

Cases in Clinical Mass Spectrometry Abstract Example

Case Description:

A 54-year-old male presented to his endocrinologist with persistent hypercalcemia (Serum Calcium: 11.5-12.5 mg/dL; Reference Interval (RI): 8.7-10.2 mg/dL) and suppressed intact parathyroid hormone (PTH) (5 pg/mL; RI: 15-65 pg/mL). The patient had suffered from recurrent kidney stones and had passed ~10 kidney stones during the last twenty years of his life. He also reported an increased frequency of kidney stone events while he was an avid runner a few years ago and now more recently after taking high doses of vitamin D (cholecalciferol, 5000 IU/day) supplement during the last three months prior to his current presentation. The patient also reported that his father was a recurrent kidney stone former. The underlying etiology of his persistent hypercalcemia had remained unknown.

Background:

Vitamin D and PTH are principal regulators of calcium homeostasis in humans. 25(OH)D is utilized to form the active hormone, 1,25(OH)₂D during calcium demand by the 25-hydroxylase (CYP27B1). Whereas 24-hydroxylation by CYP24A1 converts 25(OH)D to 24,25(OH)₂D. Loss-of-function mutations in CYP24A1 have been identified as the underlying cause of hypercalcemia previously considered to be idiopathic. Diagnosis of aberrant calcium regulation is commonly made by measuring serum concentrations of calcium, PTH, 25(OH)D and 1,25(OH)₂D. Until a few years ago, 24,25(OH)₂D measurements were not available in clinical laboratories. LC-MS/MS methods have now facilitated the measurement of this analyte in many clinical laboratories.

MS Method and Results:

25(OH)D, 1,25(OH)₂D and 24,25(OH)₂D concentrations were measured by LC-MS/MS. LC-MS/MS methods for 25(OH)D and 24,25(OH)₂D involved protein precipitation followed by solid phase extraction for sample clean up. The dried sample extracts were derivatized with 4-phenyl-1,2,4,-triazoline-3,5-dione (PTAD) and analyzed and quantified by LC-MS/MS. 1,25(OH)₂D sample preparation involved a solid phase antibody coupled immune-enrichment step prior to derivatization by PTAD. His serum 25-(OH)D levels were 50 ng/mL (RI: 30-80 ng/mL), 1,25(OH)₂D = 90 pg/mL; 24,25(OH)₂D = 0.5 ng/mL and 25(OH)D/24,25(OH)₂D ratio = 100 [RI: 7-35].

Discussion and Conclusion:

Mutations that cause reduction in CYP24A1 function cause hypercalcemia, hypercalciuria, and elevated 1,25(OH)₂D concentrations. CYP24A1 mutations result in low or undetectable serum 24,25(OH)₂D or increased 25(OH)D/24,25(OH)₂D ratio (RI:7-35). A 25(OH)D/24,25(OH)₂D >99 has been described to identify candidates for confirmatory genetic testing. The patient's persistent hypercalcemia was shown to be caused by a CYP24A1 mutation. The measurement of 25(OH)D/24,25(OH)₂D ratio in this case helped delineate the pathogenesis of the patient's persistent hypercalcemia.