

Urinary Proteomic Analysis for Assessment of HIV Clinical Status

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Med-into-Grad Research Proposal
3/6/09

Background

In recent years, proteomic-based approaches have led to the discovery of numerous new biomarkers. Urine is an ideal biological sample for the discovery of new biomarkers due to the ease and noninvasive nature of collection. However, subsequent to collection, difficulties arise in utilizing this biologic sample for analysis. High concentrations of albumin, globulins, and interfering salts make it difficult to identify other proteins of interest. Recent improvements in fractionation techniques allow us to better exploit the urine fractions for study [1].

Diseases of the kidney readily show changes in the makeup of urine, with the classic example of diabetic nephropathy and albuminuria. Many other biomarkers more sensitive than albumin can be found in the urine for diabetes. Systemic diseases also may affect urinary makeup. They may do so by directly affecting the kidney, or by causing the amount of a marker molecule to increase to such a level in the plasma that it overcomes the ability of the tubules to reabsorb. The urinary profiles of these molecules/biomarkers may eventually be useful for diagnostic or therapeutic monitoring purposes.

Renal disease is a relatively common complication of HIV infection. Clinical manifestations include HIV associated nephropathy (HIVAN), IgA nephropathy, cryoglobulinemia, amyloidosis, and a lupus-like immune complex called glomerulopathy. The role of HIV in HIVAN has been thought to be direct infection of tubular and glomerular epithelium, possibly triggering apoptotic pathways. Inflammatory cytokines may also play a role in the pathogenesis of these renal processes. The advent of Highly Active Antiretroviral Therapy (HAART) has led to a marked decrease in the incidence of HIVAN.

Clinical management of HIV revolves around measurements of the patients' CD4 count and HIV viral load. Although long established, these measurements require regular blood draws, which are tiresome for patients, and not without risks for the phlebotomists. In the developing world, where most individuals currently living with HIV are located, these regular blood draws and laboratory tests are very resource intensive, and well beyond the financial means of many of the patients requiring them.

Open Question

We aim to determine if a urinary proteomic profile can be used as a predictor of the clinical state of an HIV infected individual.

Hypothesis

We hypothesize that the immunomagnetic evaluation of urinary exosomes will help 1) find urinary biomarkers that differ between HIV positive subjects and healthy controls, 2) determine differences in urinary levels of the inflammatory cytokines TNF alpha, IL-6, and CRP between groups, and 3) measure quantities of p24 antigen in the urine of HIV infected individuals.

Experimental Approaches and Alternatives

Specific Aim #1: To assess the differences in the proteomic profiles in each group of study subjects. Using our recently developed techniques to study urinary exosomes, we aim to create proteomic profiles of each of the 6 subjects in the three groups we are analyzing in the study. Using measures of protein concentration, we will identify 5 proteins whose levels differ most significantly between the untreated HIV positive subjects and those on therapy with viral suppression, and the untreated HIV subjects and the healthy controls. We will then attempt to identify these proteins using standard analytic techniques.

Specific Aim #2: To determine if the urinary levels of the inflammatory cytokines TNF alpha, IL-6, and C reactive protein differ between groups of subjects that are HIV positive and receiving therapy with viral suppression, are HIV positive but have not yet received therapy, and healthy controls. We will determine the levels of the above cytokines in the total urines of 6 subjects from each of the above groups, and compare the levels of these 3 inflammatory cytokines between the groups.

Specific Aim #3: To determine if p24 antigen can be detected in the urinary exosomes of subjects with HIV who are not yet receiving antiretroviral therapy. We will determine if subjects with HIV that are not yet receiving therapy have consistent levels of p24 antigen present within their urinary exosomes. We will then use the urinary exosomes of subjects with HIV who are currently on therapy with viral suppression to determine if p24 antigen is detectable in the exosome fraction.

In this prospective case-control study, we will collect urinary samples from six individuals with HIV not on therapy, six individuals with HIV currently on treatment with undetectable viral loads, and six healthy controls for analysis. Our goal will be to determine if the relative distributions of the inflammatory cytokines TNF-alpha, IL-6, IL-1, and potentially other metabolic markers will be present in higher concentrations within the exosomes of individuals with HIV who are not on effective therapy compared to those on therapy, and healthy controls.

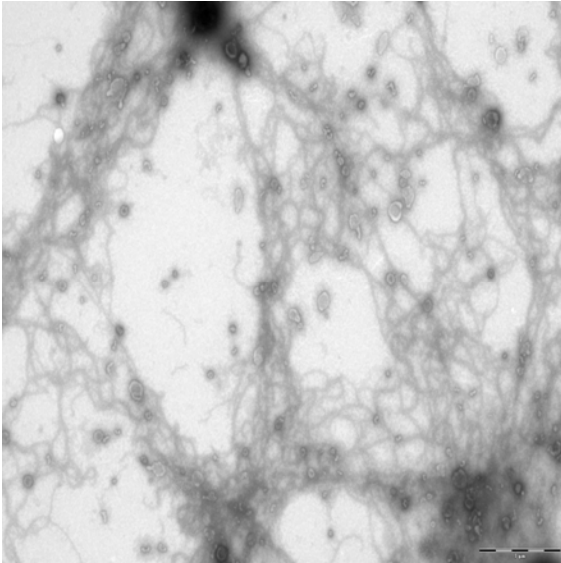
Subjects presenting to the Antiviral Research Center at UCSD for follow up for other studies will be presented with the option to donate urine for a small compensation. The study will be discussed in detail with the volunteer, and if informed consent is obtained, the individual will be asked to sign a consent form. Urine will then be collected from that individual and stored at -80C.

After transportation to Stein Clinical Research Building, the urine samples will be spun down, and the protein precipitated using a lab developed fractionation protocol for exosome isolation. An aliquot of the urine will be taken for analysis of its metabolite profile. Urines from healthy volunteers will also be obtained using the same consent method, and analyzed in the same way.

A profile of the proteins present in the subject's exosomes will be developed using two-dimensional gel electrophoresis as well as a gel-free method. After comparing the profiles of each of the study groups, most up/down regulated urinary proteins in exosomes derived from these patient samples will be subjected to liquid chromatography/tandem mass spectrometry to establish the identity of the proteins. This up/down regulation will be validated using non-proteomic techniques such as western-immunoblotting and ELISA for the protein under investigation. ELISA will also determine the relative concentrations of the protein(s) under investigation in normal and HIV/AIDS patient urine samples.

Electron Microscopy of Normal Male Human Urine Exosomes (13,500x)

[images taken in our lab]

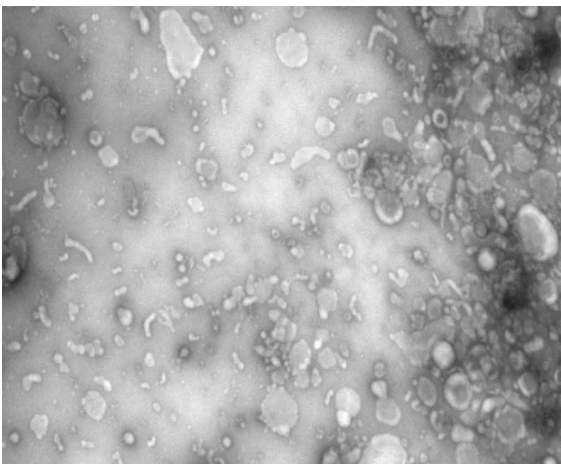


Male urine sample exosomes contain:

1. Membrane-bound particles 14-200 nm in diameter,
2. Filaments that are 7-10 nm in diameter and can form braided structures, with 2 or 4 single filaments wound around one another.

The filaments are polymers of Tamm-Horsfall protein and interact with the membrane-bound particles. The particle concentration is 1×10^{10} particles/ml in unconcentrated urine.

Electron Microscopy of Normal Female Human Urine Exosomes



Female sample contains:

1. Membrane-bound particles 10-300 nm in diameter
2. No filaments

References

1. Thongboonkerd, V., et al., *Proteomic analysis of normal human urinary proteins isolated by acetone precipitation or ultracentrifugation*. *Kidney Int*, 2002. **62**(4): p. 1461-9.