Novel urine test developed to diagnose human kidney transplant rejection

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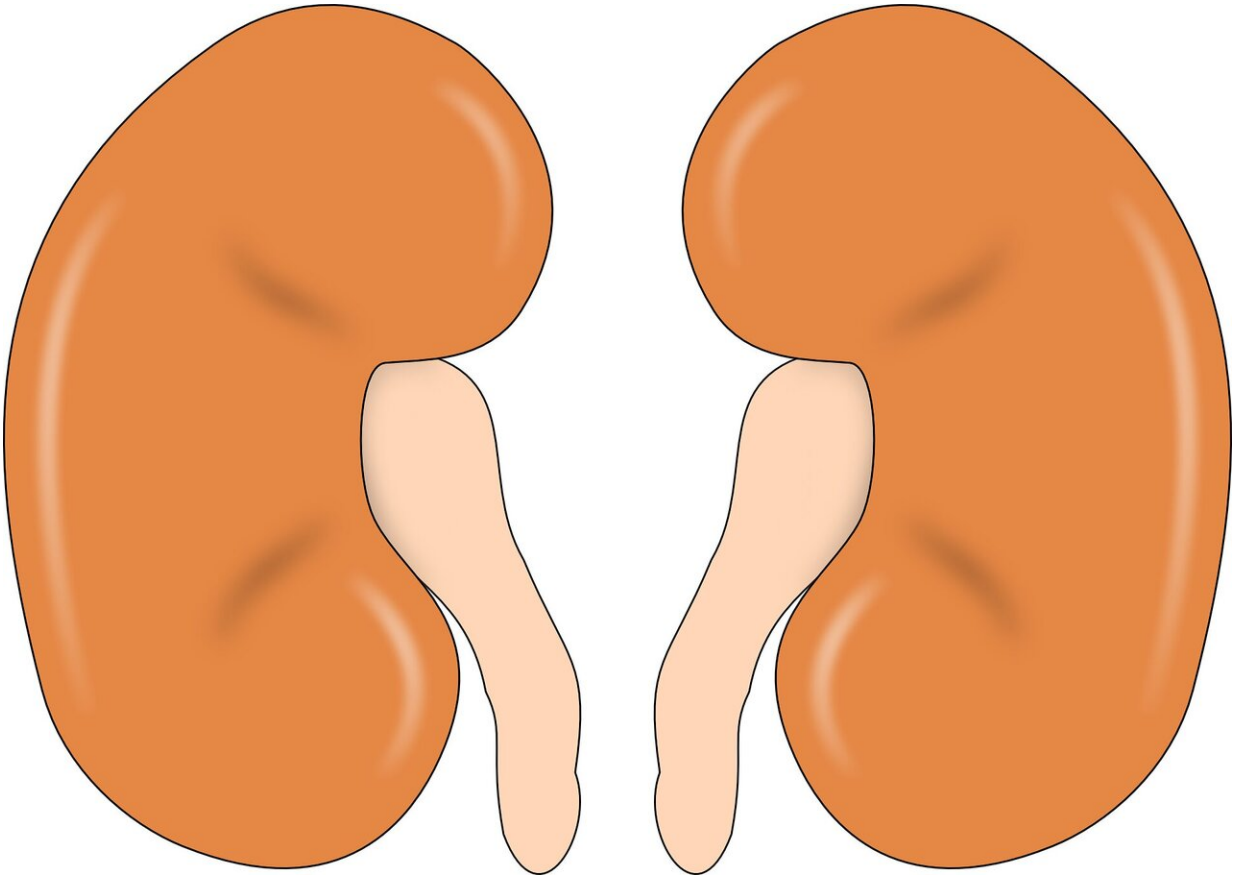

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Patients can spend up to six years waiting for a kidney transplant. Even when they do receive a transplant, up to 20 percent of patients will

experience rejection. Transplant rejection occurs when a recipient's immune cells recognize the newly received kidney as a foreign organ and refuse to accept the donor's antigens. Current methods for testing for kidney rejection include invasive biopsy procedures, causing patients to stay in the hospital for multiple days. A study by investigators from Brigham and Women's Hospital and Exosome Diagnostics proposes a new, noninvasive way to test for transplant rejection using exosomes—tiny vesicles containing mRNA—from urine samples. Their findings are published in the *Journal of the American Society of Nephrology*.

"Our goal is to develop better tools to monitor patients without performing unnecessary biopsies. We try to detect rejection early, so we can treat it before scarring develops," said Jamil Azzi, MD, associate physician in the Division of Renal Transplant at the Brigham and an associate professor of Medicine at Harvard Medical School. "If rejection is not treated, it can lead to scarring and complete kidney failure. Because of these problems, recipients can face life-long challenges."

Before this study, physicians ordered biopsies or blood tests when they suspected that a transplant recipient was rejecting the donor organ. Biopsy procedures pose risks of complications, and 70-80 percent of biopsies end up being normal. Additionally, creatinine blood tests do not always yield definitive results. Because of the limitations surrounding current tests, researchers sought alternate and easier ways to assess transplant efficacy.

In this study, researchers took urine samples from 175 patients who were already undergoing kidney biopsies advised by physicians. From these samples, investigators isolated urinary exosomes from the immune cells of the newly transplanted kidneys. From these vesicles, researchers isolated protein and mRNA and identified a rejection signature—a group of 15 genes—that could distinguish between normal kidney

function and rejection. Notably, researchers also identified five genes that could differentiate between two types of rejection: cellular rejection and antibody-mediated rejection.

"These findings demonstrate that exosomes isolated from urine samples may be a viable biomarker for kidney transplant rejection," said Azzi.

This research differs from prior attempts to characterize urinary mRNA because clinicians isolated exosomes rather than ordinary urine cells. The exosomal vesicle protects mRNA from degrading, allowing for the genes within the mRNA to be examined for the match rejection signature. In previous research, mRNA was isolated from cells that shed from the kidney into urine. However, without the extracellular vesicles to protect the mRNA, the mRNA decayed very quickly, making this test difficult to do in a clinical setting.

"Our paper shows that if you take urine from a patient at different points in time and measure mRNA from inside microvesicles, you get the same signature over time, allowing you to assess whether or not the transplant is being rejected," said Azzi. "Without these vesicles, you lose the genetic material after a few hours."

One limitation to this research is that these tests were done on patients undergoing a biopsy ordered by their physician, who already suspected that something was wrong. In the future, Azzi and his colleagues aim to understand whether a test such as this one can be used on kidney transplant recipients with normal kidney activity as measured in the blood to detect hidden rejection (subclinical rejection). They are currently doing a second study on patients with stable kidney function, looking to see if the same signature they identified in this current study could be used on patients without previously identified issues but still detect subclinical rejection.

"What's most exciting about this study is being able to tell patients who participated that their effort allowed us to develop something that can help more people in the future," said Azzi. "As a physician-scientist, seeing an idea that started as a frustration in the clinic, and being able to use the lab bench to develop this idea into a clinical trial, that is very fulfilling to me."

More information: Rania El Fekih et al, Discovery and Validation of a Urinary Exosome mRNA Signature for the Diagnosis of Human Kidney Transplant Rejection, *Journal of the American Society of Nephrology* (2021). DOI: 10.1681/ASN.2020060850

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