Are Changes in Renal Papillary Gene Expression the Cause or the Result of Stone Presence: A Chicken and Egg Analysis of Calcium Hydroxyapatite Kidney Stones

Introduction: Kidney stones affect approximately 5% of the American population each year and there is an approximately 12% likelihood that you develop a kidney stone during your lifetime. [1-2] Approximately half of these stones are entirely or in part made of a mineral called calcium hydroxylapatite (CHA). [3] This CHA appears commonly at 2 other locations within the kidney mineralization cascade. It is not yet known how these stones form. Many attempts have been made to work backwards from the tip of the renal pyramid to the cortex of the kidney in an attempt to follow mineralization upstream to its origin. This attempt to reduce the complexity of this biological system into a number of inputs that lead to downstream mineralization is not currently feasible as the technology has not progressed enough to provide researchers sufficient or accurate data. There are simply too many potentially influential unknown factors at play in CHA mineralization such as: pH, ion density and gradient, cell presence, gene expression, pressure, and architecture of different kidneys. Without a proper understanding of at least some of these factors it appears evident that it will be inordinately difficult to extract the nitus of the CHA mineralization.

Hypothesis: This research project will attempt to obtain a better understanding of how first time stone former's renal papillae evolve as a result of CHA stone presence.

Research Plan: To better understand if gene expression can be linked to the renal pyramid's stone presence, we will run both in-vivo (surgical) and ex-vivo (laboratory) studies of epithelial cells and H2K cells. The reason for running both in-vivo and ex-vivo studies is for the in-vivo data to better inform the accuracy of our ex-vivo model, and the ex-vivo model will be used to simulate a more controlled experimental environment. Epithelial cells and H2K are the cells on the surface of the papilla (there are 8-12 papilla's per kidney) and one or both of these cell types are believed to be the source of the mineralization that becomes a CHA kidney stone.

Our study will analyse 50 patients that currently have their first kidney stone during the 2020 calendar year. This first kidney stone must be of CHA composition as defined by a computed-tomography (CT) scan's mineral density reading, and of dimensions less than 5 millimeters across it's longest axis, as determined by CT-scan. Limiting the size of the stone will ensure that the surrounding tissue is less disrupted by the presence of the stone, because it is believed that stone size is related to mineralization time. Additionally, x-ray diffraction data will confirm that the mineral composition of this stone is predominantly CHA. Patients must be otherwise healthy and have no confounding kidney related diseases.

Selected patients must agree to three biopsies of their renal papilla. Patients will be biopsied prior to stone removal, adjacent to the stone, far from the stone on the same papilla and on a "healthy" papilla with minimal Randall's Plaque. The stone will then be extracted and be subject to x-ray diffraction for the aforementioned confirmatory reasons.

Using gel-electrophoresis on each biopsy, we can identify differences in gene expression between our "control" biopsy on the healthy papilla, and gene expression located adjacent and far from the stone.

For the laboratory portion of this study we will culture epithelial and H2K cells in petri dishes. Next, we will biopsy the developed cultures to obtain an ex-vivo control. This laboratory control will be compared with the in-vivo control to identify experimental accuracy with regards to gene expression. Then, we will take whole kidney stones from the above patients and set them into the cultured petri dishes. Finally, we will biopsy adjacent to the stone and at several radial distances from the stone.

We will then run gel electrophoresis on all of the petri biopsies and compare the resulting gene expression with that of the in-vivo gene expression. We expect to complete gene expression analysis near the end of the 2021 calendar year.

Anticipated Results: This research should be able to indicate if a change in gene expression is responsible for or the product of CHA stone formation. Future studies could then attempt to identify the trigger for that specific gene expression and potentially develop a treatment.

Conclusion: It is critical that this research be completed immediately to ensure a more rapid analysis and identification of the causes of CHA stone formation. Once gene expression is either ruled out or shown to be a factor in CHA stone formation, the entire field of kidney stone research can move closer to helping treat patients. This research has the potential to identify a cause for up to half of all kidney stones.

[1] Romero V, Akpinar H, Assimos DG. Kidney stones: a global picture of prevalence, incidence, and associated risk factors. *Rev Urol*. 2010;12(2-3):e86–e96.

[2] Tilahun Alelign and Beyene Petros, “Kidney Stone Disease: An Update on Current Concepts,” Advances in Urology, vol. 2018, Article ID 3068365, 12 pages, 2018. https://doi.org/10.1155/2018/3068365.

[3] Tilahun Alelign and Beyene Petros, “Kidney Stone Disease: An Update on Current Concepts,” Advances in Urology, vol. 2018, Article ID 3068365, 12 pages, 2018. https://doi.org/10.1155/2018/3068365.