Leveraging Machine Learning to Understand the Role of Trace Elements in Kidney Stone Pathogenesis for Enhanced Early Detection

Naina Kumar, Nishita Paruchuri
Louis J. Vaickus MD PhD, Joshua J. Levy PhD
Emerging Diagnostic and Investigative Technologies, Department of Pathology, Dartmouth Hitchcock Medical Center

Dartmouth Health

ABSTRACT

- Kidney stone disease affects 1/500 in US; costed $9 billion in 2021
- Crystallization of stones influenced by bioaccumulation of trace elements
- Role of zinc in stone formation is not widely understood
- Protein markers: TRPV4, Piezo1, MFN2, HIFα shown to be involved in stone formation
- Machine learning models can be used to analyze correlations between protein expression and zinc accumulation

INTRODUCTION

Trace Elements: Trace elements such as zinc (Zn), copper (Cu), nickel (Ni), aluminium (Al), strontium (Sr), cadmium (Cd) and lead (Pb) form poorly soluble salts with phosphate and oxalate ions and therefore play an important role in kidney stone formation

- influence the crystallization process by acting as inhibitors or promoters of crystal growth; directly acts on crystal surface
- Zinc plays an important role in nucleation yet its function as a promoter or inhibitor is not widely understood

Protein Markers: TRPV4, Piezo1, MFN2, HIFα play a role in kidney stone formation

- TRPV4 and Piezo 1 are transport ion channels that regulate the flow of calcium ions and respond to mechanosensitive stimuli; dysregulation can lead to build up of calcium within the kidney
- MFN2 is an essential protein in the function of mitochondria and can be linked to the active transport of ions like calcium
- HIFα is a protein activated under hypoxic conditions (generally present in the papillae), helping to reduce oxidative stress and potentially decrease kidney stone formation

Machine Learning: neural networks can predict protein expression from zinc intensity data to determine how zinc affects stone-forming pathways

- Multilayer Perceptron (MLP) is a Feedforward Neural Network with nonlinear activation functions to learn complex patterns in data
- Final layer - fully connected layer - has X output neurons where X is the number of protein markers

METHODS

Goal: develop ML/DL model to investigate correlation between bioaccumulation of trace elements (such as zinc) and protein expressions in stone formers vs. non-stone formers

Experimental Design:

- Data collected by Sunita Ho Lab at University of California San Francisco
- 8 patients: RG, CW, YH, KA, WF, WB, DP, BS
- Sections were stained with antibodies for TRPV4, Piezo1, MFN2, HIFα markers; same sections were stained for H&E
- Whole slide images (WSIs) with IHC stains were coregistered using VALIS with Piezo1 used as the reference image instead of H&E (due to poor quality)
- Reference image converted to .tiff and co-registered with Zn elemental map using TRACE to get zinc intensity data (y)
- Reference image (czi) divided into patches of size 500 using binary mask to separate background and foreground; coordinates of patches were used to retrieve corresponding protein marker expression data (X)
- Correlations performed using zinc intensity data and average nuclei intensity, positive cell count, % positive cell count (nuclei detection performed by lab)

Algorithmic Methods:

- ML Models: Leveraged 3-layer MLP and Random Forest with 100 estimators
- Statistical Models: Spearman's Rank Correlation, Robust Linear Regression, 0-inflated negative binomial

RESULTS

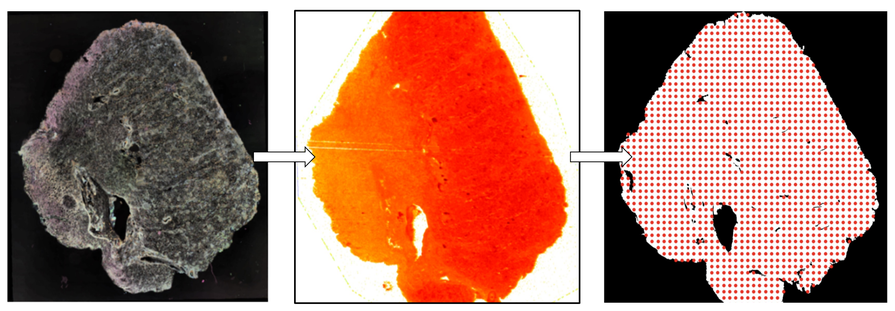

Figure 1: Image Processing

Table 1: Patient BS, Average Nuclei Intensity

Protein Marker	β	z	p
Piezo1	$\beta = 0.12$	$z = 2.91$	$p < .001$
MFN2	$\beta = -.63$	$z = -27.02$	$p < .001$
HIFα	$\beta = 0.18$	$z = 11.15$	$p < .001$

Table 2: Patient RG, Average Nuclei Intensity

Protein Marker	β	z	p
MFN2	$\beta = .19$	$z = 8.64$	$p < .001$
HIFα	$\beta = 0.65$	$z = 35.63$	$p < .001$
TRPV4	$\beta = 0.46$	$z = 46.82$	$p < .001$

Table 3: Patient DP, Average Nuclei Intensity

Protein Marker	β	z	p
Piezo1	$\beta = 3.65$	$z = 6.37$	$p < .001$
MFN2	$\beta = 2.05$	$z = 4.96$	$p < .001$
HIFα	$\beta = -0.18$	$z = -0.633$	$p = 0.527$
TRPV4	$\beta = -0.35$	$z = -5.32$	$p < .001$

Table 4: Mean Absolute Error (MAE) - Neural Network

Patient Name	Piezo1	MFN2	HIFα	TRPV4
BS	311.43	172.21	119.34	N/A
RG	374.92	361.69	165.05	92.52
DP	N/A	4967.757	5688.57	2236.251

- Patients DP and BS both had significant positive association between average zinc intensity and Piezo1 average nuclei intensity
- Associations for MFN2, HIFα, TRPV4 were mixed across patients
- Relatively high errors for MLP and Random Forest compared to the ranges of average nuclei intensity of proteins
- Random Forest produced higher MAE and higher R^2 values than MLP

DISCUSSION

Main Findings

- Zinc might act as agonist in sheer stress activated passive transport pathways that could lead to kidney stone formation
- Zinc's role in active transport, mitochondrial activity, and oxidative-stress pathways that could lead to kidney stone formation is not conclusive
- Mixed findings for associations and models could be a result of whole tissue analysis instead of zonal analysis and missing data

Limitations:

- Missing Zn elemental maps for patients CW, YH, KA
- Sections were cut at different locations for patient WF, resulting in misaligned stains
- Staining and cell detection inaccuracies

Future Directions:

- Perform zonal instead of whole-slide correlations
- Use Micro MS Software for nuclei detection
- Leverage Graph Neural Networks (GNNs)
- Expand existing work to other trace elements, such as Mn, Mg, Ni, K, Fe, Na, Se, As, Cu, Ca, and Cr

Potential for Clinical Impact:

- Understanding the role of trace elements in kidney stone formation will enable early detection and prevention
- Greater understanding of kidney stone pathogenesis

Data and Code Availability:

- Data/code available on reasonable request, privacy/ethical restrictions.

Acknowledgements: EDIT, Sunita Ho Lab at University of California San Francisco.

References: Available using QR code:

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