



**REGION 6 PRIORITY ACTIVITY 1 PROJECT
Laboratory Quality Assurance
(HRSA 07-016)**

ELEVATION OF TYROSINE IN NEWBORNS

Tyrosine is one of the amino acids monitored by expanded newborn metabolic screening using tandem mass spectroscopy. The rationale for measuring tyrosine is two-fold. A normal or low tyrosine level in an infant with elevated phenylalanine and high phenylalanine/tyrosine ratio is highly specific for phenylketonuria. Also, an elevated tyrosine is seen in older patients with tyrosinemia type I (hepatorenal tyrosinemia) and it was hoped that elevations in newborns would allow identification of affected individuals at a pre-symptomatic stage. Unfortunately, this is not the usual case. Rarely, infants with tyrosinemia type I have elevated tyrosine levels in the newborn period. Tyrosine levels can be elevated in other inborn errors of metabolism, and two of these, tyrosinemia type II and III, can be identified in the neonate.

Tyrosine is an aromatic amino acid derived from the hydroxylation of phenylalanine by phenylalanine hydroxylase (Figure 1). It is also abundant in natural proteins. Inborn errors of metabolism affect the activity of four of the enzymes involved in the catabolism of tyrosine. The degree of elevation is related to the proximity of the metabolic block to the substrate, tyrosine. Clinical symptoms, severity, and treatment differ for each of these conditions.

Tyrosine levels can be elevated in the presence of any of the following situations:

- Intravenous hyperalimentation
- Impaired hepatocellular function for any reason
- Transient tyrosinemia of the newborn
- High protein intake
- Tyrosinemia type I (hepatorenal)
- Tyrosinemia type II (oculocutaneous)
- Tyrosinemia type III

Following detection of an elevated tyrosine level, the clinician can suspect one or more of these conditions depending upon the degree of elevation and associated historical, laboratory, and clinical findings. Recommendations for confirmatory testing include liver function tests, quantitative serum or plasma amino acids, and urine organic acids especially to measure succinylacetone, the marker of tyrosinemia type I. Other than the presence of jaundice and hepatomegaly, which suggest primary liver disease, the physical examination is usually not helpful in identifying the cause of a high tyrosine.

Intravenous hyperalimentation may raise the level of many amino acids, among them, tyrosine. If the infant is receiving hyperalimentation, it should be indicated on the specimen, but failure to complete the cards is common. Clues to the possibility of hyperalimentation include low birth weight, prematurity, and, from an analytical standpoint, the elevation of multiple amino acids.



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Liver disease, when severe, causes an elevation of tyrosine along with other metabolites, especially methionine. The pattern of amino acid abnormalities is not diagnostic for specific causes of hepatocellular dysfunction. Several metabolic disorders can impair hepatocellular function, but some of them do not usually present this early. These include tyrosinemia type I (see below), galactosemia, hereditary fructose intolerance, citrin deficiency, peroxisomal disorders, mtDNA depletion syndromes, alpha-1-antitrypsin deficiency, and some lysosomal storage disorders (notably Niemann-Pick type C). The degree of elevation seen in each of these conditions does not allow differentiation, although in general tyrosinemia type I has a significant overlap with normal values; tyrosinemia type II and transient tyrosinemia of the newborn produce tyrosine levels ranging from mildly elevated to over 900 micromolar. A single case of tyrosinemia type III in the Region 4 MS/MS collaborative project database (http://region4genetics.org/msmsda_ta_project/msms_data_project/data_project_home.a.spx) had a level above 900 micromolar. High levels of tyrosine and other amino acids can be seen with DGUOK deficiency, one of the mtDNA depletion syndromes, even at birth. There is inadequate information to compare initial and later screening levels from states with two mandatory newborn screens.

Transient tyrosinemia of the newborn is probably the most common cause of an elevated tyrosine level in a newborn screening specimen. Phenylalanine may also be elevated. The infant appears clinically well and liver function is normal. The cause of this condition is most likely multifactorial, resulting from a combination of 4-hydroxyphenylpyruvate dioxygenase (4HPPD) deficiency due to immaturity, high protein intake, and a relative deficiency of ascorbic acid. It is most commonly seen in premature infants or in term infants on a high protein diet. It resolves over the first few months of life, often within weeks. Resolution can sometimes be hastened by treating with ascorbic acid (100-400 mg/day). Most children with transient tyrosinemia are asymptomatic and have normal development. In states with two newborn screens, the fall in level on the second screen is virtually diagnostic of this condition.

A high protein intake can cause tyrosinemia, but most newborn screening specimens are obtained prior to large protein loads, thus this is an unlikely explanation for an early elevation. We have seen markedly elevated tyrosine on second screens of infants fed adult milk products at birth.

Tyrosinemia type I or hepatorenal tyrosinemia is due to a defect in fumarylacetoacetate hydrolase, the terminal step in the conversion of tyrosine to fumarate and acetoacetate. Succinylacetone is a byproduct formed when maleylacetoacetate and fumarylacetoacetate accumulate as a consequence of FAH deficiency. These metabolites are very toxic and carcinogenic, and are responsible for most of the pathology of tyrosinemia type I. They can disrupt sulfhydryl metabolism by forming adducts with glutathione. They can cause secondary deficiency of 4-hydroxyphenylpyruvate dioxygenase and methionine adenosyl transferase, leading to increased tyrosine and methionine. Succinylacetone acts as a poison to the cells of the renal tubule causing



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impaired reabsorption of nutrients and minerals (renal Fanconi syndrome) and resulting in rickets. It inhibits the enzyme delta-aminolevulinic acid dehydratase leading to diminished production of porphobilinogen and porphyria-like crises and causes liver carcinoma. The presence of succinylacetone is specific for tyrosinemia type I and is thus a better marker for this condition than measurement of tyrosine.

Tyrosinemia type I shows a wide spectrum of severity even within the same family. In the acute form the presentation is within weeks of birth with liver failure. Jaundice is usually mild and despite the severity of liver dysfunction, transaminase levels are only moderately elevated. Synthetic function is affected with prominent coagulation defects and edema. Hypophosphatemic rickets may occur early. Neurologic problems often complicate the course of the disease and include porphyria-like attacks of abdominal pain, peripheral neuropathy and muscle weakness. Hepatocellular carcinoma is a major complication of all survivors with tyrosinemia type I, occurring even in some cases successfully managed with NTBC (2-(2-nitro-4-trifluoro-methylbenzoyl)-1, 3-cyclohexanedione) treatment from an early age.

Most patients with tyrosinemia type I do not have elevated levels of tyrosine on the first newborn screening specimen. The actual sensitivity of the neonatal screening for tyrosinemia type I using tandem mass spectrometry is related not to technical considerations, but to the biological fact that the levels of tyrosine rise only after a few weeks of age in most patients. Measurement of succinylacetone on the dried blood specimen can determine whether infants have tyrosinemia type I, independently from the level of tyrosine.

Tyrosinemia type II is caused by deficiency of tyrosine aminotransferase, the first step in the catabolic pathway of tyrosine. Due to proximity of the metabolic block, tyrosine levels are higher than in other forms of tyrosinemia. Clinically this disease is characterized by eye lesions (75%), skin manifestations limited to the palms and soles (80%), and neurological complications (60%). The eye findings include herpetic-like corneal ulcerations and conjunctival inflammation with pain, tearing, and photosensitivity. Scarring and visual impairment may occur. The skin lesions begin as blisters which become erosions and then hyperkeratotic areas which are also painful. These symptoms usually do not appear in the newborn period, but after several months. They are due to the deposition of tyrosine crystals and the consequent inflammatory reaction. Neurological complications and mental retardation have been reported in some patients, although it is not established whether they were only concomitant findings. These symptoms have been reported more frequently in patients with late diagnosis. Our patients with tyrosinemia type II treated from birth or shortly after have done very well in terms of development. Plasma tyrosine levels in the newborn period range from 370-3300 micromolar with normal levels of other amino acids. Tests of renal and hepatic function are normal but the urine contains elevated levels of multiple tyrosine metabolites (4-hydroxyphenylpyruvate, 4-hydroxyphenyllactate, 4-hydroxyphenylacetic acid, n-



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acetyltyrosine, and 4-tyramine). In states where two newborn screens are performed, it is expected that the level of tyrosine will be higher in the second screen than in the first.

Tyrosinemia type III is caused by homozygous deficiency of 4-hydroxyphenylpyruvate dioxygenase. There are relatively few cases described and those identified by newborn screening are too young to draw conclusions from their course. Most patients described have had various neurological symptoms, but whether this reflects an ascertainment bias or is actually related to the disorder is unknown. The plasma levels of tyrosine overlap those seen in tyrosinemia type II. The levels of plasma tyrosine reported have ranged from 500-1200 micromolar. The role of tyrosine restriction in treating this condition is unclear. As with type II disease, tyrosine metabolites are seen in the urine. Like the two other forms of tyrosinemia, this is an autosomal recessive condition.

In summary, newborn screening using tyrosine as the primary marker is not adequately sensitive to detect all cases of tyrosinemia type I. Primary testing for succinylacetone in all samples would be required to identify this disease. Tyrosinemia types II and III can be detected if an adequate cut-off for tyrosine is used. More experience is needed with the latter two conditions given their relative rarity. The most common causes of an elevated tyrosine in the newborn are hyperalimentation, transient tyrosinemia, and liver insufficiency or immaturity. Some questions remain; what is the duration of transient tyrosinemia and should this condition be considered benign; should persistent cases be treated with ascorbic acid; what is the sensitivity of tyrosine testing for specific conditions; what is the role of second tier testing for succinylacetone; and what is the possible use of routine second screening tests to sort out underlying causes and increase sensitivity for tyrosinemia type I?

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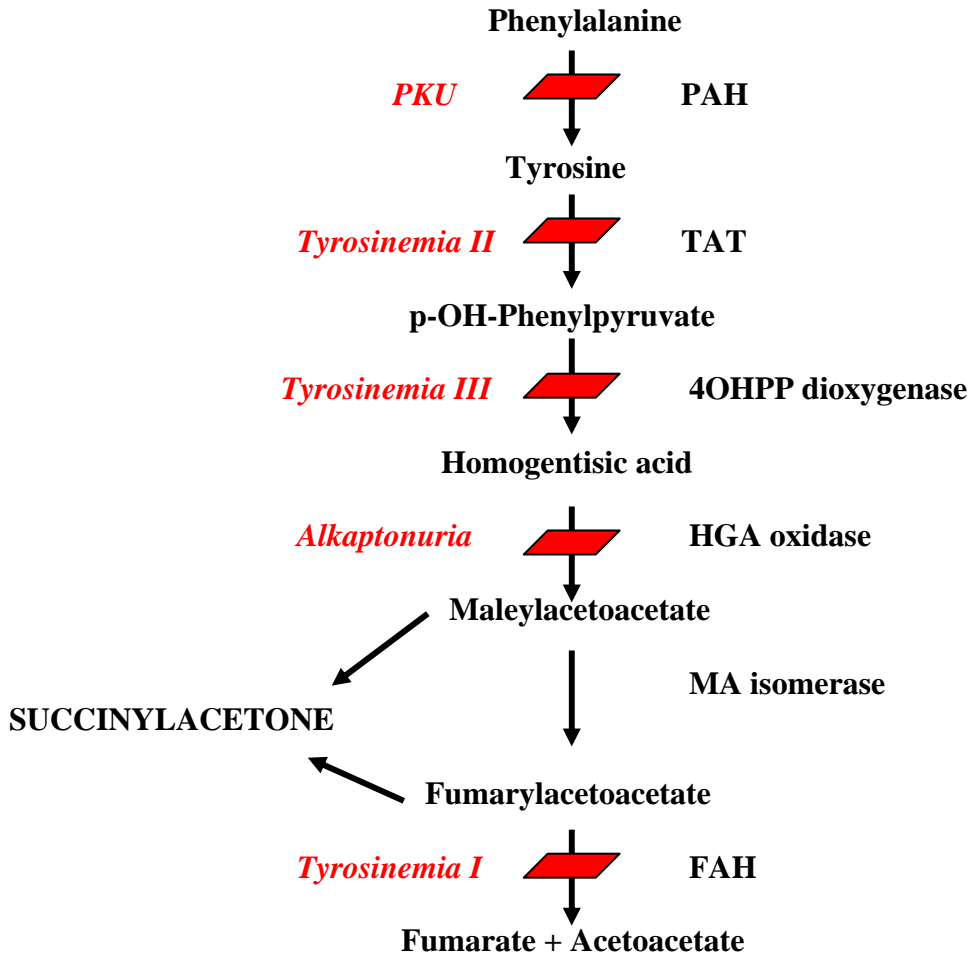


FIGURE 1. Phenylalanine/tyrosine metabolism. Phenylalanine, an essential amino acid, is hydroxylated to tyrosine by the enzyme phenylalanine hydroxylase (PAH), the deficiency of this enzyme causes phenylketonuria (PKU). Tyrosine is converted to p-hydroxyphenylpyruvate by the enzyme tyrosine aminotransferase (TAT); deficiency of TAT is the cause of tyrosinemia type II. 4-Hydroxyphenylpyruvate (4OHPP) dioxygenase converts 4-hydroxyphenylpyruvate to homogentisic acid. The cofactor of this enzyme is vitamin C (ascorbate). Deficiency of 4OHPP dioxygenase is responsible for tyrosinemia type III. Transient tyrosinemia of the newborn is also caused by delayed maturation of this enzyme. Homogentisic acid is converted to maleylacetoacetate by homogentisic acid (HGA) oxidase. Alkaptonuria is caused by impaired activity of this enzyme. Maleylacetoacetate is isomerized to fumarylacetoacetate by maleylacetoacetate (MA) isomerase, and then fumarylacetoacetate is converted to fumarate and acetoacetate by fumarylacetoacetate hydrolase (FAH). A deficiency of FAH causes tyrosinemia type I.



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SUGGESTED READING

1. Oculocutaneous tyrosinemia/tyrosine aminotransferase deficiency. Chapter 25 in *Atlas of Metabolic Diseases*; Nyhan WL, Barshop BA, Ozand PT Eds. 2nd Edition, 2005 Oxford University Press Inc.; New York, NY
2. Hepatorenal tyrosinemia/fumarylacetoacetate hydrolase deficiency. Chapter 26 in *Atlas of Metabolic Diseases*; Nyhan WL, Barshop BA, Ozand PT Eds. 2nd Edition, 2005 Oxford University Press Inc.; New York, NY
3. Scott CR. The genetic tyrosinemias. *Am J Med Genet C Semin Med Genet*. 2006 May 15;142C(2):121-6. Review