutes of Health,
University of the Philippines Manila
625 Pedro Gil Street, Ermita Manila 1000, Philippines
Telephone: +63 2 536 7002
Email: scestrada@post.upm.edu.ph

newborn screening. Neonatal screening for PKU was performed using the standard Guthrie bacterial inhibition assay.⁷ Samples with PHE concentrations ≥ 200 $\mu\text{mol/L}$, or that were the largest on the plate, or exhibited no growth or abnormal growth underwent second-tier testing by thin layer chromatography (TLC) to improve screening sensitivity by obtaining a more quantitative PHE concentration. Because the laboratory set up the TLC procedure to include standards for both LEU and PHE, samples with abnormal elevations of LEU were incidentally detected even though a preliminary LEU screen was not performed.

The newborn screening database of the Newborn Screening Center (NSC)-NIH was reviewed specifically looking for cases of LEU elevations incidentally observed during PKU second-tier screening. Data collected included patients' demographics, initial PHE concentrations (reason for second-tier testing), LEU concentrations (by TLC) and PHE concentrations (by TLC). The final diagnoses of patients with MSUD was confirmed by elevations in LEU/isoleucine on new blood samples (using TLC) and clinical observations.

Results

Between September 1, 1996 and June 12, 2008, 966,096 babies underwent newborn screening for PKU (Figure 1). Second-tier testing was done on 28,248 (2.9%) samples and of these, 403 (1.4%) had significantly elevated PHE concentrations. Nine of the 403 samples were confirmed clinically to have PKU or HPA. Fifty three (0.19%) of the 28,248 newborns had normal PHE concentrations but exhibited elevated LEU concentrations (from 300 to 4000 $\mu\text{mol/L}$). These babies were recalled through the follow-up network of the newborn screening system and a second dried blood spot was obtained. Of these, 15 had elevated LEU concentrations, 24 had normal LEU concentrations, specimens were not obtained for 12, and 2 had died by the time of recall. Two babies had concurrent elevations of PHE and LEU; however, confirmatory testing was not possible since both had died before recall.

Discussion

Classic MSUD is a devastating metabolic disorder that manifests early in the neonatal period with feeding difficulties and irritability progressing to lethargy and coma within 48-72 hours of birth.¹ If undetected, the full neurologic syndrome with seizures, ophthalmotonus, and progressive encephalopathy are evident within the first week and soon after, a fatal outcome can occur due to respiratory failure.^{2,3}

Patients with elevated blood LEU concentrations are presumed to have MSUD until proven otherwise. Hence, even without MSUD as part of the Philippine newborn screening program, a protocol was included that would result in recalling certain screened newborns for LEU retesting to eliminate other conditions that may elevate LEU concentrations. Catabolism, sepsis, presence of other

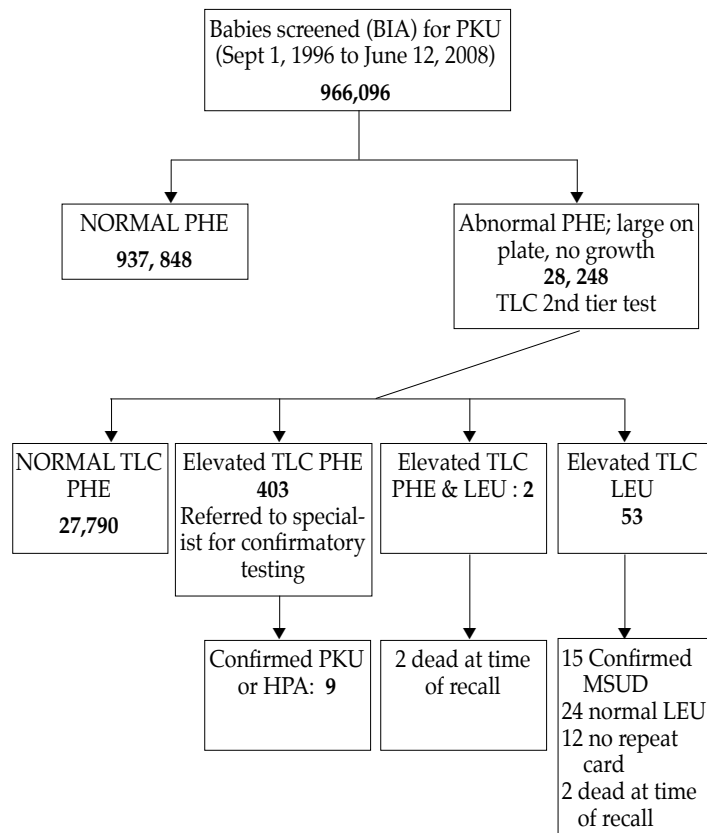


Figure 1. PKU neonatal screening and second tier testing, n=966,096

metabolic disorders, and liver dysfunction may cause LEU elevations in neonates.^{2,8,9} Premature infants given corticosteroids and parenteral nutrition may also have increases in LEU.⁸ In our series, 2 patients with simultaneous elevations in LEU and PHE did not undergo confirmatory testing because they had died by the time of recall; but their clinical data showed liver enzyme elevation and jaundice, suggesting that liver dysfunction may have been the underlying pathology rather than PKU or MSUD.

The detection of 15 cases of MSUD was incidental as a result of a procedural step in PKU screening. We routinely perform second-tier screening for PKU because the initial bacterial inhibition assay is subject to various interferences (e.g. antibiotics) and subjective reading variations. Agar quality and time of specimen collection may also influence screening results.¹⁰ These factors have caused us to utilize a more quantitative assay on all specimens exhibiting an initial PHE concentration ≥ 200 $\mu\text{mol/L}$. Because TLC can simultaneously detect LEU, we have elected to use LEU calibrators along with PHE calibrators to look for possible indications of MSUD.

From our data over 12 years, MSUD (15/966,096) is more prevalent than PKU (9/966,096) in a newborn population with initial elevations of PHE. This population does not represent an 'at risk' population for MSUD, but rather is

an opportunistic cohort conveniently evaluated because of the ease of the analytical 'add on' procedure. The incidence of MSUD in this group strongly suggests a similar or higher incidence in the overall newborn population. Initial screening studies with LEU are needed to further confirm these findings.

Conclusion

The incidental detection of MSUD in our newborn screening program provides preliminary data that its occurrence is not as rare as PKU. The detection of 15 cases of MSUD among the 966,096 babies screened is almost twice the 9 PKU/HPA cases confirmed. Because MSUD presents more acutely in the neonatal period with severe metabolic crises and death, there is a pressing need to re-examine the possibility of including MSUD in the Philippine newborn screening panel. The evidence here indicates an incidence of MSUD higher than PKU (currently part of the screening panel). We suggest validation of these data through a pilot study. It should be relatively inexpensive to institute the relatively inexpensive bacterial inhibition assay for LEU, which has been used in other programs, as the primary screening method. The second-tier TLC for PKU can be expanded to verify elevated or inconclusive screens. Until then, an elevated LEU concentration at the time of second-tier PKU screening should alert the newborn screening staff to the possibility of MSUD. Recall of these patients for confirmatory testing should be treated with haste since catastrophic consequences can occur at a few days of age if untreated.

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