

Urinary Glucose Tetrasaccharide Assay using Rapid Ultrapformance Liquid Chromatography Tandem Mass Spectrometry for Pompe Disease

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Pompe disease is the type II glycogen storage disease involving heart and skeletal muscle which is caused by the deficiency of enzyme named lysosomal acid alpha glucosidase (GAA). Although the enzyme assay for deficiency of GAA should be needed for definite diagnosis, this enzyme assay is not appropriate for screening Pompe disease due to low capability of discriminating Pompe disease patients from GAA pseudodeficiency patients. Urinary glucose tetrasaccharide (Glc₄) has been reported as supportive biomarker for the screening and therapeutic response monitoring of many glycogen storage diseases including Pompe disease.

Various assays for urinary Glc₄ have been developed including thin-layer chromatography and high performance liquid chromatography (HPLC). Recently urinary Glc₄ assay using ultrapformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) has been developed because of high resolution of UPLC-MS/MS and labor-intensiveness of existing assays such as extraction step and relatively long chromatographic runs of HPLC. Thus we developed and evaluated the urinary Glc₄ assay using UPLC-MS/MS (AB SCIEX Triple Quad™ 6500 system, CA, USA).

We collected 31 urine samples which consist of 27 normal control, 3 GAA pseudodeficiency patients who were confirmed by GAA gene mutation and 1 patient who was diagnosed with Pompe disease by GAA enzyme assay and had received enzyme replacement therapy (ERT). Each sample was injected to 0.1% of ammonium hydroxide solution containing internal standard acarbose, then the filtrates of samples were identified and quantified by UPLC-MS/MS.

We identified and quantified Glc₄, M₄, and acarbose using the ion pairs m/z 665/179, 665/161, 644/161 respectively. All three analytes were successfully separated. Calibration curve was linear over a range from 5 to 500 $\mu\text{mol/L}$ with linear regression value (R^2) > 0.999. The mean concentration of urinary Glc₄ of 27 normal controls was 1.5 mmol/mol creatinine (mmol/mol Cr), and of 3 pseudodeficiency patients was 12.1 mmol/mol Cr. Urinary Glc₄ concentration of the Pompe disease patients was 171.3 mmol/mol Cr, and it decreased to 130.9 mmol/mol Cr after ERT. Within- and between-day precision CVs were 14.6% and 13.2% (10 $\mu\text{mol/L}$ Glc₄), and 6.52% and 11.5% (200 $\mu\text{mol/L}$ Glc₄).

The urinary Glc₄ assay using UPLC-MS/MS can be a reliable biomarker for discriminating Pompe disease patient with pseudodeficiency patients. This assay also successfully verified the response of ERT in a patient with Pompe disease.