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Application of Tandem Mass Spectrometry to the diagnosis of Tyrosinemia Type-I in the Saudi population

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SUMMARY

Tyrosinemia type-I (Hereditary Tyrosinemia; HT-I) is an autosomal recessive inherited metabolic disorder. It is the most serious and common of the genetic defects in tyrosine catabolic pathway. The disorder results in extensive clinical and pathological manifestations involving mainly the liver, kidney, and peripheral nerves. HT-I is caused by deficient fumarylacetoacetate hydrolase (FAH) activity, which leads to the accumulation of succinylacetone (SA), the key marker of the disorder, in the urine and plasma of affected individuals. As HT-I may now be effectively treated and early diagnosis has proven to yield a better clinical outcome, its inclusion in newborn screening programs is being considered in many countries.

Current methods for the detection of the disease are based on the measurements of the SA in urine, plasma, and amniotic fluid by gas chromatography mass spectrometry (GC-MS). A semiquantitative spectrophotometric assay, whereby inhibition of δ -aminolevulinic dehydratase by SA in dried blood spot (DBS) is used to rule out the disorder, however possible problems with this strategy is false-positive results if the DBS was exposed to high temperatures, in cases of hereditary δ -ALA dehydratase deficiency, or in cases of lead exposure via cord blood. The disease can be detected by determining FAH deficient activity in human liver, lymphocytes and fibroblast. However, the demonstration of FAH activity alone in any tissue may not be conclusive for the diagnosis of HT-I due to the presence of pseudodeficiency gene of FAH that results in enzyme activity nearly as low as the HT-I gene.

A stable isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the determination of SA in urine for the diagnosis of HT-I was

developed, where the ¹⁵N-5(3)-methyl-3(5)-isoxazole propionic acid is used as an internal standard (IS). To urine samples, hydroxylamine HCl was added under acidic condition and heat to yield the methyl-isoxazole propionic acid derivative; this was followed by ether extraction and then the addition of butanolic HCl to give the butylated derivative. The butyl isoxazole derivatives of SA and its IS were detected and quantified using positive-ion Electrospray LC-MS/MS with selected reaction monitoring. The turn around time between injections was 10 min. Calibrations were linear over the range of 0.0633-63.3 µmol/L. The intra- and inter-day assay variations were less than 7%. Mean recoveries of SA at three different concentrations ranged from 96% to 109%. During the course of this study we identified 12 new patients with HT-I and applied this method to follow-up the treatment of four of these patients.