Detection of other inborn errors of metabolism in hyperphenylalaninemic babies picked up on narrow-spectrum screening programs

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In many countries, neonatal screening programs have been unable to expand and have been limited to a few diseases. We highlight herein the opportunity available for the early detection of some inborn errors of metabolism (IEMs) in those countries in which newborn screening programs are limited. All the newborns that are referred to us for hyperphenylalaninemia are examined physically and their blood samples are checked by both high-performance liquid chromatography (HPLC) for blood phenylalanine levels and by amino acid analyzer for the measurement of blood amino acid concentrations. Seven patients who had been referred to our unit for hyperphenylalaninemia were eventually diagnosed with another IEM. A careful physical examination of the babies sent for positive screening test result combined with the utilization of low expense screening techniques at the experienced referring centers might facilitate otherwise missed opportunities for the early detection of some IEMs.

Key words: galactosemia, hyperphenylalaninemia, newborn screening, thin layer chromatography, tyrosinemia type I, missed opportunities.

Guthrie bacterial inhibition assay, developed by Dr. Robert Guthrie in 1962, has been an important starting point for neonatal screening programs[1]. Since then, in approximately 50 years, more than 50 congenital conditions have become available for neonatal screening[2]. Thus, thousands of children with genetic and metabolic diseases have had an opportunity for a healthy life with early diagnosis and treatment. Unfortunately, in many countries, the screening programs have been unable to expand further, and thus have been limited to a few diseases. The need for an established organization for screening and costs are some reasons for the limited number of screened disorders in these countries[3].

In Turkey, the neonatal screening program was started for phenylketonuria (PKU) on a nationwide basis in 1986 in collaboration with the Ministry of Health[4]. The program was expanded at the end of 2006, and it has continued successfully since then for three diseases, namely PKU, hypothyroidism and biotinidase deficiency. Blood levels of phenylalanine (Phe) and thyroid stimulating hormone (TSH) and biotinidase activity are measured in all newborns within the program that is carried out by the Ministry of Health, and newborns with positive test results are referred to the experienced pediatric endocrinology and metabolism centers. However, because Turkey is one of the countries with high rates of consanguineous marriages and inborn errors of metabolism (IEMs), a screening program covering only three diseases is unsatisfactory, and studies are in progress to expand the program to cover more inherited disorders.

In our clinic, all the newborns that are referred to us for hyperphenylalaninemia (HPA) are examined physically, and their blood samples are checked by both high-performance liquid chromatography (HPLC) for blood Phe levels

and by amino acid analyzer (Amicus, Germany) for the measurement of blood amino acid concentrations. Hence, the physical examination along with the chromatographic screening is expected to create a chance for the incidental detection of some disorders that lead to a secondary increase in blood Phe in the neonatal period, such as galactosemia, tyrosinemia and some other IEMs.

Here, we present seven patients who had been referred to our unit for a positive test result for HPA and were diagnosed eventually with maple syrup urine disease (MSUD, 4 patients), galactosemia (2 patients) and tyrosinemia type I (1 patient).

Case Reports

Case 1

A 27-day-old male baby was referred to our center for the evaluation of HPA detected on our national newborn screening program. His blood Phe level was measured as 28.3 μmol/L (N: ≤120 μmol/L). The next day, amino acid chromatography showed elevated leucine, isoleucine and valine levels. On the physical examination, mild axial hypotonia, spasticity in the arms and characteristic maple syrup urine odor were noted. Branched-chain amino acid levels were as follows: leucine: 1867 μmol/L (N: 46–147 μmol/L), isoleucine: 414 μmol/L (N: 12–77 μmol/L) and valine: 728 μmol/L (N: 79–217 μmol/L). Ketones were negative on urine analysis. He was diagnosed with MSUD and a special dietary treatment was started.

Case 2

A 28-day-old male baby was referred to our center for the evaluation of HPA detected on our national newborn screening program. His blood Phe level was 100.6 μmol/L. Because the amino acid chromatography showed elevated blood levels of leucine, isoleucine and valine, the patient was re-called. He had reportedly been admitted to another hospital because of vomiting and subtle seizure activity before the neonatal screening program results were known. On the physical examination, mild axial hypotonia, spasticity in both extremities and maple syrup odor were noted. On quantitative amino acid analysis, serum levels of branched-chain amino acid concentrations were: leucine: 1700 μmol/L, isoleucine: 530 μmol/L and valine: 1160 μmol/L. Ketones were negative, but α-ketoacids were positive on urine analysis and urine organic acid analysis by gas chromatography-mass spectrometry (GC-MS). He was hospitalized with the diagnosis of MSUD and placed on a special dietary treatment.

Case 3

A 2.5-month-old female baby was referred to our center for the evaluation of HPA detected on our national newborn screening program. The parents report feeding difficulties, vomiting, lethargy, and failure to thrive from birth, and the baby had been hospitalized at another center for these signs and symptoms. On the physical examination, she had growth retardation, axial hypotonia, spasticity in the extremities, and characteristic body odor. Amino acid chromatography showed elevated blood levels of leucine (2643 μmol/L), isoleucine (562 μmol/L) and valine (542 μmol/L). Ketones were negative, but α-ketoacids were positive on urine analysis and urine organic acid analysis by GC-MS. She was diagnosed with MSUD and special dietary treatment was commenced.

Case 4

A 40-day-old male baby was referred to our center for the evaluation of HPA detected on our national newborn screening program. Tandem MS analysis ordered by another center for the confirmation of the initial results revealed elevated blood branched-chain amino acid levels. On quantitative amino acid analysis, branched-chain amino acid concentrations were: leucine: 1790 μmol/L, isoleucine: 623 μmol/L and valine: 917 μmol/L. He had respiratory insufficiency on the physical examination, and mechanical ventilation was initiated. Urine ketones and α-ketoacids were found elevated on urine organic acid analysis by GC-MS, and he was diagnosed with MSUD and then given special dietary treatment.

Case 5

A 23-day-old male baby was referred to our center because of HPA detected on our national newborn screening program and jaundice. Tyrosinemia type I was suspected in the patient at another center because the

tyrosine level was also found elevated. On the physical examination, he had jaundice and mild hepatomegaly. Routine biochemical investigations revealed elevated liver enzymes and conjugated bilirubin levels. Reducing substance test in urine was positive, and a subsequent urine sugar chromatography showed elevated galactose levels in urine. Molecular genetic analysis yielded a homozygous Q188R mutation in the *GALT* gene, and thus the baby was diagnosed with galactosemia and a lactose-free dietary treatment was started.

Case 6

A one-month-old female baby was referred to our center because of HPA detected on our national newborn screening program. Her blood Phe level was found normal. On the physical examination, she had jaundice and moderate hepatomegaly. Routine biochemical investigations revealed elevated liver enzymes and conjugated bilirubin levels. Reducing substance test in urine was positive, and a subsequent urine sugar chromatography showed elevated galactose levels in urine. Molecular genetic analysis yielded arg231His homozygous mutation in the *GALT* gene. The baby was diagnosed with galactosemia and placed on a lactose-free dietary treatment.

Case 7

A 45-day-old male baby was referred to our center because of HPA detected on our national newborn screening program. His blood Phe level was 64.5 μmol/L, while tyrosine level was 862.8 μmol/L (N: <165 μmol/L). On the physical examination, mild jaundice and hepatomegaly were noted. Routine biochemical investigations revealed normal liver enzymes, mildly elevated conjugated bilirubin levels and elevated alkaline phosphatase (ALP) levels (1937 U/L, N: ≤450). Reducing substance test in urine was positive, and a subsequent urine sugar chromatography showed elevated galactose levels in urine. Alpha-fetoprotein (AFP) level was found highly elevated (686200 IU/ml, N: 0–5.8). Galactose-1-phosphate uridylyltransferase (GALT) enzyme activity was found normal. Urine organic acid analysis by GC-MS revealed the presence of succinylacetone in urine. Molecular genetic analysis yielded val166gly homozygous mutation in the *FAH* gene. The baby was diagnosed with tyrosinemia type I, and special dietary treatment was commenced.

Discussion

Expanded neonatal screening for all IEMs is not conducted worldwide routinely; instead, screening for a limited number of diseases is practiced more often in many countries. In Turkey, PKU, congenital hypothyroidism and biotinidase deficiency are the three disorders included in the currently ongoing screening program. Since Turkey is one of the countries in which consanguineous marriages and IEMs are most frequent, screening for only three diseases can be considered insufficient. Although the number of screened diseases is limited, referral of these babies to a center that is experienced in IEMs may provide the baby with an opportunity for screening for many other metabolic diseases and for early diagnosis and treatment in the case of an IEM other than the disease for which the baby was referred. This opportunity can only be provided by carefully noting the findings suggestive of IEM on the physical examination or by making use of the simple screening tests like thin layer paper chromatography (TLC) technique for blood amino acids apart from the test that is used for the confirmation of the disease for which the patient was referred. Here, we reported seven patients who were referred to our center for HPA but were diagnosed with other IEMs, namely MSUD (4 patients), galactosemia (2 patients), and tyrosinemia type I (1 patient).

All the patients presented here had been referred to us because of HPA detected on our national newborn screening program. HPA is defined as the presence of blood Phe levels above the established cut-off (2 mg/dl or 120 μmol/L). Phe levels may increase primarily due to phenylalanine hydroxylase (PAH) deficiency or to the deficiency of its cofactor tetrahydrobiopterin. On the other hand, elevation of the Phe level may be seen secondarily especially in the course of the diseases causing liver dysfunction. Newborn screening for HPA sometimes reveals a disease that can lead to secondarily elevated Phe levels, like tyrosinemia, galactosemia or other liver diseases. Galactosemia may lead to HPA through liver dysfunction. Tyrosinemia may

present with elevated levels of both tyrosine and Phe.

Camargo et al.[5] evaluated the frequency of transient neonatal tyrosinemia, with or without secondary HPA observed through neonatal screening for metabolic disorders. In their study, 409 patients showed high tyrosine levels, and in 118 of these cases, serum Phe level was also increased. In the study of Neto et al.[6], by using a low-cost TLC for amino acids, they detected a high frequency of transient tyrosinemia with secondary HPA in some newborns. Serial blood Phe determinations made it necessary to introduce appropriate dietary treatment in these babies. Shakespeare et al.[7] investigated the proportion of dried blood spot samples that gave a positive screen result due to clinically significant conditions other than PKU. Out of 438,674 babies who were screened, 67 had Phe concentration >210 μmol/L (15 per 100,000). Of these, 40 had PKU or persistent HPA with a Phe concentration identified by screening of 270-2350 μmol/L. A further 11 were diagnosed with another clinically significant disorder: galactosemia (n=8), biopterin defects (n=2), and tyrosinemia type I (n=1). In addition, 16 had transient blood Phe elevations. In total, nine cases of galactosemia were identified, of whom three had Phe concentrations <240 μmol/L, with one asymptomatic individual having a concentration <210 μmol/L. In these studies, most of the IEMs other than HPA that were detected in the hyperphenylalaninemic patients were transient. In our study, all the other IEMs detected in the babies who were referred to us because of HPA were not transient. These diseases were confirmed by the conventional diagnostic work-up and molecular genetic analyses.

In this study, four HPA babies were found to be affected by MSUD. Specific body and urine odor and the other physical examination findings like axial hypotonia, lethargy and failure to thrive were noticed on the thorough physical examination. The slightly elevated blood Phe levels in these babies on the screening program were deemed to be incidental. Amino acid chromatography revealed elevated branched-chain amino acid levels in the plasma. In the patients with galactosemia, physical examination findings were compatible with cholestasis, and they were diagnosed with galactosemia based on the results of urine sugar chromatography, GALT enzyme activity measurement in erythrocytes, and then molecular genetic analysis.

A careful physical examination of the babies sent for positive screening test result for HPA combined with the utilization of low-cost screening techniques like TLC for amino acids at the experienced referring centers might facilitate opportunities otherwise missed for the early detection of some IEMs in those countries in which an expanded newborn screening program has not yet been established.

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