Is Succinylacetone a pathognomonic marker for diagnosing Tyrosinemia type 1?

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Abstract

Tyrosinemia type 1 (Tyr 1) is an autosomal recessive condition caused by fumarylacetoacetate hydrolase (FAH) deficiency encoded by *FAH*. The incidence of Tyr 1 is approximately 1 in 100,000 live births, resulting in severe liver disease, hypophosphatemic rickets, renal tubular dysfunction, and neurologic crises. If left untreated, most patients die of liver failure in the first years of life. Typical biochemical findings include increased succinylacetone concentration in the blood and urine, elevated plasma concentrations of tyrosine; methionine, and phenylalanine; and elevated urinary concentration of tyrosine metabolites and the compound δ-ALA. Pathognomonic marker of tyrosinemia type 1 is succinylacetone, however succinylacetone being a volatile compound is not detected in urine sample of all the patients with Tyrosinemia type 1. At our centre we have received 98 urine samples with a clinical suspicion of Tyrosinemia type 1 between 2008 and 2012, all these samples were analyzed by Gas chromatography—Mass spectrometry for urine organic acids. Only 42 samples showed elevation of succinylacetone and out of these 42 samples, 38 samples showed significant elevation of tyrosine metabolites which is usually expected in tyrosinemia. In the remaining 56 samples succinylacetone was not detected and out of these 56 samples, 47 samples showed significant elevation of tyrosine metabolites. Succinylacetone being a volatile compound can be lost during shipment of the sample if not transported in proper conditions and since it is the pathognomonic marker for diagnosing Tyrosinemia type 1, it is recommended to send a frozen urine sample to the laboratory for analysis in case of clinical suspicion of tyrosinemia type 1.

Index Terms : Tyrosinemia type1, Succinylacetone, Fumarylacetoacetate hydrolase, 4-hydroxy phenyllactate, 4-hydroxy phenylpyruvate, Gas chromatography-Mass spectrometry.

INTRODUCTION

Tyrosinemia type 1 (Tyr 1) is an autosomal recessive condition caused by fumarylacetoacetate hydrolase deficiency. The enzyme block results in accumulation of fumarylacetoacetate and possibly maleylacetoacetate, which are alkylating agents thought to cause hepatorenal damage [1]. These metabolites are precursors of succinylacetoacetate and succinylacetone, which accumulate in plasma and urine of the patients. Succinyacetone is a potent inhibitor of δ-aminolevulinate dehydratase which results in elevation of δ-aminolevulinate[2].Other intermediates of tyrosine metabolism also accumulate, namely 4-hydroxyphenylpyruvate and its derivative, 4-hydroxyphenyllactate.The gene for fumarylacetoacetase is located on chromosome 15. More than 20 mutations causing tyrosinemia have been reported [3], [4], [5], [6], and the most common mutation, IVS_{12} +5(g-a), is only found in about 25% of alleles [7]. The incidence of Tyr 1 is approximately 1 in 100,000 live births and the carrier rate has been estimated to be between 1 in 20 and 1 in 31 [8]. Type 1 tyrosinemia typically presents in infancy as failure to thrive and hepatomegaly. The primary effects are progressive liver and kidney dysfunction. The liver disease is characterized by cirrhosis, hyperbilirubinemia, elevated levels of serum alpha-fetoprotein, hypoglycemia and prolonged prothrombin and partial thromboplastin time [9]. There is also an increased risk of hepatocellular carcinoma. The kidney dysfunction presents as Fanconi syndrome. Cardiac, neurologic and dermatologic manifestations are also possible. If left untreated, most patients die of liver failure in the first years of life.

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Biochemical findings of tyrosinemia type 1 include increased succinylacetone concentration in the blood and urine, elevated plasma concentrations of tyrosine, methionine, and phenylalanine; and elevated concentration of tyrosine metabolites and the compound δ-aminolevulinic acid [9]. The hallmark of the diagnosis is elevated levels of succiylacetone in urine and blood. Succinylacetone being a volatile compound may be lost during transport of the samples and hence storage and shipment of urine samples to the laboratory for diagnosis of tyrosinemia type 1 has always been a concern due to bacterial growth and instability of succinylacetone. Alternately shipment of urine samples on absorbent filter papers has been evaluated for reliability and reproducibility of metabolites in urine samples. Since succinylacetone is a volatile compound and some patients with tyrosinemia type 1 excrete only small amounts of succinylacetone in urine[1], it is not detected in all the patients with Tyrosinemia type 1.

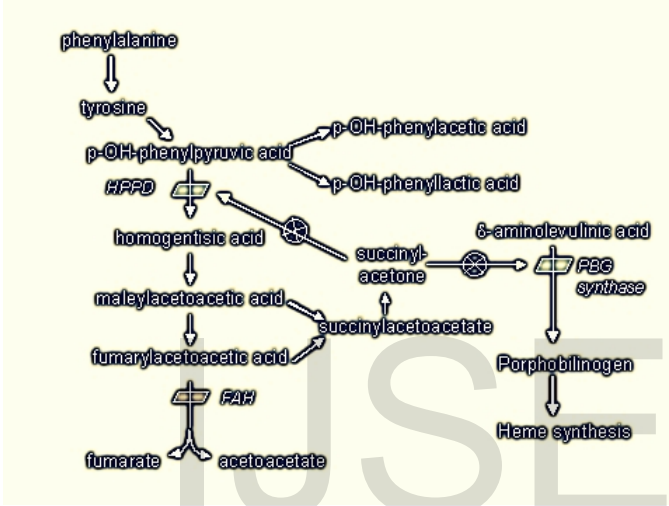

Figure1. The tyrosine catabolic pathway

MATERIALS AND METHODS

Urine samples from 98 patients with a strong clinical suspicion of tyrosinemia type 1 were analyzed at our centre in south India by Gas chromatography-Mass Spectrometry (GCMS) for urine organic acids between May 2008 and September 2012. The method of measuring the level of succinylacetone in urine by Gas chromatography-Mass Spectrometry as follows:

Creatinine was estimated by Jaffe's method. To a volume of urine, equivalent to a consistent amount of creatinine (e.g., 0.2

mg) 20µl of urease was added and was incubated for 30 min at 37°c which helps in hydrolysis of the sample. Internal standards I & II were added which include the components of margaric acid, Tetracosane & tropic acid respectively, these compounds help to measure the percentage of organic metabolites in the sample. After adding internal standards to the samples, it was made to 2000µl by adding MQ water. aqueous hydroxylamine hydrochloride and sodium hydroxide were added and incubated at room

temperature for 60 min, during this period carbonyl groups are converted to oximes(oxidation of 2-ketoacids) . The pH of the processed sample was adjusted using 6N HCl, vortex it completely for proper mix. Oxidized organic acids were extracted by adding ethyl acetate, centrifuge it for 5min at 3000rpm at 4°c, collect the supernatant of the centrifuged sample and repeat the extraction step once more. Anhydrous sodium sulphate was added which helps to absorb the moisture content in the extracted sample.

The samples were evaporated by inert gas which concentrates the sample to estimate organic acids. The processed sample was derivatized by BSTFA+TMCS (N,O-bis(trimethlsilyl) trifluroacetamide with trimethylchlorosilane). The residue (acidic metabolites) was derivatized at 80 °C and heating for 30min which helps in stabilization of organic metabolites and makes them volatile. 1μL aliquot of the derivatized samples and 1 μL of mixture of hydrocarbon were injected simultaneouy into Gas chromatography-Mass spectrometry for further analysis of the sample.

RESULTS

98 urine samples with a clinical suspicion of Tyrosinemia type 1 were analyzed by Gas chromatography—Mass spectrometry for urine organic acids between 2008 and 2012. Our method for detection of urine organic acids has a cut off of 0.4μmol/l for succinylacetone.

Out of 98 samples, 42 samples showed elevation of succinylacetone and out of these 42 samples, 38 samples showed significant elevation (more than 200 fold) of 4-hydroxy phenyllactic acid and 4-hydroxy phenylpyruvic acid which is usually expected in tyrosinemia. In the remaining 56 samples succinylacetone was not detected and out of these 56 samples, 47 samples showed significant elevation (200 to 300 fold) of 4-hydroxy phenyllactic acid and 4-hydroxy phenylpyruvic acid.

Discussion and Conclusion

Tyrosinemia type I is a metabolic disorder in which an enzyme critical for the breakdown of the amino acid tyrosine is missing and as a result abnormal amounts of tyrosine accumulate in the body causing damage to the liver. Patients also suffer from renal tubular dysfunction (Fanconi syndrome), hypophosphatemic rickets and neurologic crises. Diagnosis is based on the observation that plasma levels of tyrosine and methionine and urine levels of succinylacetone, 4-hydroxy phenyllactic acid and 4-hydroxy phenylpyruvic acid are significantly elevated. Succinylacetone is a potent inhibitor of δ-aminolevulinic acid dehydratase which results in elevation of δ-aminolevulinate. The pathognomonic marker for diagnosis is elevated levels of succinylacetone in urine and blood [9] however this

compound being volatile, may be lost during transport of the samples and hence caution has to be executed to make sure that the urine samples sent to the laboratory for detection of Succinylacetone are reached within 24 hours of collection in dry ice box to maintain the stability of samples.

To conclude, Succinylacetone is the pathognomonic marker for diagnosis of Tyrosinemia type 1 [9], however absence of this compound in urine and blood of patients with a strong clinical suspicion does not rule out the diagnosis. Estimation of succinylacetone in urine by gas chromatography-mass spectrometry may yield a false-negative result for tyrosinemia type 1 hence it is recommended to determine the levels of succinylacetone in the dry blood spots using tandem mass spectrometry which is a good method for diagnosis of tyrosinemia type 1[10] and to monitor the levels of δ-aminolevulinate and tyrosine metabolites like 4-hydroxyphenylpyruvic acid and 4-hydroxyphenyllactic acid which are significantly elevated in this disorder.

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