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Determination of urinary stone composition using biochemical analysis of fluid samples taken during ureterorenoscopic laser lithotripsy

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Abstract

Purpose The present study aims to biochemically analyze the fluid samples containing stone dust taken during the perioperative period to determine the role of fluid in the prediction of stones in patients treated with ureterorenoscopic procedures. Our secondary aim is to investigate the role of both fluid analysis and stone analysis in predicting the results of the metabolic analysis.

Methods Comparative analyses were performed using fluid samples containing stone dust from 93 patients. Biochemical analysis of fluid containing stone dust was conducted; stone fragments were examined at a separate location using X-ray diffractometry(XRD). Metabolic analysis was performed to patients who provided stone-free status 1 month later. The results of chemical analysis were compared with the results of the XRD analysis.

Results Patients' stone type was determined with high accuracy using biochemical analysis. Differences were noted in ten patients following biochemical analysis and XRD analysis. Biochemical analysis predicted metabolic disorders in more patients than XRD analysis, particularly for those patients with multiple stone compositions. However, no significant differences between the results of biochemical and XRD analysis methods were found ($\kappa = 0.27$; $p = 0.002$). Moreover, biochemical analysis results revealed metabolic disorders in five patients; these findings were missed by XRD analysis.

Conclusion Biochemical analysis of fluid taken perioperatively during ureterorenoscopic laser lithotripsy to treat urinary system stone disease was found to determine stone composition with high accuracy. Biochemical analysis of fluid samples taken during the perioperative period is, thus, an easy, reliable and cost-effective test to assess stone composition in patients undergoing ureterorenoscopic procedures.

Keywords Ureteroscopy · Laser lithotripsy · Stone dust · Renal calculus biochemistry · X-ray diffractometry

Introduction

In recent years, laser lithotripsy with flexible ureterorenoscopy (fURS) has been applied to upper urinary system stones for most patients, alongside contemporaneous developments in technology, such as laser devices, and increased surgical experience. In ureterorenoscopic surgery of the upper urinary tract, stones are fragmented and made millimetric to ensure 'stone-free status'. Therefore, following a ureterorenoscopic approach, stones that can be analyzed cannot be obtained in most of the patients [1].

The stone recurrence rate is 50% in 5–7 years after a patient is determined to have 'stone-free status' [2]. To prevent recurrence, metaphylaxis is recommended, according to a complete urine metabolic evaluation [3]. In a urinary metabolic evaluation, stone analysis is essential [4, 5]. Stone analysis, in a sense, has characteristics of a biochemical biopsy in the urinary system as the detection of special stone components can occasionally indicate a specific metabolic disease [6]. In recent years, studies on predicting of stone composition have been increasing [7, 8].

The gold-standard technique of stone analysis is X-ray diffractometry (XRD) and infrared spectroscopy (IRS). While biochemical analysis provides information about stone composition, it does not provide information regarding

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stone structure. However, it is sufficient to understand the stone composition at the metabolic evaluation required for stone metaphylaxis for most urinary systems stones. In this study, it was hypothesized that stone composition results could be obtained using biochemical analysis of fluid, containing stone dust, taken during the operation in ureterorenoscopic stone surgery, without the need for a stone fragment. Further, the relationship between biochemical analysis and metabolic analysis was investigated.

Materials and methods

This study was prospectively designed and approved by the Ethics Committee of the Faculty of Medicine at Uludag University (06/07/2017). Funding was given from Uludag University Scientific Research Projects Unit (Project No: HDP(T) 2017/43) to conduct this study.

A total of 109 consecutive patients, who underwent ureterorenoscopic laser lithotripsy due to renal or ureteral stones, were included in this study between the 31st July 2017 and 31st December 2017. Only retrograde intrarenal surgery (RIRS) was carried out for 85 patients, while combined ureterorenoscopy (URS) and RIRS were carried out in 24 patients. Stone dust samples were taken from all patients; stone fragments could not be obtained from 16 patients due to ureter stenosis. Comparative analyses of the 93 patients with stone fragments and fluid containing stone dust were undertaken. Pure stones (stones that included no more than a single stone composition) and non-pure stones (stones that included more than one stone composition) were stratified, and the compatibility between the two methods was assessed. Patients had a kidney, ureter and bladder (KUB) X-ray and non-contrast computed tomography (NCCT) scan preoperatively. Fluid containing stone dust samples and stone fragments was taken perioperatively from patients.

This study was double-blind; two laboratories were used for biochemical analysis of the fluid containing stone dust and the analysis of stone fragments using an XRD method. Both centers were blinded to each other's results.

Surgical technique

At the end of the ureter or kidney stone operation, the fluid containing the stone dust was aspirated with a 10-ml syringe; a 3–4-mm fragment was removed from the collector system with a basket catheter. We did not use a ureteral access sheath (UAS). A Double J (DJ) stent was placed and a Foley catheter was inserted. The Stonelight™ 30, Holmium–YAG laser and 273-micron laser probe were used for the laser lithotripsy. A 7.5 Fr Richard Wolf™ was used as the semi-rigid ureterorenoscope, while a Flex X^2 Karl Storz™ was used as the flexible URS. The fluid containing stone dust, taken perioperatively, was evaluated using biochemical analysis in a biochemistry laboratory. The stone fragment taken at the end of the operation was examined using XRD method.

Stone analysis using a biochemical method

Qualitative, colorimetric determination of carbonate, calcium, magnesium, ammonium, oxalate, phosphate, uric acid and cystine was completed for urinary system stones by visual inspection [9]. To accomplish this outcome, a small quantity of urinary system stones is shattered to dust. Different rates were added to different solutions; the chemical reactions that develop allow qualitative means of identifying the presence or absence of individual components. This method enables identification (presence or absence) of eight components of urinary calculi. All analyses were verified with reference results [9].

Stone analysis using an X-ray diffractometry method

XRD is a technique used for identification of minerals with a small particle size that cannot be assessed using optical microscopy due to their crystalline structure and properties. The sample was ground until the ideal grain size was reached; the powder was analyzed using the Bruker D8 Advance, Panalytical X'Pert Powder and Philips PW 1830 ™ [10]. This technique is based on the principle that X-rays with angstrom-size wavelengths can enter atomic planes, forming the surfaces of the crystals (inside the crystal lattice) and reflect diffractively through crystal surfaces (Bragg Equation). Minerals can be defined by determining the unit-cell parameters (d values) that compose the fingerprints of each mineral crystal [11]. In addition to natural minerals, urinary system stones were examined using this XRD method. Powdered samples were analyzed between 2° and 40° using devices with Ni filter and Cu X-ray tube, and so the type of urinary system stones was determined [10].

Statistical analysis

Patient age, stone size and stone density were reported with mean and standard deviation values. The concordance between the results of the biochemical and XRD analysis was examined with Cohen's kappa coefficient. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy rates were reported. SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) was used for statistical analysis; the level of significance was set at $\alpha = 0.05$.

Metabolic analysis

Metabolic analysis was performed to patients who provided stone-free status 1 month later. Three months after the operation, stone-free status was checked using non-contrast computed tomography. All of the patients included in our study were able to obtain stone dust containing fluid, but the stone fragment could not be obtained from all patients. Therefore, at first, we targeted the patient group on whom we performed metabolic analysis to obtain stone dust that contained fluid and also stone fragments. Unfortunately, 28 of the patients could not come for the metabolic analysis because they were living away from the hospital. Independent of the operation, three patients died after 2 months and stone-free status was not provided in two patients. Thus, metabolic analysis was performed on 60 patients.

The parameters we looked at in metabolic analysis were as follows:

(1) blood tests: calcium (ionized), albumin, creatinine, uric acid, potassium, PTH, C-reactive protein, blood cell count and blood gas analysis,
(2) 24-h urine measurement: urine volume, pH, specific weight, uric acid, creatinine, calcium, magnesium, inorganic phosphate, sodium, potassium, oxalate, citrate, cystine, ammonium.

Results

93 patients participated in this study. The mean age of patients was 51.4 ± 18.2 years; 59 patients were males. The mean stone size was 15 ± 6.3 mm and mean stone density was 1085.9 ± 273.2 HU. Stones were non-opaque, semi-opaque and opaque in 9, 15 and 69 patients, respectively. One patient presented with a horseshoe kidney anomaly, while another patient presented with a solitary kidney. No perioperative complications occurred in any of the patients. However, postoperative fever (Clavien grade 2) was present in two patients. Comparison of the results of biochemical analysis of the fluid containing stone dust and the results of stone fragment analysis using XRD method is shown in Table 1.

Biochemical analyses of the fluids containing stone dust determined struvite stones, calcium oxalate stones and uric acid stones with accuracies of 100%, 99%, and 99%, respectively, according to the analyses using XRD method. Biochemical analyses of the fluids containing stone dust assessed mixed composition stones containing calcium

Table 1 Comparative results and sensitivity (Sens.), specificity (Spes.), positive predictive value (PPV), negative predictive value (NPV) and accuracy rate of the biochemical analysis of fluid containing stone dust

Stone analysis results with XRD		Biochemical analysis results	Success rate	Patients who have different results from XRD method with biochemical analysis	Sens. (%)	Spes. (%)	PPV (%)	NPV (%)	Accuracy rate
Calcium oxalate (pure)	64	63	63/64	In addition to CaOx stone, extra infection stone was detected in one patient	98	100	100	97	99
Calcium oxalate + calcium phosphate	13	7	7/13	Extra uric acid stone was detected in four patients and extra calcium carbonate stone was detected in two patients	54	100	100	93	94
Uric acid (pure)	4	3	¾	In addition to Uric acid stone, extra CaOx stone was detected in one patient	100	99	75	100	99
Calcium oxalate + uric acid	10	8	8/10	Extra cystine stone was detected in one patient and extra infection stone was detected in one patient	80	100	100	98	98
Struvite (pure)	2	2	2/2	–	100	100	100	100	100
Pure stones	70	68	68/70		97	100	100	92	98
Non-pure stones	23	15	15/23		65	100	100	90	90

oxalate and uric acid, mixed composition stones containing calcium oxalate and calcium phosphate with accuracies of 98% and 94%, respectively, according to analyses using XRD method. When analysis for pure and non-pure stones was undertaken, pure stones were identified with 98% accuracy, while non-pure stones were identified with 90% accuracy (Table 1). The results of the stone dust biochemical analysis and XRD analysis were found to be highly compatible based on Cohen's kappa Coefficient (Table 2).

Table 2 Compatibility of the results of biochemical analysis of fluids containing stone dust with the results of analysis using XRD method

XRD method and biochemical analysis	κ	p value
Calcium oxalate (pure)	0.98	< 0.001
Calcium oxalate + calcium phosphate	0.67	< 0.001
Uric acid (pure)	0.85	< 0.001
Calcium oxalate + uric acid	0.88	< 0.001
Struvite (pure)	1.00	< 0.001
Pure stones	0.95	< 0.001
Non-pure stones	0.74	< 0.001

κ cohen's kappa coefficient

The XRD stone analysis method and biochemical analysis were compatible with metabolic analysis; no significant difference was found between the tests ($\kappa = 0.27$; $p = 0.002$). Of note, 24 patients had metabolic disorders; the disorders of 18 of these patients were consistent with their XRD results, while 23 were consistent with the results of biochemical analysis. For five of the ten patients whose biochemical analysis results were considered incorrect based on the XRD method, the metabolic analysis supported that the stone types found in the biochemical analysis may, indeed, be present in the patient (Table 3). The remaining five patients presented with normal metabolic analysis. Thus, it is difficult to declare the results of the biochemical analysis are incorrect.

Discussion

This study investigated the role of biochemical analysis of the fluid containing stone dust that was taken perioperatively in the prediction of stone types in ureterorenoscopic stone surgery. Further, this study evaluated the relationship between biochemical stone analysis and metabolic disorders. The gold-standard method for stone analysis includes IRS

Table 3 Incompatible results between the stone analysis with XRD method and biochemical analysis, properties of these patients, and metabolic analysis results

Patient number	Stone analysis using XRD method	Biochemical analysis	NCCT (HU)	Opacity	Preop. UTI history	Stone size (mm)	Metabolic analysis result
1	Brushite + whewellite + hydroxylapatite	Calcium oxalate, phosphate, **uric acid**	1050	Opaque	Not available	7	Hyperuricemia + **hyperuricosuria**
2	Uric acid + weddellite + whewellite	Calcium, oxalate, uric acid, **cystine**	920	Non-opaque	Not available	30	**Cystinuria** + hyperuricosuria
3	Uric acid	**Calcium oxalate**, uric acid	850	Semi-opaque	Not available	14	**Hypercalciuria** + hyperuricosuria
4	Uric acid + whewellite	Calcium, oxalate, uric acid, **carbonate**, **ammonium (infection stones)**	720	Semi-opaque	**Available**	15	Normal
5	Whewellite	Calcium oxalate, **phosphate**, **ammonium (infection stones)**	1550	Opaque	**Available**	9	Normal
6	Whewellite + hydroxylapatite + weddellite	Calcium, oxalate, phosphate, **uric acid**	750	Non-opaque	Available	13	Hypercalcemia + **hyperuricosuria**
7	Whewellite + weddellite + whitlockite	Calcium, oxalate, uric acid	730	Non-opaque	Not available	12	Normal
8	Whewellite + Weddellite + Hydroxylapatite	Calcium, oxalate, phosphate, carbonate	1570	Opaque	Not available	25	Normal
9	Whewellite + weddellite + hydroxylapatite	Calcium, oxalate, phosphate, **uric acid**	890	Semi-opaque	Available	15	Hypercalciuria + **hyperuricosuria**
10	Whewellite + weddellite + hydroxylapatite	Calcium, oxalate, phosphate, carbonate	940	Semi-opaque	Not available	18	Normal

Bold indicates the extra findings that obtained in biochemical analysis and the findings that supporting them in metabolic analysis

XRD X-ray diffractometry, *NCCT* non-contrast computed tomography, *HU* Hounsfield units, *Preop* preoperative, *UTI* urinary tract infection

and XRD [4]. However, biochemical stone analysis, another stone analysis method, provides information regarding the biochemical content of the stone but does not provide information about the structure [12]. Alternative methods were investigated for stone analysis in patients whose stone fragment could not be obtained after surgery. In the determination of the stone composition after ureterorenoscopic laser lithotripsy, the spectroscopic (IRS) examination of the fluid containing the stone dust, taken perioperatively, was defined as a rapid and highly sensitivity method in detecting the stone types [1, 8]. However, this method is expensive and does not exist in even large health centers mostly. Like the IRS, XRD is also expensive and not available in many centers. For biochemical analysis, laboratory conditions is present in almost every hospital but XRD is performed in only very few special centers. Therefore, more applicable and accessible methods are needed to obtain the stone analysis. Thus, the examination of stone dust with biochemical analysis was advantageous in terms of accessibility as it is undertaken under basic laboratory conditions. Although biochemical analysis provides information about stone composition, which is the basis of metabolic evaluation, there is no study showing that biochemical analysis of the fluid containing stone dust, taken perioperatively, is reliable.

Obtaining a fluid containing stone dust is much easier than obtaining a stone fragment and can easily be obtained from all operated patients. The fluid containing stone dust is taken quickly by aspirating from the channel of the URS and FURS with the help of an injector during operation, but it is necessary to use a basket catheter to obtain the fragment, which sometimes causes the operation time to prolong, or it may cause jam in of the fragment taken in the narrow ureter segment, creating a risk of complications.

There is no difference in cost between conventional chemical analysis and biochemical analysis of fluid containing stone dust. However, in comparison with XRD, biochemical analysis of the fluid is both more economical and more accessible method. Furthermore, biochemical analysis laboratory conditions are present in almost every hospital, but XRD is performed in only very few special centers.

Ray et al. analyzed and compared both stone fragments and fluid containing stone dust using the IRS method [8]. Their study included 97 patients; however, fluid containing stone dust could only be obtained from 67 patients, thus limiting the comparison. In the present study, the fluid containing stone dust was obtained from all patients; thus, the comparative analyses were able to be performed with 93 patients. This situation could be an advantage to the biochemical analysis of fluid containing stone dust when compared to analyses using XRD and IRS methods that evaluate stone fragments. Only 16 patients, whose stone fragment was not obtained because of ureteral stenosis, were excluded from this study. In our research, we consciously did not collect stone fragments from these patients because ureteric stenosis was observed in the 16 patients during the insertion of URS. We faced with difficulties, particularly narrow segment, during the insertion of the URS. If we had used the basket for the stone fragments when passing the narrow segment, this action might have damaged the ureter. Thus, we did not prefer to obtain a stone sample.

Although using ureteral access sheath (UAS) could help to obtain the stone fragments with a basket catheter, we did not prefer to use UAS because previous studies showed that using UAS may cause some important problems. For example, in Lallas et al.'s study with a swine animal model, the size of UAS was 12/14 Fr and 14/16 Fr. The ureteral perfusion reduced [13]. In Traxer et al.'s study, the size of UAS was 12/14 Fr and their findings showed that 13.4% serious acute ureteral wall injury was present [14]. In Lildal et al.'s research, there was slight ureteral wall injury independent of prestenting when compared to the patients on whom UAS was not used with the patients who used UAS. In other words, the patients who used UAS had more ureteral wall injury [15]. Also, late period results of the use of ureteral access sheath are unknown.

Stone composition is the basis for the diagnosis and treatment of stone disease. Kidney stones are often seen as mixed composition stones, containing more than one component [16]. If a stone sample is desired when using laser lithotripsy, fragmenting or dusting causes small stone fragments. Therefore, it is often not possible to obtain and analyze the whole stone in ureterorenoscopic laser lithotripsy, unlike open or laparoscopic stone surgeries. In these cases, the fragment obtained with ureterorenoscopic laser lithotripsy can lead to incomplete results as the fragment may not represent all stone subtypes in the patient. Contrastingly, samples taken for biochemical analysis may represent the stone(s) in the patient with greater accuracy as they contain dust of multiple parts of the stone(s).

In the present study, a high rate of success in finding single stones was evidenced with biochemical analysis, as compared to the XRD method (Table 1). Following comparison of the XRD method and biochemical analysis, the metabolic analyses were performed for 60 patients, including ten patients whose XRD method and biochemical analysis results differed. The results of ten patients who were detected extra stone type in the biochemical analysis were considered to be incorrect. However, as a result of the metabolic analyses, the results of these ten patients were consistent with the results of the metabolic analysis. The fact that the results of the metabolic analysis in multi-subtype stones are consistent with the results obtained using the biochemical analysis supports that biochemical analysis is a reliable method for clinical use.

Table 3 summarizes results of a detailed evaluation of patients whose biochemical analysis results differed from the

XRD method. As shown in patient 3, the XRD method identified a uric acid stone, while biochemical analysis revealed both a calcium oxalate stone and uric acid stone. When the NCCT, obtained preoperatively, is taken into consideration, stone density was 850 HU, and hypercalciuria was found in the metabolic analysis. This supports that the stone may be a mixed composition stone and may contain calcium oxalate. In patient 2, while a mixed composition stone result was obtained using the XRD method, a cystine stone was found in biochemical analysis. A high level of cystine in urine was detected in this patient's metabolic analysis. The opacity of the stone was found to be very low in imaging. These supporting that the patient had a cystine stone. Similarly, a mixed composition stone was detected using XRD in patients 1, 6 and 9; however, the uric acid stone composition was also detected using biochemical analysis. When the metabolic analysis of these patients was performed, hyperuricosuria was detected, supporting the presence of uric acid stones. In patients 4 and 5, XRD results were reported as mixed composition stones, but biochemical analysis revealed extra ammonium, carbonate (infectious stone) and ammonium, phosphate (infectious stone). Although metabolic analysis did not provide additional information in these patients, the presence of UTI (Urinary Tract Infection) history in preoperative periods supported the possibility of infectious stone in these patients. As a result, the data supported that the extra detected stone composition using the biochemical analysis could be present in patients. This situation may arise because while RIRS or URS are performed, the outer layers of the stones become more powdery and fragments break away from the central parts. Typically, fragments of the outer layers that were formed by epitaxy could not have taken. In consequence, the XRD method may be inadequate to detect different types of stone combinations as the fragments examined with XRD may be based on a less representative stone sample as compared to the stone dust examined using biochemical analysis. The definitive decision on this topic can be made through the biochemical analysis of the fluid containing stone dust; adequate amounts and appropriate fluids containing stone dust can be analyzed using the XRD method, and the results are compared. At present, in addition to the XRD method, the biochemical analysis of fluid containing stone dust may be an important complementary assessment for more accurate evaluation in patients with stone, as compared to analysis with the XRD method alone.

There are limitations in this study. Although the metabolic analysis was undertaken for 65% of the patients, metabolic analysis was performed in all patients who had a difference between their XRD and biochemical analysis results. Other limitations to this study include that differentiation calcium oxalate monohydrate and dihydrate is not possible with the biochemical analysis, and also information about stone structure and possible stone formation mechanisms is not provided by this particular method. Larger series of studies are needed as some stone types are a small number of and metabolic analysis is not performed in all patients. This may be an important avenue for future research.

Conclusions

In the treatment of urinary system stone disease with ureterorenoscopic laser lithotripsy, the biochemical analysis of the fluid containing stone dust obtained perioperatively determines stone composition with high accuracy and is compatible with metabolic analysis. Therefore, biochemical analysis of such fluid is recommended. This is a simple, cost-effective and reliable method for this patient group.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in our study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Ethics Committee of the Faculty of Medicine at Uludag University (Verdict Number: 2017-11/31).

Informed consent General consent for data usage was obtained from all individual participants included in this study.

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