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이식환자 소변세포 전사체에서 비침습적 진단 마커를 위한 droplet digital PCR과 real-time PCR 비교

경희대학교 의과대 신장내과

서정우, 문해나, 이아라, 김양균, 정경환, 문주영, 이상호

A Comparison of Droplet Digital PCR and Real-time PCR for Noninvasive Diagnostic Marker of Renal Allograft Rejection in Urinary-cell mRNA

Jung-Woo Seo, Haena Moon, Arah Lee, Yang-Gyun Kim
Kyung-Hwan Jeong, Ju-Young Moon, Sang-Ho Lee

Division of Nephrology, Department of Internal Medicine, College of Medicine, Kyung Hee University

Background: The diagnosis of acute rejection in renal transplantation is still being standardized by an invasive renal biopsy. Non-invasive diagnostic tests and biomarkers that can replace this invasive procedure for detection of rejection in renal transplantation are required to improve the outcome of renal allograft. A previous study has suggested that the expression analysis of urinary-cell mRNA for CD3ε chain, interferon-inducible protein 10 (IP-10), and 18S rRNA provides a less invasive mean for diagnosing AR in US renal transplant recipients. Recent study reported that droplet digital PCR (ddPCR), allowing higher sensitivity, reproducibility, and more absolute quantification of gene expression levels without standard curves, could be a very useful tool for various researches. We investigated whether these three genes from urine specimens are useful to diagnose AR in Korean renal transplant patients and compared the results from ddPCR and qPCR.

Methods: 65 urine specimens (25 stable, 27 acute cellular rejection (ACR), and 13 acute antibody-mediated rejection (AMR)) were collected after transplantation. Isolated RNA from urine specimens was eluted in 30 ul nuclease-free water. Then DNA was synthesized with equal volume of 10 ul total RNA in all samples without quality control. The expression levels of transcripts for CD3ε, IP-10, and 18S rRNA were determined in urinary cells using qPCR and ddPCR and correlated transcript levels with renal allograft rejection.

Results: In the analysis of qPCR, CD3ε and IP-10 were not detected, 30.8% and 35.4%, respectively, whereas these genes were absolutely quantified in all samples by ddPCR. In ddPCR, the expression levels of CD3ε and IP-10 were significantly increased in ACR and AMR groups. Increased expression levels of these two genes were also observed from qPCR but only ACR was significant.

Conclusion: From this study, only CD3ε among the three genes that were previously found as novel biomarkers for acute rejection was validated in Korean renal transplant patients in both qPCR and ddPCR assays. With more sensitive and accurate quantification, ddPCR could be a useful tool to monitor acute rejection by urine mRNA analysis after renal transplantation.

Key Words: Droplet digital PCR, 신장이식, 거부반응
Droplet digital PCR, Kidney transplantation, Acute rejection