RESUMEN

El rechazo agudo a aloinjerto es el principal obstáculo para la supervivencia de un riñón trasplantado. La biopsia renal todavía es la prueba de referencia en el diagnóstico de la disfunción del injerto, pese a ser un procedimiento invasivo. Diversos estudios indican que los linfocitos T reguladores, que expresan el gen FOXP3, aumentan en el aloinjerto durante el rechazo agudo. En esta investigación, se evaluaron sistemas de RT-qPCR con la química TaqMan® para analizar la expresión del biomarcador urinario FOXP3 ARNm y del gen de referencia 18S ARNr en muestras clínicas de ARN de orina de pacientes no nefrópatas y de pacientes trasplantados renales, proporcionadas por el Hospital Carlos Andrade Marín en Quito, Ecuador. Para ello, se optimizaron los sistemas de RT-qPCR a partir de ARN de sangre total, y paralelamente se estandarizó un método de extracción de ARN a partir de sedimento urinario. El aislamiento de ARN de muestras clínicas de orina tuvo rendimientos de 4.15-13.85 ng de ARN por mL de orina, baja calidad y tendencia del ARN a degradarse. El sistema 18S en muestras clínicas de orina es preciso y reproducible, con una variabilidad intraensayo de 1.6% y sin diferencias entre las variaciones de los ensayos. Se comprobó una mayor cantidad del gen 18S en muestras de pacientes trasplantados respecto a muestras de pacientes no trasplantados. El sistema FOXP3 no detectó el gen en muestras de ARN de pacientes no trasplantados, pero sí lo hizo en una de cuatro muestras de pacientes trasplantados, demostrando que este sistema necesita mayor optimización para poder detectar el gen en todos los pacientes trasplantados. Pese a una sensibilidad analítica del sistema FOXP3 de hasta 1 pg/µL de ARN a partir de sangre total, en muestras de ARN a partir de orina se dificultó la detección y amplificación del gen debido a una cantidad de ARNm del gen FOXP3 menor en muestras de orina y presencia de inhibidores de PCR. Por lo tanto, el sistema FOXP3 necesita aumentar su sensibilidad analítica para ser validado en muestras de orina, y se recomienda mejorar la calidad de las muestras de ARN e implementar una etapa de preamplificación.

PALABRAS CLAVES: RECHAZO AGUDO RENAL, BIOMARCADOR, FOXP3, 18S, RT-qPCR.

ABSTRACT

Acute allograft rejection is the main complication in kidney transplantation. Despite being an invasive procedure, renal biopsy is still the gold standard in the diagnosis of graft dysfunction. Several studies indicate that regulatory T cells, which express the gene FOXP3, increase in the allograft during acute rejection. In this research, RT-qPCR TaqMan® systems were evaluated in order to study the potential applicability of FOXP3 mRNA as a urinary biomarker for acute kidney rejection. A housekeeping gene, 18S rRNA was used as reference gene for quantitation of gene expression. For both systems, RNA was extracted from urine samples from patients without kidney disease and from kidney transplant patients. Biological samples were kindly provided by the Transplant Unit and the Laboratory of Molecular Biology of Carlos Andrade Marin Hospital in Quito, Ecuador. RT-qPCR systems were optimized with whole blood extracted RNA. A method of RNA extraction from urine epithelial cell pellet was also standardized. Yields of extracted RNA from urine were 4.15-13.85 ng of RNA per mL of urine, low quality and RNA tended to degradate. The 18S system on urine samples is accurate and reproducible, with intra-assay variability of 1.6%, and no significant differences in variation between essays. More quantity of the 18S gene was proved in transplanted patient samples compared with non-transplanted patient samples. The FOXP3 system did not detect the gene in RNA from non-transplanted patient samples, but it detected the gen in one of four transplant patient samples, showing that this system needs further optimization in order to detect the gene in all transplant patients. Despite an analytical sensitivity of FOXP3 system of 1 pg/mL of RNA from whole blood, detection and amplification of the gene became more difficult in RNA samples from urine due to a less amount of mRNA of FOXP3 gene in urine samples and the presence of PCR inhibitors. Therefore, the system FOXP3 needs to increase analytical sensitivity to be validated in urine samples, and it is recommended to improve the quality of the RNA samples and implement a preamp stage.

KEYWORDS: ACUTE ALLOGRAFT REJECTION, BIOMARKER, FOXP3, 18S, RT-qPCR.