

**Abstract Type : Poster**

**Presentation No. : PTL 015**

## **Urinary Proteomic Biomarkers for Prediction of Chronic Antibody-mediated Rejection in Kidney Transplant Recipients**

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**Objectives:** Chronic antibody-mediated rejection (CAMR) is a challenging issue associated with allograft loss in kidney transplant recipients (KTRs) undergoing long-term follow-up. Changes in the urinary proteomes precede the elevation of serum creatinine concentration, the development of donor-specific antibodies, and histopathologic changes in KTRs with CAMR. The aim of this study was to discover potential proteomic biomarkers for prediction of CAMR in KTRs.

**Methods:** Nine KTRs with CAMR and 33 KTRs with long-term good survival (LGS) were included in this study. We used the proteomic approach to measure the changes of urinary exosomes of KTRs. The urinary exosomes were trypsin-digested using a gel-assisted protocol, and quantified by label-free LC-MS/MS, using a DDA mode.

**Results:** Analysis of the isolated exosomal proteins showed that 102 and 125 proteins were identified in CAMR and LGS, respectively. The detected proteins were quantified using the software Peaks 7. Identically detected proteins in a large amount in each group were excluded for candidate biomarkers and high-significance proteins with the fold change of at least 1.5 were selected as candidate biomarkers. Finally, 8 proteins (PIGR, CP, GPRC5A, APOA1, TSPAN1, TTR, AZGP1 and HPX) that were distinguishable from LGS were select as biomarker candidates to predict CAMR. We confirmed the 8 proteomic biomarkers by using immunoblot analysis in urinary exosomes which were independently acquired from 17 CAMR and 24 LGS.

**Conclusions:** We discovered and validated 8 specific proteins to predict CAMR in KTRs. Further studies are needed to apply the rejection-specific biomarkers for early prediction, diagnosis, and monitoring of clinical response of treatment of CAMR in KTRs.