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GENERAL UROLOGY

Original Article

Laser-induced breakdown spectroscopy is a reliable method for urinary stone analysis

Lazer kaynaklı dağılım spektroskopisi üriner sistem taşlarının analizinde güvenilir bir yöntemdir

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ABSTRACT

Objective: We compared laser-induced breakdown spectroscopy (LIBS) with the traditionally used and recommended X-ray diffraction technique (XRD) for urinary stone analysis.

Material and methods: In total, 65 patients with urinary calculi were enrolled in this prospective study. Stones were obtained after surgical or extracorporeal shockwave lithotripsy procedures. All stones were divided into two equal pieces. One sample was analyzed by XRD and the other by LIBS. The results were compared by the kappa (ϰ) and Spearman's correlation coefficient (rho) tests.

Results: Using LIBS, 95 components were identified from 65 stones, while XRD identified 88 components. LIBS identified 40 stones with a single pure component, 20 stones with two different components, and 5 stones with three components. XRD demonstrated 42 stones with a single component, 22 stones with two different components, and only 1 stone with three different components. There was a strong relationship in the detection of stone types between LIBS and XRD for stones components (Spearman rho, 0.866; $p<0.001$). There was excellent agreement between the two techniques among 38 patients with pure stones (κ index, 0.910; Spearman rho, 0.916; $p<0.001$).

Conclusion: Our study indicates that LIBS is a valid and reliable technique for determining urinary stone composition. Moreover, it is a simple, low-cost, and nondestructive technique. LIBS can be safely used in routine daily practice if our results are supported by studies with larger numbers of patients.

Keywords: Laser spectroscopy; stone analysis; urinary calculi; X-ray diffraction

ÖZ

Amaç: Üriner sistem taş analizinde, yeni bir yöntem olan lazer kaynaklı dağılım spektroskopisini (LKDS) geleneksel olarak kullanılan X ışını toz kırınım (XTK) tekniği ile karşılaştırmak.

Gereç ve yöntemler: Toplamda 65 hasta bu prospektif çalışmaya dahil edildi. Taşlar hastalardan cerrahi ya da şok dalga tedavisi sonrasında elde edildi. Tüm taşlar 2 eşit parçaya ayrıldı. Parçalardan biri LKDS, diğeri ise XTK ile analiz edildi. Sonuçlar kappa (ϰ) ve Spearman korelasyon katsayı testleri kullanılarak karşılaştırıldı.

Bulgular: LKDS yöntemi ile 95, XTK tekniği ile 88 taş bileşeni saptandı. LKDS yöntemi ile 40 taş tek bileşenli, 20 taş iki bileşenli, 5 taş ise üç bileşenli olarak tanımlandı. XTK tekniği ile 42 taş tek bileşenli, 22 taş iki bileşenli ve sadece 1 taş üç bileşenli olarak tanımlandı. Taş tiplerini belirleme açısından LKDS ile XTK testleri arasında güçlü bir ilişki vardı. (Spearman korelasyon katsayısı=0,866; $p<0{,}001$). Her iki teknikte de tek bileşen saptanan 38 taş için yapılan karşılaştırmada, her iki teknik arasında mükemmel bir uyumluluk olduğu gözlendi (κ indeksi=0,910; Spearman korelasyon katsayısı=0,916; $p<0{,}001$).

Sonuç: Çalışmamız LKDS'nin üriner sistem taş analizinde geçerli ve güvenilir bir yöntem olduğunu göstermektedir. Bunun yanı sıra, daha basit, ucuz ve tahrip edici olmayan bir tekniktir. Daha fazla hasta sayısının olduğu çalışmalarla da desteklendiği takdirde, LKDS üriner sistem taş analizi için günlük pratikte güvenle kullanılabilir.

Anahtar kelimeler: Lazer spektroskopi; taş analizi; üriner sistem taşları; X ışını toz kırınım

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Introduction

Urolithiasis is a painful and recurrent urological disorder with considerable morbidity worldwide. Its prevalence is 1- 5% in Asia, 5- 10% in Europe, and 13% in Northern America [1] Urolithiasis is common in Turkey, and it affects almost 15% of the population.[2] Treatment requires different surgical techniques, such as extracorporeal shockwave lithotripsy (ESWL) or medical management alternatives, but the recurrence rate may reach 50% during the 5-year follow-up period if appropriate stone analysis and management are not carried out.[3] Determination of the urinary stone composition is important to establish the etiology of stone disease, to select the most appropriate treatment, and to prevent recurrence of urolithiasis. After surgery or ESWL, removed stones can be analyzed by various techniques to identify their constituents. Determination of the chemical composition of urinary calculi has been studied since 1950s to overcome problems associated with recurrence. Various techniques can be used for this purpose. Chemical analysis, X-ray diffraction (XRD), and infrared spectroscopy (IRS) have been evaluated and recognized as useful techniques in the analysis urinary calculi. [4-6] However, in the 1990s, XRD and IRS were conclusively preferred over chemical analysis, which is now considered obsolete. [7,8] IRS is used to examine the molecular structure of the stone, while XRD is used to detect the crystalline structure of a substance. XRD requires technical skill, stone preparation, and use of X-rays and can be used in only a small number of specialized laboratories (5–9%).[7,9] Additionally, IRS has been used in clinical chemistry, whereas it often provides a complex spectra with contributions from a sizeable number of unknown interfering substances.[10] Especially, in mixed type urinary stones , since increasing the calcium concentration reduces the crystal size and inhibits the struvite growth, or affects struvite crystallization and leads to the formation of an amorphous substance rather than crystalline struvite, XRD results have shown a more noisy pattern with reduction in peak size.[11] These limitations have encouraged investigators to search for the optimal stone analysis method.

Laser-induced breakdown spectroscopy (LIBS) is a more recently defined technique used in urinary stone analysis.[12,13] However, there are insufficient data to support the routine use of LIBS for this purpose. To our knowledge, LIBS has not been compared with traditionally used and recommended techniques such as XRD or IRS. In this study, we compared LIBS with XRD with respect to their abilities to define stone constituents in urinary stone analysis. We also assessed trace elements, as one of the major advantages of LIBS is the ability to detect trace elements and their spectral emission lines, albeit in a non-quantitative manner.

Material and methods

Overall, 65 patients with urinary calculi were enrolled in this prospective study which lasted from 2011 to 2014. Patients were treated with ureterorenoscopic surgery (n=43), open surgery (n=6), or ESWL (n=16) based on the length and localization of stones, and stone samples were obtained after these treatments. Total number of patients treated with ESWL was higher than the number of the patients treated with the other methods during the study period, but only 16 of them underwent successful retrieval of stones from their urine samples after ESWL procedure. All stones were washed with deionized water to remove urine, blood, and other contaminants. Stone samples were then dried and equal amounts of each stone samples were stored in sealed pots. One sample was sent to the Scientific and Technological Research Council of Turkey-Marmara Research Center (TUBITAK MAM) to define the elemental composition and molecular structure of the stones. A Shimadzu-6000 instrument was used for XRD analysis. Classification of the kidney stones was performed using a pregenerated XRD kidney stone software library. The other stone sample was sent to the Kocaeli University, Laser Technologies Research and Application Center (LATARUM) for analysis of their elemental composition and molecular components using LIBS. LIBS analysis was performed with a BAKI-LIBS setup developed at Kocaeli University, LATARUM. In BAKI-LIBS system, a neodymium-doped yttrium aluminium garnet (Nd:YAG) laser pulsed laser beam is focused on the surface of the urinary stone in order to produce high-density plasma. BAKI-LIBS experimental set-up was described in detail in our previously published article.[14] We also evaluated the trace elements detected by LIBS analysis and compared the presence of these elements with the stone types. For the whole cohort, we used LIBS to detect Ca, Na, P, Mg, K, S, Si, Ti, and Zn as well as organic elements C, H, N, and O.

This study was approved by the Ethics Committee of our institution. All patients provided written informed consent before the collection of samples.

Statistical analysis

All collected data were analyzed using the Statistical Package for the Social Sciences (SPSS Inc.; Chicago, IL, USA) 17.0 statistical software. A kappa ($\varkappa$) test was used to compare XRD and LIBS in terms of identification of stone types. Although there is no formally established standard, $\varkappa$ values are often segmented into ranges of values indicative of a low ($\kappa<0.4$), medium ($0.4\leq\kappa\leq0.75$), or high correlation ($\varkappa>0.75$). The Spearman's rank correlation (rho) test was used to detect correlations between the LIBS and XRD stone analysis techniques. Statistical significance was set at a p value of <0.05.

Results

In total, 65 patients were included in the study (45 male, 20 female; median ages of 42 [range, 4–60] and 39 [range, 5–78] years, respectively). LIBS technique revealed only one (n=40), two (n=20), and three (n=5) in respective number of stones. XRD technique disclosed only one (n=43), two (n=21), and three (n=1) different components in indicated number of stones. Numbers (%) of stone types with pure or mixed components according to both methods are summarized in Table 1. There was a strong relationship in the detection of stone types between LIBS and XRD as for stone types with pure and mixed components (Spearman's rho, 0.784; p<0.001). The statistical power of this study according to Spearman's rho coefficient of 0.784 was 1.

X-ray diffraction demonstrated a total of 88, while LIBS 95 stone components from 65 patients.. The most common stone component for both XRD and LIBS was calcium oxalate (Ca-ox) with rates of 63.63% and 60.00%, respectively. The stone components demonstrated by both techniques are summarized in Table 2. There was strong agreement between LIBS and XRD, with a ϰ index of 0.761. Comparison of the detection level of LIBS with that of XRD revealed an excellent Spearman's rho coefficient of 0.866 (p<0.001) (Table 2).

Table 1. The number of stone types according to LIBS and XRD

	LIBS	XRD
No. of patients	65	65
Ca-oxalate, n (%)	33 (50.8)	35 (53.8)
Ca-oxalate and Ca-phosphate, n (%)	4 (6.2)	4 (6.2)
Ca-oxalate and uric acid, n (%)	13 (20.0)	13 (20.0)
Ca-oxalate and cysteine, n (%)	1 (1.5)	1 (1.5)
Ca-oxalate and xanthine, n (%)	1 (1.5)	2 (3.1)
Ca-oxalate, Ca-phosphate, and uric acid, n (%)	1 (1.5)	0 (0.0)
Ca-oxalate, Ca-phosphate, and struvite, n (%)	4 (6.2)	1 (1.5)
Ca-phosphate and uric acid, n (%)	1 (1.5)	1 (1.5)
Uric acid, n (%)	6 (9.2)	7 (10.8)
Struvite, n (%)	1 (1.5)	1 (1.5)

Data are presented as n (%). LIBS: laser-induced breakdown spectroscopy; XRD: X-ray diffraction technique

Next, we compared LIBS with XRD among 38 patients with only one type of stone (pure stone). There was excellent agreement between the two techniques (ϰ index, 0.910; Spearman's rho coefficient, 0.916; p<0.001) (Table 3).

We also assessed the trace elements C, Ca, Mg, Na, P, K, H, N, O, Zn, S, Si, and Ti identified by LIBS. Spectral emission lines of the trace elements C, Mg, Na, K, H, N, and O were detected in whole stones. Ca was detected in all stones, with the exception of pure uric acid stones. The trace element S was not detected in five stones, and the presence of S was not associated with the stone type. Zn was detected in only 51 stones. There was no significant correlation between the presence of Zn and the stone type. The trace element Si was detected in one stone with Ca-oxalate and uric acid components. Ti was detected in only 14 stones. There was no significant correlation between the presence of Ti and the stone type.

Discussion

Definition of the stone composition is very important in selecting the optimal treatment type for patients and providing accurate stone-prevention information to clinicians.[15,16] Chemical analysis is deemed obsolete, whereas physical analysis techniques such as XRD and IRS are currently recommended as the preferred techniques.[7,8] The use of LIBS in stone analysis has been described recently [12,13] but data supporting its use for this purpose are limited. In the present study, 65 urinary stones were comparatively assessed and analyzed using both XRD and LIBS.

Tablo 2. Comparison of stone components detected by LIBS and XRD (Kappa test*)

		XRD						
		Ca-oxalate	Uric acid	Ca-phosphate	Struvite	Cysteine	Xanthine	None
LIBS	Ca-oxalate	55	1	0	0	0	0	1
	Uric acid	1	20	0	0	0	0	0
	Ca-phosphate	0	0	5	0	0	1	4
	Struvite	0	0	0	1	0	0	4
	Cysteine	0	0	0	0	1	0	0
	Xanthine	0	0	0	0	0	1	0
	None	0	0	1	1	0	0	0

*p<0.001; κ index: 0.761. LIBS: laser-induced breakdown spectroscopy; XRD: X-ray diffraction technique

Table 3. Comparison of types of pure stones detected by LIBS and XRD (Kappa test*)

		XRD		
		Ca-oxalate	Uric acid	Struvite
LIBS	Ca-oxalate	31	1	0
	Uric acid	0	5	0
	Struvite	0	0	1

*p<0.001; κ index: 0.910. LIBS: laser-induced breakdown spectroscopy; XRD: X-ray diffraction technique

Fang et al.[17] reported the first use of LIBS for urinary stone analysis. They analyzed seven urinary stone samples using both LIBS and Fourier transform infrared spectroscopy. Their results suggested that LIBS could be successfully used for elemental analysis of urinary stones. Singh et al.[12] used LIBS to analyze five urinary stone samples and found that it can successfully identify urinary stone types. Anzano and Lasheras[13] studied two LIBS strategies to analyze kidney stones, and their results suggested that LIBS is a reliable method for analysis of urinary calculi. The findings of the present study support the results of the above-mentioned studies with respect to the reliability of LIBS. Furthermore, we used XRD for comparison, and our study had a larger number of patients than did that of Fang et al.[17]

Laser-induced breakdown spectroscopy has some advantages that make it a useful technique. One of these advantages is that prior sample preparation is not necessary,[17] while it is required for XRD and IRS.[18] Analysis using LIBS is easy and rapid. Small stones can be analyzed using LIBS, whereas only large stones can be analyzed using XRD. Small stones cannot be accurately analyzed by XRD. However, the exact size of stone appropriate for analysis in both techniques has not been sufficiently studied and well established in the literature, yet. Moreover, LIBS is not a destructive method; thus, stones can be reanalyzed using other applicable techniques.

X-ray diffraction also cannot adequately detect amorphous stones such as carbonite or struvite.[19] As shown in Table 2, LIBS detected five struvite stone types, while XRD detected only one. Although this difference was statistically significant ($p<0.01$), the $\varkappa$ index showed weak agreement with a value of 0.350. We believe that this weak agreement was due to the low number of patients with struvite stones. Nevertheless, the number of patients is very low to arrive at a decisive conclusion for this stone type. We also found that Ca-oxalate and uric acid stones were correctly identified by LIBS in 55 of 56 (98.2%) and 20 of 21 (95.2%) patients, respectively (Table 2). In patients with pure stones, we found that Ca-oxalate stones were correctly identified by LIBS in all 31 patients (100.0%) and uric acid in 5 of 6 patients (83.3%) (Table 3). According to previous epidemiological studies, these two stone types comprise 67- 90% of all urinary stones,[20] and our results support the use of LIBS for these types of stone, with higher agreement with XRD findings.

The other advantage of LIBS is its cost-effectiveness.[21] In our country, the cost of analysis for one stone is 10 $ in LIBS technique, while it is 30$ in XRD. The costs of both the device and analysis of the stones are lower than those of XRD. The cost of the LIBS device, which was devised at the University of Kocaeli, LATARUM and called the BAKI-LIBS,[14] is one-third that of the XRD instrument. Furthermore, the cost of analysis of each stone using LIBS is one-fourth that of XRD. Additionally, the LIBS instrument is small in size and can be easily transported and placed in an office or operation room. Finally, lack of exposure to X-rays is another advantage of LIBS.

Laser-induced breakdown spectroscopy also detects the trace elements within stones. Therefore, it is possible to detect elements that may be a part of the stone, but do not contribute to the stone type. The role of trace elements in lithogenesis of urinary calculi is still debated. Detection of trace elements is an advantage of LIBS because trace elements are considered to affect the formation of stone crystals.[22] Zinc is thought to be an inhibitor of kidney stone formation, but it could also be present in calcium-containing stones.[23] Atakan et al.[24] found lower serum and urine zinc levels in patients who previously had stones when compared with stone-free population. Their results indicated that zinc has an inhibitory role in stone formation. However, the findings of other studies are not consistent with these results.[25,26] Our results indicate that although zinc can be seen as an elemental component of urinary calculi, no conclusion can be reached regarding the relationship of zinc with stone types. We found that zinc can be detected in all stone types containing uric acid and struvite, which are non-calcium–containing stones. Similar to our results, Giannossi et al.[27] found that zinc can be detected in uric acid and struvite stones.

Magnesium is a major component of struvite stones. Although it is considered by some authors to be one of the most important inhibitors of lithogenesis, [22,24] this is a controversial issue due to conflicting results.[28] Moreover, its inhibitory role in this process has not been clearly explained. Singh et al.[12] used LIBS to analyze five urinary stone samples and found that Mg was detected in every part of all stones. We also found that Mg was detected in all urinary stones, supporting the findings of Singh et al.[12] According to our results, the inhibitory effect of Mg on lithogenesis is unclear. Our findings do not support conclusions regarding other trace elements because of their low detection rates. However, it is clear that LIBS has an advantage over XRD with respect to more accurate detection of trace elements.

One of the limitations of LIBS is its inability to determine the levels of trace elements. Additionally, in large stones, LIBS may miss the elemental component, which may be located outside of the laser beams. Moreover, the laser beams are focused on the exterior of the stone. The stone core may differ from the stone exterior; thus, this technique may underestimate the stone components. Disintegration of large stones to create stone sand prior to the procedure and application of additional laser therapy may solve this problem.

The present study was not free of limitations. There were small numbers of cysteine, struvite, xanthine, and Ca-phosphate stones. On the other hand, the statistical power of this study for Spearman's rho coefficient of 0.784 was one and this showed that the number of patients in the whole cohort was enough to make an appropriate comparison for common stone types between these two different stone analysis techniques.

To our knowledge, LIBS and XRD have not been compared previously, and our study is the first to compare these methods of urinary stone analysis. Additionally, LIBS has been insufficiently investigated in terms of urinary stone analysis. Our comparison of these two techniques suggests that LIBS has a higher accuracy rate in stone analysis. According to our results, LIBS also has higher accuracy for stones with a single component compared to XRD. These results encourage us to plan further use of LIBS using a specialized catheter at the time of stone fragmentation during endourological stone surgeries.

In conclusion, our results suggest LIBS to be a reliable method for urinary stone analysis. According to our results, LIBS can detect stone constituents with accuracy similar to that of XRD, which is a recommended technique and a reference standard. Our results should be verified by further studies with larger numbers of patients.

Ethics Comittee Approval: This study was approved by the Clinical Research Ethics Committee of Kocaeli University (Project no: KOU/KAEK 2012/15).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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