Standardization and Optimization of Urinary Extracellular Vesicle Isolation

Role of Insurance Companies in Kidney Disease Disparities

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By
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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Introduction

Each kidney contains approximately half a million glomeruli, which are specialized bundles of capillaries that serve as primary facilitators in the filtration process (Pollak et al., 2014). The glomerular filtration rate measures how well the kidneys, specifically the glomeruli, are filtering waste products and excess water from the blood. Chronic kidney disease is defined as the presence of kidney damage for an extended period of time. This damage is classified as end-stage renal disease (ESRD) when a glomerular filtration rate less than 60 ml/min persists beyond three months (Vaidya & Aeddula, 2022).

Approximately 14.9% of the overall population in the United States is impacted by chronic kidney disease (Gupta, 2021). This chronic condition disproportionately impacts individuals of lower socioeconomic status and minority groups. For instance, “black people make up about 13% of the total population but account for 30% of the people with ESRD in the United States" (United States Renal Data System, 2022). Social determinants of health, including financial, environmental, and social factors, can and often do contribute to kidney disease progression. These determinants can begin to explain disparities in ESRD, but the progression of chronic kidney disease often cannot be attributed to one single cause.

Several treatment methods exist to manage the progression of ESRD. Approximately 63% of patients receiving treatment for ESRD undertake in-clinic hemodialysis, 29% engage in at-home peritoneal dialysis, and 8% are recipients of transplantation (Gupta, 2021). Hemodialysis is a time-consuming demand, as treatment occurs three times a week for roughly 3-5 hours a session, and fatigue and discomfort are persistent symptoms between sessions (Mayo Foundation, 2021). Transplantation is for those who meet extensive criteria and overcome the waitlist from national organ-shortages, and also requires a lifelong commitment to

immunosuppression management. Peritoneal dialysis allows patients to receive treatment from the comfort of their homes but requires a support system and sufficient home environment to operate the dialysis machine in a sanitary and safe manner. Despite the existence of several management methods, the lifestyle of individuals with this chronic condition is greatly altered and none of the solutions can be considered a perfect cure.

It is critical that research efforts aim to identify biomarkers that indicate the progression of kidney disease for early detection and to examine the stakeholders responsible for exacerbating the impact of ESRD on minority populations. In an effort to slow or stop the progression of kidney disease, Dr. Urban and I seek to standardize a method to isolate urinary extracellular vesicles that can be useful tools for early diagnosis and clinical application, including drug delivery or therapeutic agents. Concurrently, I intend to investigate if there are stakeholders who may exacerbate the racial and socioeconomic disparities that exist in the development and progression of chronic kidney disease, so that these sources of inequity can be appropriately addressed.

Technical Project

Urinary extracellular vesicles (uEvs) are of particular interest as sources of biomarkers that can indicate the development of kidney disease in a non-invasive and easily accessible manner (Gámez-Valero et al., 2015). However, current uEv research fails to propose a method that standardizes the isolation of uEvs. To use uEvs for diagnosis and clinical applications, we first must produce a method that standardizes uEv isolation by characterizing control samples and optimizes sample quality by identifying biomarkers in patient samples indicative of kidney injury. We hypothesize that early detection of kidney injury can mitigate disease progression.

Urine content is highly variable and dependent on several factors, including the collection time, diet and exercise, age, gender, medications, and health status (Erdbrügger et al., 2021). While uEvs are the primary Evs found in the urinary tract, circulating Evs can mediate organ crosstalk, increasing inflammation and amplifying kidney disease progression (Grange, 2022). It is essential to distinguish between uEvs and circulating extracellular vesicles when analyzing the molecular content of the sample. First, we intend to standardize collection time and hydration conditions using a control group of ten healthy volunteers between 20-25 years old. There will be five male and five female subjects in the control group for equal sex representation that reduces bias in the event that there are sex-related differences present. Each volunteer must meet the inclusion criteria and provide consent prior to sample collection. We will exclude individuals with substantial health history involving the renal system, such as transplanation, to ensure that we can classify this control group as healthy for our purposes. We seek to determine which hydration conditions produce the best sample quality by analyzing the average uEv quality and quantity across all samples in each of three scenarios:

1) Water Deprivation (12 AM - 8 AM), followed by morning (8 AM) collection
2) No Water Deprivation, followed by morning (8 AM) collection

We will use Nanoparticle Tracking Analysis (NTA) to measure and visualize the uEvs. Finally, we will use these samples to produce western blots for pro tein identification. Aquaporin and nephrin are two proteins of interest that will be useful for validating that the particles originate from the urinary tract. It is important to identify the best conditions for uEv collection as well as validate the origin of the uEvs to produce a method that not only produces the greatest yield, but that we are certain we have measured the extracellular vesicles of interest.

After standardizing a method for uEv isolation, we seek to investigate patient samples with acute kidney injury to optimize the sample quality and identify biomarkers indicative of kidney injury. Existing methods of uEv isolation include ultracentrifugation, filtration, immuno-affinity and peptide-based isolation, aggregating agents, size exclusion chromatography, microfluidic-based methods, and hydrostatic dialysis (Gámez-Valero, 2015). We will centrifuge 50 ml aliquots of urine and collect the cell-free urine supernatant. The cell-free urine will undergo centrifugation again to separate the cell fragments-free urine supernatant and cell fragments in pellet form. Then, we will analyze whether differential centrifugation alone, or a combination of differential centrifugation and size-exclusion chromatography produces the greatest quality and quantity of uEvs from the cell fragments-free urine. Specifically, we will analyze the content of two biomarkers for acute kidney injury, NGAL and Kim-1, to identify the progression of kidney disease in patient samples.

Future clinical applications of uEvs include utilization in drug delivery, diagnosis of kidney disease, or role as therapeutic agents. These applications could be life changing for individuals who are battling the physical, financial, and lifestyle barriers associated with chronic kidney disease. Additionally, the use of uEvs for early detection in groups that are at high risk for developing kidney disease that progresses rapidly prior to detection, such as those with cystic fibrosis, can ideally slow the progression of this disease and enhance the quality of life for these individuals. Overall, there is abundant potential for the clinical application of uEvs, but standard protocol for uEv isolation must first be established in order to open the doors to this biomedical development.

STS Project

Research validates that both socioeconomic and racial disparities exist in chronic kidney disease. One study examined the time elapsed from ESRD to death in over 15,000 urban and mostly poor adults with CKD. The study methodsutilized a public health safety-net system and they found "racial/ethnic minorities had a 2.2–4.0 fold higher risk of progression to ESRD compared with white persons with CKD, which was not explained by lower relative mortality, which could increase their likelihood of progressing to ESRD" (Hall et al., 2010, cited in Nicholas et al., 2015, p. 5). This study expresses that there is a correlation between both socioeconomic and racial factors and the increased likelihood of kidney disease progressing to ESRD. This is significant because ESRD demands management impacts patient quality of life, which disproportionately includes minority groups. A study that investigates quality of life (QOL) scores in ESRD found that "QOL of hemodialysis patients was found to be significantly ($P < 0.05$) impaired in comparison to the QOL of healthy individuals selected from the general population, particularly with respect to the physical, psychological, and social relationship domains" (Sathvik et al., 2008, p. 6). This is not surprising to me as hemodialysis is a time-consuming demand that subsequently hinders weekday employment opportunities, limits time for socialization, and takes a physical toll on patients after and between sessions.

Social determinants of health, or environmental factors that result from systems established by people, often contribute to kidney disease progression and can begin to explain how those of lower socioeconomic status and minority groups are propelled into ESRD at a higher rate than others with chronic kidney disease. These include housing challenges, food insecurity, and unemployment, to name a few. For instance, an individual with a lower income and job stressors may experience a worsened lifestyle, such as less time for exercise, insufficient

diet, poor quality of sleep, and so on. This lifestyle increases the risk of advancing kidney disease. Once this individual begins hemodialysis to manage ESRD, treatment is costly, and the individual has even less time to put toward employment, exercise, and sleep. This creates an often-unrecoverable cycle of physical, financial, social, and psychological burden for the underprivileged individuals with ESRD. Researchers validate the impact of social determinants of health in ESRD as they write, "Indeed, the social environment has been cited as a key determinant in the persistence of health inequities in the U.S" (Nicholas et al., 2015, p. 2). However, this is not the sole factor as the authors proceed to explain that "despite our recognized standing as a world leader in health technology and medical care, the U.S. ranks near last in preventable deaths among developed nations" (Nolte & McKee, 2008, cited in Nicholas et al., 2015, p. 2). The word 'preventable' suggests that there may be groups or individuals responsible for health inequities in the United States that emerge during the treatment phase of ESRD. The contribution of the identified social determinants is significant, but with high level health technology and medical care available in the United States, these factors do not adequately explain further disease progression for minority and poorer groups post-diagnosis

To identify the source of preventable deaths from ESRD in the United States, I propose the following research question: *What role do insurance companies have in exacerbating the socioeconomic and racial disparities in chronic kidney disease progression?* This question was motivated by initial background research regarding the insurance coverage and the subsequent standard of care in an analysis of the Third National Health and Nutrition Examination Survey, conducted by the Center of Disease Control and Prevention for inclusion in their National Center for Health Statistics database. Specifically, "investigators found that blood pressure was 59% more likely to be controlled in hypertensive participants with private health insurance than in

those without health insurance" (He et al., 2002, cited in Jurkovitz et al., 2013, p. 2); "Likewise, uninsured patients with chronic kidney disease (CKD) are less likely than insured patients to be treated for hypertension or to receive angiotensin inhibitors" (Hall et al., 2009, cited in Jurkovitz et al., 2013, p. 2). These findings indicate that conditions such as hypertension in underinsured patients were treated at a standard of care beneath that of privately insured patients. While not directly denied care for chronic kidney disease in this situation, hypertension can increase inflammation and indirectly causes further damage to the kidneys. Thus, shortcomings of public health insurance can be identified as a key stakeholder in the healthcare industry that is worth investigating in my STS project.

This approach relies heavily on the theories expressed in *There's no such thing as 'We'* by Max Liboiron. Specifically, Liboiron argues "that universalism eliminates and controls crucial aspects of difference. Evoking the universal "we" is a technique of discarding through differentiation in a way that upholds dominant power dynamics" (Liboiron, 2020). This motivates my methodology, which is to analyze insurance companies and their specific policies from a cultural and social perspective to identify how companies claiming to serve "everyone" in a community may fail to serve poorer groups and minorities.

Conclusion

Upon the completion of the experiments and analysis outlined in the technical section, Dr. Urban and I anticipate the production of a method that standardizes and optimizes urinary extracellular vesicle isolation. Through my sociotechnical research, I intend to identify how much or how little of a role insurance companies may have in exacerbating the already existing disparities in kidney disease progression. My standardized method will be useful for researchers

and clinicians who seek to utilize uEvs to facilitate drug delivery, for diagnostic purposes, or as therapeutic agents. Most importantly, my method will benefit those with biomarkers indicative of acute kidney injury as early detection or clinical intervention can slow or pause the progression of chronic kidney disease. Further, it is my hope that individuals advocating for equitable healthcare and public policy officials use the findings of the STS project to guide reform by targeting specific insurance companies and their policies that play the greatest role in worsening kidney disease progression for minorities. Earlier detection and intervention are critical for reducing the progression of kidney disease that minority groups experience disproportionately and the subsequent impact that insurance companies may have on these groups.

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