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Increased Urinary Leukocyte Esterase Distinguishes Patients With Brushite Kidney Stones

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Patients with brushite kidney stones have more severe renal papillary pathology than idiopathic calcium oxalate (CaOx) stone formers (SFs).[1] We have shown that papillary tissue from brushite but not CaOx SFs demonstrates abundant neutrophil activation including neutrophil extracellular trap formation, a neutrophil response to bacteria and other perceived pathogens.[2] This neutrophil infiltration may explain the increased scarring and inflammation observed in the papillae of brushite SFs.

Brushite stones have an increased risk of recurrence,[3,S1] are frequently large and bilateral,[4,S2] and require more stone removal procedures compared with CaOx stones.[5] Their prevalence is increasing, including among pediatric SFs.[6,S3] It would be useful to know whether a signal of the papillary inflammatory response in kidneys of brushite SFs could be detected in urine. Neutrophil proteins in urine, such as leukocyte esterase (LEU), are routinely measured by a urine dipstick. We investigated whether this signal of neutrophil elevation in the kidney could be detected in the urine of brushite SFs when not associated with infection.

RESULTS

Patients

We studied 812 24-hour urine samples collected from 215 SFs (Supplementary Table S1, Supplementary Methods).

Nitrite

Nitrite in urine may signal the presence of Gram-negative bacteria and infection. The stone type was not associated with positive urine nitrite when tested by the Pearson chi-square analysis (Supplementary Table S2).

LEU

We performed a Kruskal-Wallis test for LEU by stone type. The results were $H(3) = 83.8$, $P < 0.000001$, indicating that stone type populations have different distributions of LEU. The rank sums were 83,734, 107,123, 68,601, and 70,619 for apatite, brushite, CaOx, and uric acid SFs, respectively. The differences between groups are highly significant.

We performed analysis of variance of LEU in urine using 4 different models to look at the effects of covariates on LEU measurements (Table 1). In model 1, adjusted for sex and age, LEU differed significantly by stone type and sex. Brushite SFs had significantly higher urinary LEU than other SFs. Females had higher LEU than males (0.57 ± 0.04 vs. 0.28 ± 0.03, $P < 0.00001$), but the cross product between stone type and sex was not significant.

In model 2 (Table 1), we adjusted model 1 further for other dipstick measurements that could be associated with possible infection such as blood, protein, or nitrite. Brushite and uric acid SFs had significantly more blood in their urine than apatite or CaOx SFs (Supplementary Table S3). Uric acid SFs had elevated urine protein compared with all other groups (Supplementary Table S3). The analysis of variance results were essentially the same as for model 1, except apatite SFs had more LEU than both uric acid and CaOx SFs. Blood, protein, nitrite, and age were all significant in this model at $P < 0.05$.

Model 3 (Table 1) added adjustment for urine ammonium. High urine ammonium can indicate infection by urea-splitting bacteria. Urine ammonium was

Table 1. Analysis of variance of urine leukocyte esterase by stone type

Model	Stone type				*P* values			R^2
	Apatite	Brushite	CaOx	UA	Stone type	Sex	Stone*sex	
1	0.44 ± 0.05[a]	0.77 ± 0.05[b]	0.23 ± 0.05	0.25 ± 0.06	<0.000001	<0.000001	0.51	0.134
2	0.46 ± 0.05[a,c]	0.77 ± 0.05[b]	0.29 ± 0.05	0.17 ± 0.06	<0.000001	<0.000001	0.18	0.259
3	0.47 ± 0.05[a,c]	0.76 ± 0.05[b]	0.28 ± 0.05	0.18 ± 0.06	<0.000001	<0.00001	0.30	0.269
4	0.45 ± 0.05[c]	0.72 ± 0.05[b]	0.32 ± 0.05	0.17 ± 0.06	<0.000001	<0.00001	0.54	0.256

CaOx, calcium oxalate; UA, uric acid.
[a]$P < 0.05$ versus CaOx.
[b]$P < 0.001$ versus other stone types.
[c]$P < 0.001$ versus UA.
Values are mean leukocyte esterase ± SEM. Model 1 adjusted for sex and age. Model 2 adjusted for sex, age, nitrite, blood, and protein. Model 3 adjusted for sex, age, nitrite, blood, protein, and urine ammonium. Model 4 adjusted for sex, age, nitrite, blood, protein, ammonium, and 24-hour urine volume.

not higher in brushite SFs than in other SFs (35.3 ± 1.2, 32.0 ± 1.2, 33.4 ± 1.3, and 34.8 ± 1.6 mmol/l for brushite, apatite, CaOx, and uric acid SFs, respectively; P = not significant for all comparisons). Blood, protein, nitrite, age, and ammonium were all significant in this model at $P < 0.05$, but the addition of ammonium only slightly increased the R^2 to a final value of 0.269. Urea-splitting bacteria are unlikely to be the cause of the increased LEU in the urine of brushite SFs.

Model 4 (Table 1) further adjusted for the 24-hour urine volume. The results were essentially the same as the previous models. The increased LEU concentration in the urine of brushite SFs cannot be explained by the differences in the urine volume (Supplementary Table S1).

Stone Removal Procedures

Procedures to remove stones can injure the kidney and may lead to the infiltration of neutrophils. Brushite SFs had significantly more procedures than other SFs (Supplementary Table S4). To evaluate the contribution of stone removal procedures to urinary LEU, we performed analysis of variance of the mean LEU adjusted for sex, age, LEU, blood, protein, nitrite, ammonium, and urine volume as well as the total number of each of 5 procedures. LEU was significantly different by stone type and sex ($P < 0.05$ for each), but the cross product between stone type and sex was not significant ($P = 0.80$). Brushite SFs had higher urinary LEU than CaOx SFs, whereas all other comparisons between stone types were insignificant (Figure 1). Other significant contributors to the model at $P < 0.05$ were blood, nitrite, ammonium, and the number of cystoscopies. The R^2 for the model was 0.402.

DISCUSSION

Urine LEU was higher in brushite SFs compared with CaOx SFs. Nitrite and ammonium were not different between stone types, making infection by Gram-negative or urea-splitting bacteria unlikely as the cause of increased LEU. Adjusting the analysis of variance models for ammonium, nitrite, and other possible indicators of infection, such as blood and protein as well as the number of stone removal procedures, did not abolish this increase.

We have found that increased neutrophil infiltration and neutrophil extracellular trap formation in the renal papillae of brushite SFs differentiate them from CaOx SFs.[2] Brushite SFs also had increased neutrophil markers in stone matrix compared with CaOx SFs. Our work here showing elevated urine LEU in brushite versus CaOx SFs echoes these findings.

Others have used dipstick LEU to detect neutrophil infiltration and predict infection in other disease conditions such as cirrhosis (ascites fluid)[7] and joint infection (synovial fluid).[8] High fecal neutrophil levels have been detected in inflammatory bowel disease, and LEU activity in stool has been investigated as a promising biomarker for monitoring inflammatory bowel disease status.[9] Similarly, our results show that urine dipstick LEU may be a biomarker of inflammatory

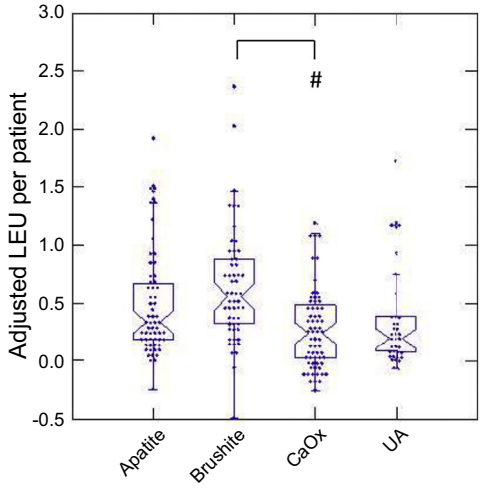

Figure 1. Dipstick leukocyte esterase (LEU) by stone type. A notched box plot of the results of analysis of variance fully adjusted for sex, age, mean blood, protein, nitrite, ammonium, urine volume, and number of stone removal procedures. The narrowing of the notch represents the median and the width of the notch the 95% confidence interval of the median. Dots are individual patients. #$P < 0.05$. CaOx, calcium oxalate; UA, uric acid.

activity of neutrophils in the kidney rather than infection per se.

The differences in urine LEU between stone types are striking and reflect what is known about renal histopathology in the various stone types. CaOx SFs produce interstitial apatite particles that form Randall's plaques but do not damage epithelial cells or cause interstitial inflammation or fibrosis.[S4] Uric acid SFs form plaque and have crystal deposits in the inner medullary collecting ducts and the ducts of Bellini but with generally absent or mild interstitial fibrosis.[S5] In contrast, brushite SFs have severe collecting duct injury and interstitial fibrosis along with prominent cortical fibrosis and tubule atrophy.[S6] Hydroxyapatite SFs have a somewhat intermediate phenotype with numerous intratubular crystal deposits that can lead to some papillary damage, but tubular atrophy and interstitial fibrosis are uncommon.[S6] The amount of papillary damage in each of these stone types is mirrored by the amount of urinary LEU, reinforcing the idea that urine LEU is a marker of inflammatory conditions in the kidney.

This study has some limitations. It is a retrospective study, so the cause of elevated LEU in the urine of brushite SFs cannot be determined, only the association. It is impossible to rule out the presence of infection in all cases, although we have tried to adjust for infection with measurements of nitrite and ammonium. Infection by organisms that are Gram positive but not urea splitting would not be detected by these assays. Likewise, it is impossible to rule out the contribution of blood in the urine as a source of LEU, although we adjusted for it statistically.

In conclusion, we have demonstrated the presence in urine of a brushite-specific molecular signature of neutrophil activation that aligns with the pathogenesis of brushite stone formation in the kidney. Our results suggest that urine dipstick LEU is informative in SFs aside from predicting infection and may serve as a biomarker of renal histopathology.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Table S1. Patients.

Table S2. Urine nitrite.

Table S3. Urine blood and protein.

Table S4. Stone removal procedures per patient.

Supplementary References.

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