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Newborn Screening for Tyrosinemia Type I: Further Evidence that Succinylacetone Determination on Blood Spot Is Essential

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Abstract Tyrosinemia type I is a genetic disorder characterized by accumulation in the blood and urine of the toxic metabolite succinylacetone (SUAC), not detectable in healthy samples. In many countries, newborns are screened for tyrosinemia type I using tyrosine as a primary marker. Unfortunately, tyrosine accumulation may take longer to occur and it may be not obvious when specimens are collected, in the first few days of life, as for newborn screening. In 2008, we reported changes to simultaneously measure acylcarnitines, amino acids, and SUAC during expanded newborn screening. We established the usefulness of this method after identifying a first asymptomatic newborn affected by tyrosinemia type I. Now we report a second infant with positive SUAC screening result (14.1 μmol/L, n.v.$<$2) and normal tyrosine concentration (74 μmol/L; n.v.$<$250). We also performed molecular analysis of *FAH* gene in both patients after diagnosis at newborn screening. They had consanguineous parents and were both homozygous for two known disease-causing mutations of the *FAH* gene. The outcome of patients detected in the MS/MS screening is significantly favorable. We also report our results of newborn screening for tyrosinemia type I before and after inclusion of SUAC as a primary marker for this disease.

Keywords Newborn screening · Succynilacetone · Tyrosinemia type I

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Introduction

Tyrosinemia type I (MIM 276700) affects 1 in every 100,000 to 120,000 babies worldwide, although the real incidence could be higher. It is an autosomal recessive disorder caused by mutations in the *FAH* gene that leads to deficiency of the fumarylacetoacetic hydrolase (FAH; EC 3.7.1.2), the last enzyme in the tyrosine degradation pathway. The consequence of the metabolic block is the conversion of the catabolic

intermediates maleylacetoacetate and fumarylacetoacetate to the toxic metabolites SUAC and succinylacetoacetate. The presence of SUAC in urine or blood is pathognomonic for tyrosinemia type I (Mitchell et al. 2001), which, if untreated, is usually fatal within age 10 years. Affected children may exhibit diarrhea, vomiting, jaundice, liver or kidney failure, neurological crisis, rickets, failure to thrive, and hepatocellular carcinoma (Mitchell et al. 2001). With treatment, many patients can lead normal lives with few restrictions. Treatment usually involves 2-(2-nitro-4-3 trifluoro-methylbenzoyl)-1,3-cyclohexanedione (NTBC) administration and a diet low in tyrosine and phenylalanine (Lindstedt et al. 1992). Early diagnosis and initiation of therapy may thus be crucial in improving outcome (Joshi and Venugopalan 2004).

In many countries, newborns are screened for tyrosinemia type I using tyrosine as a primary marker (US Department of Health and Human Services, Maternal and Child Health Bureau 2005). An elevated concentration of tyrosine, however, is not sensitive enough to detect all cases (Wilcken et al. 2003). Some newborn screening programs have recently introduced the determination of SUAC as a reliable and valid marker for the identification of tyrosinemia type I (Allard et al. 2004; Sander et al. 2006; la Marca et al. 2008, Turgeon et al. 2008; Al-Dirbashi et al. 2008, Adam et al. 2009).

In 2008, we reported an efficient method to simultaneously measure acylcarnitines, amino acids, and SUAC during expanded newborn screening (la Marca et al. 2008). We established the usefulness of this method after identifying a first asymptomatic newborn with by tyrosinemia type I (la Marca et al. 2009). We now present a second infant with positive SUAC screening and tyrosine within the normal range. We also report our results of newborn screening for tyrosinemia type I before and after inclusion of SUAC as a primary marker for this disease.

Case Report

The patient was a Moroccan boy, born at full term after an uneventful pregnancy and delivery with a weight of 3,620 g. He was the first child of first degree cousins. Both parents were healthy, but the family reported a positive history for deaths (three babies) in early childhood of unknown causes. Blood for newborn screening, collected on the third day of life, revealed an elevated SUAC level of 14.1 μmol/L (n.v.<2). Tyrosine value was normal (74 μmol/L; n.v.<250). On the sixth day of life, the boy was hospitalized for further testing. The diagnosis of tyrosinemia type I was confirmed by detection of SUAC in the urine (70 mmol/mol of creatinine) and plasma (11 μmol/L). Plasma tyrosine level was increased (791 μmol/L; n.v.<123). The child was in good condition and no clinical manifestations were apparent. Liver transaminases, alkaline phosphatase, bilirubin, coagulation factors, and ammonia were within normal levels. NTBC treatment was started at a dose of 1 mg/kg/die body weight combined with dietary restriction of tyrosine and phenylalanine.

Results and Discussion

In many countries (Canada, USA, Latin America), to reduce hospital costs, newborn screening specimen collection is done within 24–48 h after birth in response to the early discharge of mothers and their infants. This tendency causes a serious problem for metabolic newborn screening since accumulation of some metabolites, used as markers, may occur only in some days after delivery. The success of newborn screening depends on the type of marker used. Hence, the challenge is to find and to include in newborn screening programs appropriate diagnostic markers for the early detection of metabolic disorders.

Up to now, many newborn screening programs worldwide use tyrosine levels as a marker for tyrosinemia type I. Unfortunately this metabolic condition cannot be detected when specimens are collected in the first few days of life, as tyrosine accumulation may take longer to occur (Mitchell et al. 2001). The increased SUAC levels, the metabolite immediately upstream of the enzymatic block, is a reliable, appropriate, and early marker of this disorder.

The case presented here, as also the previously reported case (la Marca et al. 2009), provides further evidence on the importance of using SUAC as the primary metabolic marker for detecting tyrosinemia type I in newborn screening.

Experience in various laboratories, including our own, suggests that tyrosine was not a sensitive diagnostic marker for the timely identification of patients and decreased the specificity of the test while increasing the unnecessary medical cost for false-positive recall rate. Indeed, the most common cause of increased blood tyrosine levels is benign transient tyrosinemia of the newborn.

The inclusion of SUAC in our newborn screening program dates back to January 2007, subsequently to a false-negative result. Among about 136,000 newborns screened, two patients with tyrosinemia type I were identified. No false positive was on record. Both patients had consanguineous parents (from Turkey and Morocco) and were homozygous for the known c.709C>T (p.Arg237X) and IVS6-1G>T disease-causing mutations of the *FAH* gene (Rootwelt et al. 1996; Ploos van Amstel et al. 1996). The IVS6-1G>T leads to a splicing defect and has been reported as common in the European and Mediterranean area (Arranz et al. 2002). The results for tyrosinemia type I in the Tuscan newborn screening program are reported in the Tables 1 and 2.

Table 1 Expanded newborn screening for tyrosinemia type I in Tuscany before and after inclusion of SUAC as a primary marker

	Newborn examined	Cases diagnosed	Cases false negatives
Before SUAC inclusion	113,090	0	1
After SUAC inclusion	136,075	2	0
Total	249,165	2	1

Table 2 Patients with tyrosinemia type I born in Tuscany between 2006 and 2010

	Ethnicity	Tyrosine, μmol/L (n.v. < 250)	SUAC, μmol/L
Patient 1[a]	Moroccan	142	14.9[b]
Patient 2	Turkish	126	7.6
Patient 3	Moroccan	74	14.1

[a]False-negative result on newborn screening before SUAC inclusion
[b]Original newborn screening card was analyzed 2 years after collection

It is very difficult to estimate the incidence of this disease in the population but, based on our own experience, we are inclined to believe that it is generally underestimated. Furthermore, in Turkey as well as Morocco and other North African and Arabic countries, the elevated rates of consanguinity may have an impact on the incidence of rare autosomal recessive disorders (Al-Gazali et al. 2006; Jaouad et al. 2009).

There are various methods for SUAC determination on DBS, with significant modifications in the newborn screening procedures. The method we had previously proposed (la Marca et al. 2008) consists of a simple and fast protocol for newborn screening sample preparation and allows identifying this metabolic defect in the neonatal period with 100% sensitivity. No cost for additional equipment or staff members is required for applying such testing.

Synopsis

Succinylacetone determination on dried blood spot.

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