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Infrared Spectroscopic Analysis of Urinary Calculi: A Retrospective Study in Argentinean Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Author LVM designed the study, managed the literature searches, wrote the protocol and wrote the first draft of the manuscript. Author VCDO performed the statistical analysis. Author GLG managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate, by infrared spectroscopy (FT-IR), the chemical composition of urinary calculi obtained from patients of Buenos Aires, Argentina.
Duration of Study: The composition of the urinary calculi was evaluated in a retrospective study from March 1993 to September 2013.
Methodology: Infrared spectra of the urinary calculi were recorded in a Bruker IFS-25 FT-IR and in a Nicolet 380 FT-IR spectrophotometers. We included 440 urinary stones (286 from men and 154 from women). The samples were obtained by spontaneous passage, shockwave lithotripsy, ureteroscopy or percutaneous nephrolithotripsy mainly from patients treated at Hospital de Clínicas, University of Buenos Aires.

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Results: Calcium oxalate (both in pure or mixed samples) was detected in 326 cases (74.09%). Anhydrous uric acid (8.41%) was observed most frequently, followed by struvite (2.05%) and cystine (2.50%). For some chemical compounds, a significant gender-related difference was found. Applying the second derivative spectra allowed to distinguish between the presence of whewellite, weddellite and their mixture. More than 70% of recurrent urinary stones were of the same chemical composition.

Conclusion: FT-IR analysis of urinary calculi over a period of 20 years gave an outlook of the prevalence of certain stone components in patients from Buenos Aires, Argentina, which in some cases were found to be gender-related. The results obtained are in accordance with statistics from other industrialized countries, except for uric acid (15.93%), even pure or combined in other forms, which was more frequent than the world prevalence (up to 10%). FT-IR spectroscopy combined with the second derivative method of analysis proved to be a powerful tool to discriminate mixed oxalates whose composition only differed in one water molecule.

Keywords: Urolithiasis; chemical composition; FT-IR; second derivative; recurrence.

1. INTRODUCTION

Urolithiasis is a common, recurrent disorder with a worldwide increasing prevalence over the past few decades. Up to 5% of the general population is affected by renal stones, with a life risk estimated at 8-10% [1]. In Argentina, if asymptomatic stones are considered, the prevalence of nephrolithiasis can reach up to 12% [2]. The reasons for this trend are not entirely clear. Factors such as obesity, diabetes, endocrinopathies (hyperparathyroidism), urinary infections, diet and extrinsic conditions such as dehydration, drug excess and isohydruria may lead to the genesis of urolithiasis [3].

Urinary calculi are mainly made up of calcium, uric acid, magnesium ammonium phosphate or cystine, but some rare types such as xanthine, triamterene or indinavir stones can also be found [4].

To help with the identification of treatable risk factors to prevent recurrence, it is necessary to study the chemical composition of urinary calculi. Chemical and physical methods can both be used for analysis. Chemical methods show a significant error rate and are not able to distinguish between different mineralogical phases. Among physical methods, scanning electron microscopy (SEM), microscopy, X-ray diffraction and Fourier transform infrared spectroscopy (FT-IR) have been used for stone analysis [5]. However, only X-ray diffraction and FT-IR meet the criteria for use in a routine laboratory. The analysis by SEM is time-consuming and the equipment is very expensive. The microscopic examination of urinary stones requires very highly qualified personnel. If the constituent shows a crystalline phase, X-ray diffraction is the most reliable; however, this method has specific limitations such as the failure to detect amorphous phosphates, among others, and the equipment is also very expensive. FT-IR spectroscopy has been established as one of the most effective methods and the most widely used to determine the urinary stone composition; it is in fact, a fingerprint of the constituent materials. IR spectroscopy offers many advantages since it is a sensitive and selective analytical technique. Results can be achieved rapidly (the total analysis time including sampling, measurement, identification and report generation is about 10 minutes), only requires a very tiny amount of a substance for analysis (1 mg) and the equipment is less expensive than X-ray diffraction and SEM [6]. In this context, the present study was conducted to investigate, by FT-IR analysis, the chemical composition of 440 urinary calculi from patients of Buenos Aires, Argentina.

2. MATERIALS AND METHODS

The analysis of the urinary stones was performed in 440 patients (286 males and 154 females) with a mean age of 45 (range 3-77). Only 3 stones were from children aged 3, 4 and 11 years. The stones were obtained by spontaneous passage, shockwave lithotripsy, ureteroscopy or percutaneous nephrolithotripsy mainly from patients treated at Hospital de Clínicas, University of Buenos Aires, between March 1993 to September 2013. The stone samples were washed with distilled water, air-dried; ground with a mortar to a homogeneous powder and about 1 mg mixed with 200 mg of potassium bromide (UvasolR, Merck) and pressed to nine tons to generate a KBr disk with 13 mm diameter. The FT-IR spectra were recorded in a Bruker

IFS-25 FT-IR and in Nicolet 380 FT-IR spectrophotometers in the range between 4,000-400 cm^{-1} with a resolution set at 4 cm^{-1}. Results were analyzed with Bruker OPUS/IR 6.0 and Bruker OPUS/IR 7.5.18 software. In the case of whole calculi, samples were obtained from the nucleus (core) and the shell (surface) and when the stones were fragmented, all the fragments were ground to powder and analyzed separately. The stone composition was classified as pure or mixed, and samples spectra were compared with standard ones [7].

2.1 Statistical Analysis

Data were analyzed by the chi-square test using Statistix 8 TM software. P values < 0.05 were considered significant.

3. RESULTS

3.1 FT-IR Study of the Chemical Composition of Stones

A total of 440 urinary calculi were available for FT-IR evaluation. Table 1 gives the complete list of components identified. The analysis showed that 240 (54.54%) were pure stones. The most prevalent substance was whewellite, the monohydrate crystalline form of calcium oxalate, which was detected in 31.14% of the stones. Weddellite (the dihydrate form of calcium oxalate) was present in 7.72% and anhydrous uric acid was detected in 8.41% of the spectra. Less frequent pure stones were struvite ($MgNH_4PO_4.6H_2O$, 2.05%), cystine (2.50%) and brushite ($CaHPO_4.2H_2O$, 0.68%).

The remaining 200 stones (45.45%) were heterogeneous in composition. In this way, calcium oxalate (both in pure or mixed samples) was detected in 326 stones (74.09%).

The frequency of anhydrous uric acid in combination with other compounds (mixed stones) was 3.64%. This percentage increased up to 4.79% if the other forms of uric acid (uric acid dihydrate and ammonium urate) were present. Occasionally, calcium salts and uric acid are present in the same stone. We found an incidence of 2.04% of anhydrous uric acid mixed with whewellite.

In 31 urinary calculi, the nucleus and the shell were separately analyzed. We found that in 67.74% (n=21) of the samples, the nucleus and the shell had the same chemical composition.

3.2 Chemical Composition and Gender

Out of the 440 urinary calculi analysed, 286 were obtained from men (65%) and 154 from women (35%), giving a male to female gender ratio of 1.8:1. The results are depicted in Table 2. For some chemical compounds, a significant difference with gender could be found. Weddellite (9.8% of men patients vs 3.9% of women patients), and anhydrous uric acid (11.2% of men patients vs 3.2% of women patients) were more frequent in men as pure stones (Table 2). In this study, we did not find in women a prevalence of struvite in neither pure (P=0.548) nor mixed stones. In contrast, whewellite mixed with apatite and mixed stones of carbonate apatite with weddellite, carbonate apatite with amorphous Ca-phosphate and carbonate apatite with whewellite were most frequent in women with P=0.012, P=0.007 and P=0.018, respectively.

3.3 Incidence over the Period 1993-2013

The incidence of renal stones was evaluated between 1993-2002 (period 1) and 2003-2013 (period 2). A total of 219 and 221 stones were analysed over the periods 1 and 2, respectively. The overall proportion of pure and mixed whewellite (59.4% *vs* 57.5%), pure and mixed carbonate apatite (4.6% *vs* 4.9%), cystine (2.3% *vs* 2.7%), brushite pure stones (1.83% *vs*. 0.45%) and struvite stones (6.4% *vs*. 11.3%) remained essentially unchanged with a decreasing proportion of pure and mixed weddellite stones (23.3% *vs*. 12.7%, P = 0.0037) and a rising proportion of purines (10.5% *vs*. 17.6%, P= 0.0313) in their either pure or mixed forms.

3.4 Mixture of Whewellite and Weddellite: Analysis of Second Derivative Infrared Spectra

In the FT-IR spectra, the presence of whewellite, weddellite or their mixture can be demonstrated by the position of the CO stretching band in the second derivative of the spectra. When only whewellite or weddellite is present in a sample, only a broad infrared absorption band at about 1.320 cm^{-1} is resolved in the second derivative spectra. In the case of a mixture of the oxalates, the absorption band is resolved at about 1.318 cm^{-1} for whewellite and 1.327 cm^{-1} for weddellite [8] (Fig. 1).

Table 1. Chemical composition of urinary stones

Stone composition		Men		Women		Total	
Pure		**n**	**%**	**n**	**%**	**n**	**%**
Oxalates	Whewellite	95	21.59	42	9.55	137	31.14
	Weddellite	28	6.36	6	1.36	34	7.72
Phosphates	Carbonate apatite	1	0.23	1	0.23	2	0.46
	Struvite	5	1.14	4	0.91	9	2.05
	Brushite	3	0.68	--	--	3	0.68
Purines	Anhydrous uric acid	32	7.27	5	1.14	37	8.41
	Uric acid dihydrate	5	1.14	--	--	5	1.14
	Ammonium urate	2	0.45	--	--	2	0.45
Cystine		7	1.59	4	0.91	11	2.50
Mixed							
	Whewellite + apatite	43	9.77	49	11.14	92	20.91
	Weddellite + apatite	23	5.23	6	1.36	29	6.59
	Carbonate apatite + weddellite	1	0.23	5	1.14	6	1.37
	Carbonate apatite + amorphous Ca-phosphate	2	0.45	7	1.59	9	2.04
	Brushite (S) + weddellite + apatite (C)	--	--	1	0.23	1	0.23
	Struvite + apatite	13	2.95	14	3.18	27	6.13
	Anhydrous uric acid + whewellite	7	1.59	2	0.45	9	2.04
	Anhydrous uric acid + uric acid dihydrate	5	1.14	--	--	5	1.14
	Anhydrous uric acid + apatite	--	--	1	0.23	1	0.23
	Uric acid dihydrate + whewellite	1	0.23	--	--	1	0.23
	Carbonate apatite + whewellite	--	--	3	0.68	3	0.68
	Ammonium urate + apatite	--	--	1	0.23	1	0.23
	Whewellite + Weddellite	7	1.59	1	0.23	8	1.82
	Struvite + ammonium urate + apatite	1	0.23	--	--	1	0.23
	Weddellite + amorphous Ca-phosphate + apatite	1	0.23	--	--	1	0.23
	Whewellite (S) + ammonium urate (C)	1	0.23	--	--	1	0.23
	Carbonate apatite + amorphous Ca-phosphate (S) + struvite + apatite+ ammonium urate (C)	1	0.23	--	--	1	0.23
	Anhydrous uric acid + uric acid dehydrate + whewellite +apatite	1	0.23	--	--	1	0.23
	Brushite + whewellite + apatite	1	0.23	--	--	1	0.23
	Whewellite + apatite + calcite	--	--	1	0.23	1	0.23
	Whewellite + apatite + weddellite	--	--	1	0,23	1	0.23
Total		286	65.00	154	35.00	440	100.00

(S) Shell; (C) Core

Table 2. Distribution of urinary stones according to gender

Stone composition		Men	%	Women	%	*P* value
Pure						
Oxalates	Whewellite	95	33.2	42	27.3	0.199
	Weddelite	28	9.8	6	3.9	0.027[a]
Phosphates	Carbonate apatite	1	0.3	1	0.6	0.656
	Struvite	5	1.7	4	2.6	0.548
	Brushite	3	1.0			0.202
Purines	Anhydrous uric acid	32	11.2	5	3.2	0.004[a]
	Uric acid dihydrate	5	1.7			0.099
	Ammonium urate	2	0.7			0.298
Cystine		7	2.4	4	2.6	0.924
Mixed						
	Whewellite + apatite	43	15.0	49	31.8	0.000[a]
	Weddellite + apatite	23	8.0	6	3.9	0.095
	Carbonate apatite + weddellite	1	0.3	5	3.2	0.012[a]
	Carbonate apatite + amorphous Ca-phosphate	2	0.7	7	4.5	0.007[a]
	Brushite + weddellite + apatite			1	0.6	0.173
	Struvite + apatite	13	4.5	14	9.1	0.058
	Anhydrous uric acid + whewellite	7	2.4	2	1.3	0.417
	Anhydrous uric acid + uric acid dihydrate	5	1.7			0.099
	Anhydrous uric acid + apatite	0		1	0.6	0.173
	Uric acid dihydrate + whewellite	1	0.3			0.463
	Carbonate apatite + whewellite			3	1.9	0.018[a]
	Ammonium urate + apatite			1	0.6	0.173
	Whewellite + Weddellite	7	2.4	1	0.6	0.178
	Struvite + ammonium urate + apatite	1	0.3			0.463
	Weddellite + amorphous Ca-phosphate + apatite	1	0.3			0.463
	Whewellite + ammonium urate	1	0.3			0.463
	Carbonate apatite + amorphous Ca-phosphate + struvite + apatite + ammonium urate	1	0.3			0.463
	Anhydrous uric acid + uric acid dehydrate + whewellite + apatite	1	0.3			0.463
	Brushite + whewellite + apatite	1	0.3			0.463
	Whewellite + apatite + calcite			1	0.6	0.173
	Whewellite + apatite + weddellite			1	0.6	0.173
Total		286	100.0	154	100	

P < 0.05; a Chi-square test

3.5 Recurrence

In this study, among the 440 samples analyzed, 22 (5%) were from recurrent patients and from this particular group 16 patients (72.73%) developed recurrent urinary stones with the same chemical composition: whewellite (5 cases), struvite + apatite (3 cases), cystine (3 cases), struvite (2 cases), uric acid (1 case), whewellite + apatite (1 case), and whewellite + weddellite (1 case).

4. DISCUSSION

Urolithiasis is the third most common urological pathological disease. A systematic review of the literature has shown that stone prevalence and incidence rates are increasing mainly in the United States, Germany, Spain, Italy and Scotland [9]. Pinduli et al. [10] have performed a cross-sectional study of the prevalence of urolithiasis in 1,086 subjects from the city of Buenos Aires. The authors found a 3.96%

lifetime prevalence of urolithiasis in the general population of this city.

Over the past 25 years the male to female ratio for the prevalence of renal calculi has decreased from 3:1 to less than 2:1 [11]. This data is in agreement with our study where the male-to-female ratio was 1.8:1. The increase of urinary stones in women may be due to changes in life-style factors, such as increasing obesity [12].

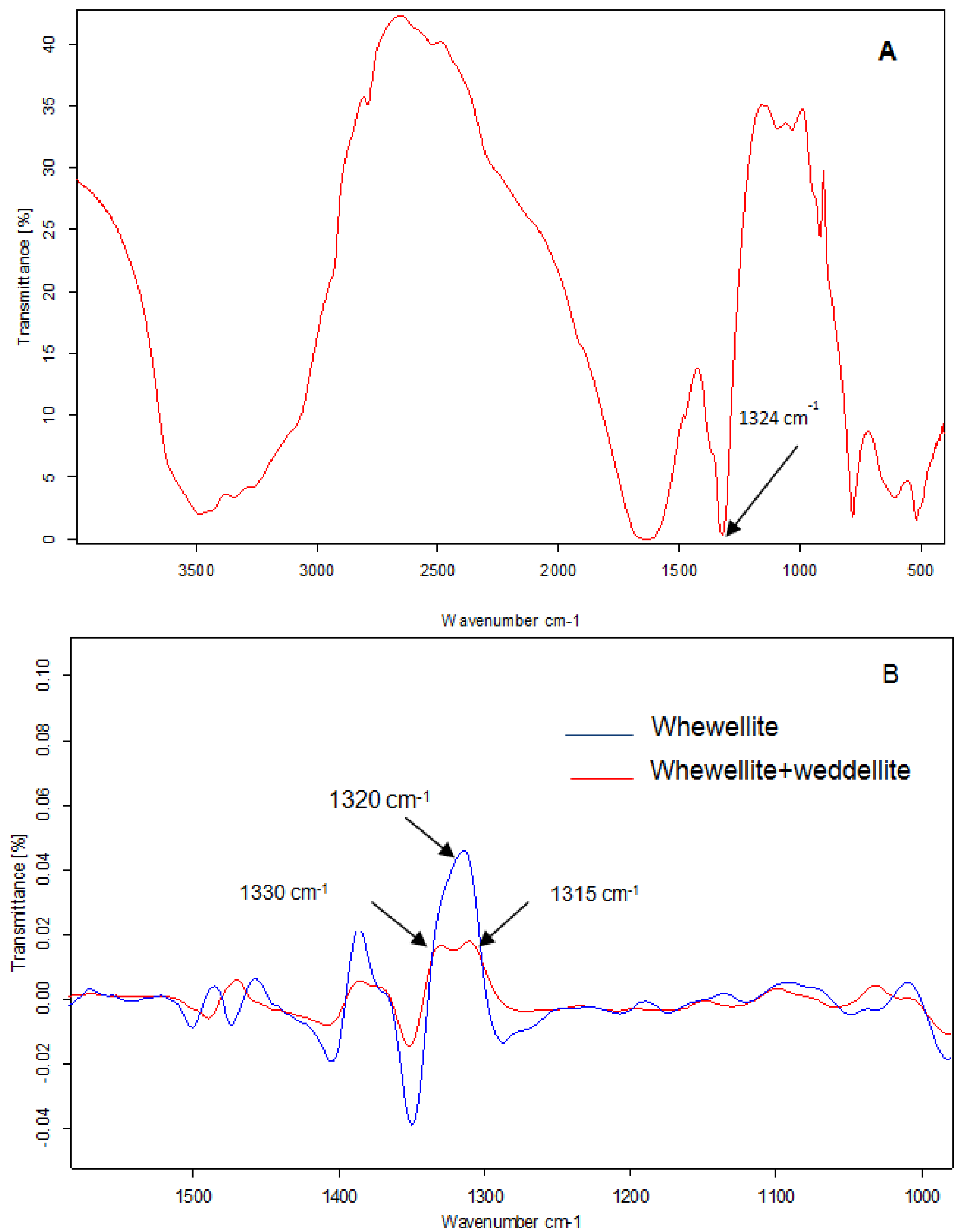

Fig. 1. A. Infrared spectra of a mixture of whewellite and weddellite. B. Infrared spectra of the second derivative of the mixture compared with pure whewellite

The objective of the identification of the chemical composition of urinary stones is to collect important information helping the physician to establish the causes of stone formation [5]. Among the different methods used for stone analysis, FT-IR is the most widely used. More than 98% of the stones can be analyzed using this technique, nevertheless it has some limitations. One of the problems relates to detect the absorption of carbonate in struvite stone because NH_4 absorption of magnesium ammonium phosphate overlaps CO_3 absorption of carbonate at 1420-1435 cm^{-1}, nevertheless this can be overcome by using Raman spectroscopy. Another limitation is the distinction between directly formed whewellite crystals from those that become from weddellite transformation, or the distinction between papillary or non-papillary whewellite calculi. In both cases the combination of SEM microscopy and FT-IR can be used.

In industrialized countries the main component of urinary calculi is calcium oxalate, (about 75–90%) and its pathogenesis may be associated with metabolic or genetic factors [13-15]. Our study showed that calcium oxalate (both in their pure or mixed forms) was detected in 74.09% of the stones. In a study carried out in 86 renal calculi from patients from Lujan and San Antonio de Areco (cities located in Buenos Aires province, Argentina), the incidence of whewellite, weddellite and whewellite combined with apatite was 36.1, 5.8 and 24.4% respectively [8]. Our findings are in agreement with these data (Table 1).

Struvite, brushite and carbonate apatite are the most important phosphates involved in urinary stone disease. Struvite occurs in about 10% to 12% of patients, more often in women due to the higher incidence of urinary tract infections [4]. Infected stones are primarily composed of struvite with smaller fractions of carbonate apatite and mono-ammonium urate and occur in the presence of urine persistently infected with urease-producing bacteria (e.g. *Proteus* and *Providencia* spp., *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) that break down urea rendering a persistently alkaline urine [16,17]. However, in this study we did not find this tendency in pure stones or in combination with other phosphate salts.

The occurrence of brushite stone disease, albeit rare (1%), has increased over the past decades [18] and due to their hardness; it has been demonstrated to respond poorly to disintegration by extracorporeal shockwave lithotripsy (ESWL) [19]. According to Siener et al. [20], urinary calcium and citrate and elevated urinary pH, but not phosphate excretion are important determinants of brushite stone formation. The increased urinary oxalate excretion, promotes the risk of mixed stone formation with calcium oxalate.

The increased urinary oxalate excretion, promotes the risk of mixed stone formation with calcium oxalate. We have identified 3 brushite pure stones (0.68%) and 1 (0.23%) mixed stone where the shell was composed of brushite and the core of weddellite and apatite (Table 1). These data are in accordance with the incidence of brushite stones among other industrialized countries [21]. Hypercalciuria, renal loss of calcium and primary hyperparathyroidism are the most frequent causes of brushite stones formation.

In certain calculi carbonate group may substitute for part of the phosphate and the compound is then referred to as carbonate apatite. Although infection is not a prerequisite for the formation of carbonate apatite stones, infective conditions favor carbonate apatite formation [21]. We found a low incidence of pure carbonate apatite stones (0.46%), but this percentage increases to 4.78% in mixed stones mainly combined with weddellite or calcium phosphate.

Uric acid stone disease is found in about 5% to 10% of stone patients [22] and occurs especially in patients with a very low urine pH (<5.0) and in those with hyperuricosuria. In our study, the frequency of pure or mixed anhydrous uric acid was 12.05%. This percentage increased to 15.93 % if the other forms of uric acid (uric acid dihydrate and ammonium urate) were present. The high incidence found is probably explained by the fact that uric acid is the result of metabolism of meat, the most consumed food in Argentina. In the other two studies carried out in our country the incidences of uric acid reported were 5.8% and 24.8% [2]. Occasionally, calcium salts and uric acid are present in the same stone. We have found an incidence of 2.04% of uric acid mixed with whewellite.

In order to evaluate if there was a difference in the incidence of urinary calculi over time, two periods were compared: period 1 (1993-2002) and period 2 (2003-2013). A decreasing proportion of pure and mixed weddellite and a rising proportion of purines in their pure or mixed forms were detected during the second period (period 2).

FT-IR spectroscopy together with the second derivative analysis provides the physician with a good method to determine the components of calculi, especially when the calculi are composed of a mixture of weddellite and whewellite [8]. This is important because the major presence of whewellite is a symptom of hyperoxaluria but weddellite advises towards the existence of hypercalciuria [23,24].

The recurrent nature of stone disease is a well-recognized clinical problem that reaches 13% in the first year and 50% at ten years of the first episode. Urinary metabolic abnormalities such as low urine volume, hypercalciuria, hyperoxaluria, hyperuricosuria and hypocitraturia predispose a patient to early recurrence [25]. We have observed that 72.7% of the patients developed recurrent urinary stones with the same chemical composition.

For many years, the analysis of urinary calculi has been controversial since it was considered that their study provided no useful information. However, an accurate analysis of the stone composition, together with blood and urine analysis may help to identify the etiopathogenic mechanisms of the lithiasis more clearly [26]. According to Hesse and Heimbach [21] during the first episode of urinary stone disease it is difficult for the physician to make a definitive diagnosis without the analysis of the stone.

In the present study, less than 1% of the cases were from children. The chemical composition of renal stones in this age group of patients has not been evaluated in our country. A relationship between the FT-IR analysis and other clinical parameters could be of relevance in the fast diagnosis of different renal and metabolic pathologies. Our future research is devoted to address this issue.

5. CONCLUSION

The FT-IR analysis of 440 renal stones obtained mostly from patients from the city of Buenos Aires, Argentina over a period of 20 years has given an outlook on the prevalence of certain stone components, which in some cases can be gender-related. The recurrence of some pathologies could also be investigated. As it is usually seen in industrialized countries, calcium oxalate was detected in the 74.09% of the stones, exhibiting a decrease over the last ten-year period for weddellite (which was more frequent in males). Either in pure or combined forms, anhydrous uric acid was found in up to 15.93% of the samples, being this prevalence value higher than that reported previously (up to 10%) and the value informed in 2010 for samples taken from a zone close to the city of Buenos Aires. FT-IR spectroscopy combined with the second derivative method of analysis proved to be a powerful tool to discriminate mixed oxalates whose composition only differed in one water molecule.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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