MSA-SRM assay for parathyroid hormone and vitamin D binding protein: Correlation with clinical immunoassay methods and application to clinical samples.

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Introduction and Aims

PTH bioactivity resides in the intact molecule, PTH(1-84), however, various truncal inactive forms also circulate. Under certain conditions such as renal disease these fragments can accumulate, leading to variable cross-reactivity with the PTH immunoassays. To accurately quantify the ratio of intact hormone to its fragments is becoming increasingly significant for the diagnosis of undiagnosed and osteological diseases. Alongside PTH, vitamin D is also critical in the control of calcium homeostasis. Measurement of serum total vitamin D alone may be misleading because vitamin D and its metabolites circulate tightly bound to vitamin D binding protein (VDBP), the concentration of which is known to be altered by pregnancy, liver and renal disease and, indeed, genotype.

Automated immunoenrichment prior to mass spectrometric analysis forms the basis of Mass Spectrometry Immunoassay (MSIA). Previously, we developed a multiplexed MSA-SRM assay for PTH concentration of which are known to be altered by pregnancy, liver and renal disease and, indeed, genotype.

MSIA-SRM Assay development: Affinity extraction of PTH and VDBP was performed using MSA pipette tips (absorbed with antibody) on a Versette pipetting robot (Thermo Scientific). Immuno-captured material was eluted from the tips and subjected to tryptic digestion followed by liquid chromatography tandem mass spectrometry analysis. This employed a HyperSil Gold column on a TQ Surf Vantage triple quadrupole mass spectrometer (Thermo Scientific) as previously described. SRM assays were developed using Thermo Scientific DataPep software. Intact PTH levels were measured using a second generation methodology using an ADVIA Centaur platform (Siemens Healthcare).

Methods

Clinical Samples: Patients were recruited following IRB-approved protocol. A total of 338 corresponding plasma and serum samples were analyzed (203 with renal impairment, 25 normal controls). Patient ages ranged from 16-88 with a mean age of 59.3 yrs. Gender distribution in the sample set was 48 % female and 51 % male.

MSA-SRM Assay development: Affinity extraction of PTH and VDBP was performed using MSA pipette tips (absorbed with antibody) on a Versette pipetting robot (Thermo Scientific). Immuno-captured material was eluted from the tips and subjected to tryptic digestion followed by liquid chromatography tandem mass spectrometry analysis. This employed a HyperSil Gold column on a TQ Surf Vantage triple quadrupole mass spectrometer (Thermo Scientific) as previously described. SRM assays were developed using Thermo Scientific DataPep software. Intact PTH levels were measured using a second generation methodology using an ADVIA Centaur platform (Siemens Healthcare).

TABLE 1. PTH variants detected in the clinical samples.

PTH variants in renal disease

Using an SRM corresponding to amino acids 34-44, those with end stage renal failure (ESRF) could be clearly separated from those with CKD stages 2-5, as well as healthy controls (Figure 5A). However, when an SRM corresponding to amino acids 33-44 was used, no difference between ESRF, CKD stages 2-5 and controls was observed (Figure 5B). Finally, when samples were stratified according to CKD stage and immunassay PTH result compared to that of the MSA-SRM intact PTH immunoassay, gross overestimate of PTH by immunoassay is demonstrated in those with ESRF (CKD stage 1) (Figure 6).

Vitamin D binding protein (VDBP). A. Sequence and surrogate peptides for SRM, B. Correlation graphs of SHM and clinical immunoassay data.

Conclusions

We have developed multiplexed, high-throughput, quantitative and precise SRM assays for both PTH and VDBP. The MSA-SRM assay allows rapid and automated enrichment to achieve high sensitivity (ng/L) and selectivity. Simultaneous measurement of intact and variant PTH species allows the precise quantification of active and inactive forms. Correlation of the PTH MSA-SRM assay using only the N-terminal peptide (aa 1-13) with the traditional immunoassay confirms that commercial immunoassays overestimate the amount of active PTH. Further, no evidence for PTH(7-84) was found in the data set. Further work will validate the utility of VDBP measurement in the routine assessment of vitamin D status.

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