Development of a Diabetes Diagnostic Tool Utilizing EV Biomarkers
(Technical project)

Investigation of the Overrepresentation of African Americans in Hemodialysis Prescription
(STS project)

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By
Molly Sander

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Isaac Heath

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.

ADVISORS

Professor MC Forelle, Department of Engineering and Society

Dr. Chris Highley, Department of Biomedical Engineering

Introduction:

While many people may not appreciate the health of their kidneys or perhaps even know what role they serve in the body, devastating impacts occur when they stop functioning properly. Kidney diseases are nicknamed “the silent killer” and are the leading cause of death in the United States (*Chronic Kidney Disease Basics*, n.d.). Among other important roles, the kidneys’ primary purpose is to filter the blood. The kidneys allow for the reabsorption of components of the blood that should be recirculated in the bloodstream, such as the plasma protein albumin, and they filter out waste products for excretion, such as creatinine (Kamińska et al., 2020). The most prominent issue facing kidney healthcare today is the late diagnosis of kidney diseases and dysfunctionality of the kidneys. Like many other organs in the body, if damage to the kidneys is detected early enough, there is often possibility for repair and restoration. There is no current medical treatment that can restore kidneys to their original function once they are damaged beyond the point of repair, and the current diagnostic methods rely on symptoms that are typically only present after the kidneys have reached this point-of-no-return such as nausea, vomiting, loss of appetite, and changes in urination, all of which are common symptoms of other diseases. The current diagnostic methods are a blood test and a urine test (*Chronic Kidney Disease Tests & Diagnosis*, n.d.). The urine test is called a protein-creatinine ratio test, in which the levels of protein, typically albumin, in the urine that should not be present are compared to expected protein in the urine, which in this test is creatinine.

Chronic kidney disease (CKD) is a condition in which the kidneys are permanently damaged and cannot filter blood as well as they should, and because of this, waste products and excess fluid may accumulate in the blood and cause other health issues (*Chronic Kidney Disease in the United States, 2021*, 2022). Many diseases affect kidney health and can lead to CKD if not

treated in time. The primary causes of CKD are diabetes and hypertension, which constitute 75% of new CKD cases (*Chronic kidney disease – Symptoms and causes*, n.d.). According to the CDC, “About 37 million US adults are estimated to have CKD, and most are undiagnosed." (*Chronic Kidney Disease Basics*, n.d.). This statistic means that 15% of the national population currently has CKD.

Once CKD has been diagnosed, the treatment options are dialysis or a kidney transplant. Dialysis is done most commonly through hemodialysis but can also be done through the peritoneal membrane in the abdomen (*What is Dialysis?*, 2015). Hemodialysis involves the use of an artificial kidney, called a hemodialyzer, to remove waste products from the blood. Patients travel to a dialysis clinic three times a week and are hooked up to the hemodialysis machine for an average of four hours while their blood gets filtered (*Dialysis*, n.d.). Hemodialysis is a demanding, painful, and exhausting process for patients, and unless they are able to receive a kidney transplant from the long kidney transplant waiting list, then dialysis is the best and only treatment they can receive until they die. The average life expectancy of a patient starting dialysis is 5-10 years (*Dialysis*, n.d.).

As detrimental and pervasive of an issue as kidney failure is in itself, the social impacts of kidney diseases are also devastating. Populations are not affected equally by kidney diseases, and examining current practices and statistics elucidates the extent to which kidney healthcare is riddled with disparities, particularly in the African American community (Norris et al., 2017). It is important to understand what factors have caused this disparity in order to understand how to best address it and to predict how technological changes will impact African Americans.

Since there is no current treatment for the kidney once CKD is developed, it is logical to examine methods of earlier detection in order to prevent kidney diseases from resulting in CKD. The development of a new diagnostic method to detect kidney diseases and dysfunctionality earlier, affordably, and site-specifically could potentially be harnessed to prevent progression to CKD and could subsequently decrease the patients who require hemodialysis; the development of this detection tool in conjunction with an investigation into the current causes of disproportionate representation of populations receiving hemodialysis could also shed light on and could help address healthcare disparities prevalent within the United States. In my technical project, I will be creating a diagnostic detection tool for diabetes harnessing extracellular vesicles and diabetes-specific biomarkers, and in my STS project, I will be investigating why African-Americans are over-prescribed hemodialysis.

Development of Diabetes Extracellular Vesicle Biomarker Detection Tool

The challenges with current diagnostic methods for kidney diseases are that they are late-stage markers of kidney damage, unspecific to the site of kidney damage, and require extensive processing of the urine that hinders accessibility for patients to receive a kidney functionality test. The aforementioned factors all likely contribute to why CKD is so prevalent, and they present the opportunity for a new diagnostic method to detect kidney damage that will address each of these weaknesses. This may be accomplished through looking from a new lens, particularly the potential use of nanoparticles in the body called urinary extracellular vesicles (EVs).

Urinary EVs are promising new kidney disease biomarkers that could be harnessed as a diagnostic tool for patients. EVs are membranous particles containing cell cargo with inherent

biological information that cells release into the extracellular space (Svenningsen, 2020). EVs not bound to a specific location and can travel throughout the body via biofluids, including blood and urine (Urabe, 2020). The contents of EVs contain important biological information about the cell it came from and its conditions, and, thus, EVs containing content from kidney cells could be used to detect kidney damage that may not be symptomatic in the patient. The first major push for analysis of EVs as biomarkers began in 2018 (van Niel, 2018), and currently, they have been proved as early markers of kidney damage (Erdbrügger, 2021). The main issue with the current method of analysis of urinary EVs is that is time consuming and is not specific to the site of damage within the kidney but just conveys information about the overall kidney health instead. We seek to develop a microchip that contains antibodies conjugated to specific known biomarkers of kidney diseases that will capture EVs in the patient sample that bind to the antibody.

Despite availability of sensitive instruments for EV analysis, most instruments require processing of EV containing samples. Following enrichment, characterization of EVs includes western blot analysis, flow cytometry, particle analysis, and omic approaches. These combined methods require large volumes of urine from the patient and long processing times to complete, limiting their use when less volume is available such as limited patient samples. The combination of methods required to process the EVs makes the procedure expensive and time-consuming, and they highlight the need for novel methods using unprocessed samples.

Since our detection tool requires the isolation and analysis of particular urinary EVs, we needed to pick a specific kidney disease to focus on for the development so that we could create a detection method for specific biomarkers present in the disease. One of the leading causes of CKD is Diabetes Mellitus (DM) (*Diabetes - A Major Risk Factor for Kidney Disease*, 2015).

DM is a chronic disease that is estimated to impact over 470 million people Worldwide by 2030 (Tabák et al, 2021). DM disables the proper functioning of the kidneys, and includes various severe long-term health complications such as progressive nephropathy, neuropathy, and cardiac damage, and it is one of the most prevalent causes of death (Lin et al., 2020). For these reasons, our detection method focuses on DM and will hopefully be extended to other diseases in the future.

Given the lack of non-invasive early detection methods, our research proposed here aims to generate a new EV detection tool for DM that requires less than 50 microliters of unprocessed sample and can be completed, without the need for enrichment, within 48 hours of sample collection. We will test and optimize a new high-throughput microchip technology (ExoviewR100) for application in urine samples (*ExoView R200 - Characterize lentivirus & exosomes*, n.d.). Our long-term goal is to develop a combination of markers specific for glomerular or tubular cells of the nephron of the kidney which characterize pathophysiological DM phenotypes. We hypothesize that this microchip technology using minimal raw urine volume can provide basic EV characterization and be used for detection of glomerular and tubular damage markers in diabetes. We will test this hypothesis in two phases.

First, we will compare basic characterization of enriched urinary EVs to ExoviewR100 based EV characterization of unprocessed urine samples**.** The basic ExoviewR100 technology utilizes tetraspanin specific antibody-based EV capture. Our second phase of the development of the EV detection tool is to utilize the basic chip and modify it with a compound linkage method for identification of multiple biomarkers. We will use these newly developed chips to create a definitive testing model designed to differentiate between EVs from the urine of healthy donors versus diabetic patients and subsequently be able to discern when a patient has an early onset of

diabetes to allow for earlier diagnosis. The development of the novel early detection diagnostic tool will have not only physiological impacts but also social, as healthcare inequities are pervasive particularly within kidney healthcare, and an earlier and affordable diagnostic method for kidney diseases may give some patients opportunities and time that they otherwise would not have had with current diagnostic practices.

Human, Social, and Technical Interactions in Hemodialysis

Healthcare disparities are pervasive in the United States, and kidney healthcare is no exception. The social and technical interplay within hemodialysis in particular is of value because of the 37 million patients currently receiving dialysis (Chronic Kidney Disease Basics, n.d.). The human and social factors within kidney health that lead to the late diagnoses of some individuals with kidney diseases and then end up being prescribed hemodialysis, such as a lower socioeconomic status and lower access to healthcare. Since populations are disproportionately affected, this leaves marginalized groups with even less time and energy available to fit into a society where they are already at a disadvantage, thus reinforcing the vicious cycle of inequity. If someone of lower socioeconomic status developed CKD due to limited access to healthcare and now cannot work due to receiving hemodialysis, the problem is only accentuated.

There are many social implications inherent within the current dialysis treatment setup, which can be observed through the employment of the STS framework known as Actor-Network Theory (ANT). Two founding authors of this framework are Bruno Latour and John Law, who each describe how people and technology are among a vast array of actors that as a whole form a network that dictates many of the understood relationships within society (Law, 1992). Understanding this network can help inform what actors are playing a role and can potentially explain why some relationships and trends have become what they have in society (Latour,

(1992). In this case, the relationships observed due to the network will be analyzed in the realm of understanding why African-Americans are overprescribed hemodialysis. Various actors in the network of hemodialysis include the health professionals, insurance companies, government policymakers, patients, dialysis machines, modality options, transportations, dialysis clinics, and many more. This framework can be used to examine how the level of comfort and function of various dialysis treatments requires the interaction of the human patients with the dialysis machinery; the assumption is made when patients are given the treatment option that they have medical knowledge and are capable of operating the non-human actors to administer dialysis treatments to themselves (Cressman, 2009). Other non-human actors that affect the patients are transportation to the dialysis sites, the supply chain for dialysate fluids, and transplant list. The institution of hemodialysis requires that patients live a reasonable distance away from the dialysis clinic, as they will need to travel there three times a week and will need to provide their own transportation there. Although dialysis is covered by Medicare, patients often must find a manner of transportation to get to the clinic, which poses a challenge to many of lower socioeconomic status (Medicare Coverage of Kidney Dialysis & Kidney Transplant Services, n.d.). Also, the dialysis clinics are typically only open during typical office hours, which is debilitating for patients who are also working. The dialysis treatment itself also is long and exhausting for the patients, and this results in the population of Americans receiving hemodialysis not feeling as well and with less time to accomplish all that they may desire due to the time spent hooked up to the machine.

The human component to dialysis is particularly evident in examining the factors that may lead to a patient needing hemodialysis, such as inequities, lifestyle choices, and genetic predispositions. The anticipated deliverable will be a comprehensive summary of data gathered

regarding hemodialysis, particularly in African American populations, and I will make sense of what factors are playing a role in the diagnosis and treatment of dialysis. There is already current research of the composition of the population of patients receiving dialysis, but there is no comprehensive summary analyzing several factors at once they may be causing some populations to be more or less likely to be prescribed hemodialysis.

Research Question and Methods

The clearest discrepancy in the realm of kidney healthcare is seen in the prevalence of kidney failure in the African American population, in which African Americans represent only 13.1% of the United States population yet constitute 36% of patients receiving hemodialysis treatments (Umeukeje & Young, 2019). The research question that I seek to examine is: why are African Americans overrepresented in the prescription of hemodialysis? This question is important to research in order to understand what has led to this discrepancy so that the factors most contributing can be addressed to minimize the disparity.

In order to execute this project, I will research statistics regarding healthcare disparities within kidney failure. I will search medical literature and national healthcare databases for what precipitates a CKD diagnosis and dialysis prescription and what the timeline of this is within different populations. I will also research databases regarding African American access to doctors and routine checkups as well as genetic, nutrition, exercise, socioeconomic factors, and systemic racism that may be contributing to a much higher incidence of hemodialysis prescriptions in African American communities than in other populations. Another important method of research will be comparing hemodialysis and other treatment options for African Americans versus other populations' typical treatments of kidney failure. I will investigate if this treatment prescription is correlated with access to transportation to dialysis clinics or access to

private home health workers. Finally, in order to compile all data gathered together, I plan to meet with a statistics pundit at the University of Virginia in order to draw conclusions from the data given and to determine the statistical significance and implications of the data found from each contributing factor.

Conclusion

The development of the novel early detection diagnostic tool will be a long-term solution in early detection of life-threatening diseases due to detecting biomarkers from low volumes of urine at routine patient check-ups rather than waiting for symptoms to appear in patients. The deliverable will be a microchip assay using diabetes site-specific antibodies on an assay to capture EVs containing the corresponding biomarker from a patient sample. The early detection would improve patients' quality of life by detecting diseases early and slowing, or potentially entirely preventing, the progression of CKD. Also, the low processing time required would make the detection method more affordable, and this will contribute to the vast social implications already anticipated from the development of the diagnostic tool.

In conjunction with the development of the diagnostic method, the current disparities within kidney disease will be analyzed through investigating why African Americans are overrepresented in hemodialysis prescription, and this deliverable will be a paper that is a comprehensive analysis of several factors that may be contributing to the overrepresentation of hemodialysis prescription in the African American population. These findings will shed light on one of the many medical inequities exist in America. I predict that conducting this research project will find that hemodialysis prescriptions are prescribed more to African Americans than other populations due to later diagnoses due to lower healthcare accessibility, which would result in worsened kidney function and would likely require hemodialysis. I predict that the lower

access to healthcare and routine checkups are due to a variety of other factors that will be explored in this project. If the root cause of this issue is unveiled, then efforts could be made by healthcare policymakers, medical workers, and everyday American citizens to change this and create a more inclusive and equitable nation.

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