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.
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, 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 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 measured as 100.6 μmol/L. Because the 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 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. 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. 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. 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), biotinidase 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.

REFERENCES

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