A SIMPLE METHOD TO TYPE THE URINARY STONES

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ABSTRACT

The main aim of this study was to find an alternative method to type the urinary stones, which do not comply with the available method. For this study 100 stones were selected and were analysed by wet chemical method. The compositions of randomly selected 10 stones each among the stones typed based on the available and the new method were crosschecked by Fourier Transform infrared Spectroscopy (FTIR) method. Among the 100 stones, 46 stones were of Category I [21 stones Uric acid/Urate, 13 stones Oxalate, 12 stones Phosphate] while five were of Category II stones. Rest 49 stones, which cannot be typed by the available method, were typed by considering the ratios between the characterizing and indicating anions. To type the Oxalate stones, Oxalate to Urate ratio between 16.8:1 and 67.7:1; Urate stones, Urate to Oxalate ratio between 0.7:1 and 101.7:1 and Non- infection Phosphate stones, Phosphate to Oxalate ratio between 0.4:1 and 24.4:1 were considered. Based on the newly proposed method majority of the stones were of Oxalate type (n=41). Based on both the methods of stone typing, of the total 100 stones, 54 stones were Oxalate type, 25 stones were Uric acid/Urate type, 16 stones were Non-infectious Phosphate stones and 05 were Infectious stones. The compositions of the randomly selected ten stones of each typed from the available and the newly proposed method were similar to the results obtained by FTIR method. This study indicated that, the new method could be used as an alternative method to type the stones.

KEYWORDS

Characterizing ions, Indicating ion, Oxalate stone, Urate stones, Urinary stone, Wet chemical method

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INTRODUCTION

Urinary stones are polycrystalline aggregates composed of varying amounts of crystalloid and a small amount of organic matrix,[1] and they are formed at any part of the urinary tract.[2,3] Urinary stone formation is one of the most prevalent urologic diseases in Asia ranging from 1-5%.[4,5] Urinary stone analysis is carried out by different methods; such as chemical analysis,[6-8] thermo gravimetry,[9] polarization microscopy,[10] scanning electron microscopy (SEM),[10] powder X-ray diffraction (XRD),[10-12] spectroscopy,[12-14] and Fourier Transform Infrared Spectroscopy (FTIR).[15,16]

The chemical analysis is commonly known as wet chemical method and is one of the most widely used approaches for stone analysis. It can determine the presence of individual ions and radicals.[6,14] The wet chemical methods usually use the quantitative analytical methods for the analysis of chemical components in the blood and urine. The components analysed by these methods are used to calculate the amounts of chemical compounds present in the stones.[6,17]

To type the urinary stones, the method described by Abdel-Halim, *et al.*[17] is commonly used. In the method, the urinary stones are typed based on characterizing and the indicating ions. Furthermore, have described a method to type the urinary stones by cluster analysis of ionic composition data.[18]

In this study an alternative method was developed to type the stones which cannot be categorized based on the method described by Abdel-Halim *et al.*[17]

MATERIAL AND METHODS

Materials: The reagent kits for the estimation of Calcium, Magnesium, Urate/Uric acid, Inorganic Phosphate and Oxalate were from Diagnosticum Zrt., Swiss Hungarian Joint Venture Company, Hungary. All the other chemicals used were of analytical grade.

Sample collection and storage: For this study, 100 urinary stones from patients who underwent surgical interventions at Genitourinary Surgical Unit of Teaching Hospital, Jaffna were collected. The collected samples were placed on sterile gauze to air dry, and transferred into a sterile glass bottle bearing the patient details. All the specimens were washed with deionized water to remove the loose debris such as blood, mucous and casts, bile and debris and then dried in an oven to 60°C for five hours or overnight[17,19] and stored at 4°C.

Ethical Approval: Ethics approval was obtained from Ethics Review Committee, Faculty of Medicine, University of Jaffna (JERC/16/75/DR/0032).

Preparation of the Urinary stones for analysis: Ground urinary stone samples (powder, 20 mg) were dissolved in 2 ml of 6N HCl with slight warming[6] and those were insoluble in 6N HCl were dissolved either in 6 g/L Li_2CO_3 [20] or in a mixture of 6 g/L Li_2CO_3 and 6N HCl. Total volume of the dissolved stone preparations was made up to 10 ml with deionized water.

Analytical Methods

Wet Chemical Methods: The stone samples were analysed by wet chemical methods for Calcium[21], Magnesium[22], Inorganic Phosphate[23], Uric acid / Urate[24], and Oxalate[25], using Diagnosticum Zrt. Reagent kits in a Semi-Automatic Biochemistry Analyser [SA-20 CLIDING Systems (UK) Co., LTD].

Calculation of the chemical compound contents of the Urinary stones: After the estimation of Calcium, Magnesium, Inorganic Phosphate, Urate / Uric acid and Oxalate; the amounts of Calcium oxalate monohydrate ($CaC_2O_4.H_2O$), Magnesium Ammonium Phosphate Hexahydrate [$Mg(NH_4)PO_4.6H_2O$] and Hydroxy apatite [$Ca_{10}(PO_4)_6(OH)_2$] were calculated.[6]

Classification of Urinary stones: The method described by Abdel-Halim, *et al.*[17] was used to type the urinary stones.

Fourier Transform Infrared Spectroscopy (FTIR) method: The fine homogenous stone powder of the samples was analysed by FTIR method.[16]

RESULTS

One hundred urinary stones collected from the Genitourinary Surgical Unit of Teaching Hospital, Jaffna were categorised based on Abdel-Halim *et al.*[17] and to the stones which cannot be typed an easy alternative method was suggested.

Typing of the urinary stones based on the available methods

Typing based on characterizing ion: The stones selected were typed by considering the characterizing ions and the indicating ion/s.[17] Among the 100 stones, 51 stones had the compositions to match the typing described by

Abdel-Halim, *et al.*[17] Among these 51 stones, 46 stones were of Category I. The Category I stones (n=46) were further typed. Of these 46 Category I stones, 21 were Uric acid/Urate (41.2%), 13 were Oxalate (25.5%) and 12 were Phosphate (23.5%) stones[17] (Table 1). The balance 5 (9.8%) stones were of Category II and contained Magnesium. The Category II stones (n=5) were with infection and known as Struvite stones (Table 1).

Sub typing based on indicating ion/s: The typed Category I stones (n=46) were further sub-typed based on the indicating ions[17]. Of the 21 Uric acid/Urate stones, majority of the stones (n=14) were of UrI 4 and all the Oxalate stones (n=13) were of the subgroup; OxI 3. Among the 12 Phosphate stones (Non-infectious), 05 were of PhI 1 and 07 were of PhI 2 (Table 1).

Typing of the stones based on the new method: Among the typed 51 stones 46 stones were of Category I and rest 5 stones were of Category II. The characterizing ion of Category I stone type had different percentage of indicating ion/s.[17] Considering the ionic compositions of the 46 Category I stones, the new method of stone typing is described by calculating the ratios between the characterizing and indicating ions (Table 2). In this new method, the ratios between the Oxalate to Urate concentrations of the stones typed as Oxalate (n=13) were calculated and was in the range from 16.8:1.0 to 67.7:1.0. Similarly the Urate stones (n=21) had the Urate to Oxalate ratios in the range from 0.7:1 to 101.7:1 and the Non- infectious Phosphate stones (n=12) had the Phosphate to Oxalate ratios from 0.4:1 to 24.4:1 (Table 2).

Based on the above ranges of the anion ratios it was possible to type the balance 49 non - typed stones. Among the stones, 41, 04 and 04 were respectively typed as Oxalate, Urate and Non- infectious Phosphate stones (Table 2). Sub

Table 1: Urinary stone typed based on the characterizing ion contents* and sub typed based on the indicating ion contents*.

Category	Stone Types	Characterizing ions	Ions (%)	Stone (No.)	Stone sub -types	Indicating ions		Stone	
						Ions	(%)	No.	%
I	Uric acid/ Urate (Ur)	Uric acid/ Urate	>/=20	21	UrI 1	Oxalate	<40->33	00	0.0
					UrI 2	Oxalate	32.9->21	03	14.3
					UrI 3	Oxalate	20.9-10	04	19.0
					UrI 4	Oxalate	< 10	14	66.7
	Oxalate (Ox)	Oxalate	>/=40	13	OxI 1	Urate	< 20	00	0.0
					OxI 2	Phosphate	< 20	00	0.0
					OxI 3	Uric acid/ Urate & Phosphate, each	< 20	13	100.0
	Phosphate (Ph)	Phosphate, Uric acid/ Urate & Oxalate	>/=10, <20 & <40	12	PhI 1	Oxalate	<40-10	05	41.7
					PhI 2	Oxalate	< 10	07	58.3
II	Magnesium	Magnesium	>/=3	05					

*The stones were typed based on Abdel-Halim, *et al.*[17] .

UrI 1, UrI 2, UrI 3 and UrI 4 indicate the Uric acid/Urate type of stones with different amounts of Oxalate, where Oxalate is said to be the indicating ion.

OxI 1, OxI 2 and OxI 3 indicate the Oxalate type of stones with Urate < 20, Phosphate < 20 and Uric acid/ Urate & Phosphate, each < 20, respectively and under this category Urate, Phosphate and Uric acid/ Urate & Phosphate are said to be the indicating ions.

PhI 1 and PhI 2 indicate the Phosphate type of stones with different amounts of Oxalate, where Oxalate is said to be as the indicating ion.

Table 2: The ratios between the concentrations of the characterizing ions and indicating ions of category I stones (n=46*) and Stones typed based on the ratios between anion concentrations (n=49).

Anion		*Ratio Between Characterising and Indicating Anions			Stones typing (No.)		Stone Type
Characterizing ion [a]	Indicating ion [a]	Min[b]	Max[c]	Mean	*Abdel-Halim, *et al.* (1993)	Ratios[d]	
Urate	Oxalate	0.7:1	101.7:1	6.5:1	21	04	Urate
Oxalate	Urate	16.8:1	67.7:1	37.1:1	13	41	Oxalate
Phosphate	Oxalate	0.4:1	24.4:1	1.3:1	12	04	Non- infectious Phosphate

*Abdel-Halim, *et al.*, 1993
[a] Characterizing ions and Indicating ions by Abdel-Halim, *et al.*[17].
[b] The minimum ratios between the characterizing and indicating anions present in the stones.
[c] The maximum ratios between the characterizing and indicating anions present in the stones.
[d] Typing of the stones based on the new method.

Table 3: Typing and sub typing of non - typed urinary stone based on the ratios between the characterizing ion and indicating ion/s.

Stone Type	Stones (Nos.)	Stone sub type	Indicating ions		Stones	
			Ions	(%)	No.	%
Uric acid/ Urate (Ur)	04	UrI 1	Oxalate	2-1.672	00	0.0
		UrI 2	Oxalate	1.622-1.072	03	75.0
		UrI 3	Oxalate	1.022-0.522	01	25.0
		UrI 4	Oxalate	< 0.471	00	0.0
Oxalate (Ox)	41	OxI 1	Urate	< 0.486	00	0.0
		OxI 2	Phosphate	< 0.486	00	0.0
		OxI 3	Uric acid/ Urate & Phosphate, each	< 0.486	41	100.0
Phosphate (Ph)	04	PhI 1	Oxalate	<4-1.044	04	100.0
		PhI 2	Oxalate	< 0.944	00	0.0

typing of non - typed stones based on the ratios between the characterizing ion and indicating ion/s.

To consider Urate sub type 1, 2, 3 and 4; Oxalate sub types 1, 2 and 3 and Non-infectious Phosphates sub types 1 and 2, the possible ranges between the characterizing and indicating ions were calculated (Table 3). The 49 stones were further sub-typed based on the ratios between the characterizing ion and indicating ion/s ranges given for the sub types by Abdel-Halim, *et al.*[17] On the basis of the calculation, of the four Uric acid/Urate stones, three stones were of UrI 2; all the Oxalate stones (n=41) were of the sub type; OxI 3 and all four Phosphate stones (Non-infectious) were of PhI 1 (Table 3). Among the stones typed based on the method suggested in this paper, the Magnesium stones were not found.

Confirming the types of the stones based on the Fourier Transform Infrared Spectroscopy (FTIR) method: To confirm the possibility of using the method proposed in this paper to type the stones, the stones were analysed by the FTIR method.[16] For this purpose, ten stones among the 51 typed [based on Abdel-Halim, *et al.*[17]] and another ten stones among the 49 non- typed stones were randomly selected for analysis by FTIR.

Table 4: Comparison of the compositions of the Urinary stones analysed by Wet chemical method and Fourier Transform Infrared Spectroscopy method (FTIR), where the stones selected were typed based on the available method* and the method described in this paper.**

Stone Typing	Stone No	Type	Compound/s	
			Wet Chemical Method (%)	FTIR method (%)
Typing*	21	Oxalate	Calcium Oxalate Monohydrate 74.54 / Calcium Oxalate Dihydrate 83.75	Whewellite and Weddellite 80 and Ammonium urate 20
	26	Oxalate	Calcium Oxalate Monohydrate 73.75	Whewellite 80 and Ammonium urate 20
	03	Phosphate	Hydroxy Apatite 77.33 and Calcium Oxalate Dihydrate 14.60	Carbonate apatite 80 and Weddellite 20
	05	Phosphate	Hydroxy Apatite 59.99 and Calcium Oxalate Dihydrate 42.56	Carbonate apatite 50 and Weddellite 50
	33	Magnesium	Magnesium Ammonium Phosphate Hexahydrate (Struvite) 48.96 and Hydroxy apatite 48.13	Struvite 50 and Carbonate apatite 50
	52	Magnesium	Magnesium Ammonium Phosphate Hexahydrate (Struvite) 59.16 and Hydroxy apatite 28.21	Struvite 60, Carbonate apatite 30 and Ammonium urate 10
	62	Magnesium	Magnesium Ammonium Phosphate Hexahydrate (Struvite) 42.84 and Hydroxy apatite 42.77	Struvite 50, Carbonate apatite 30 and Ammonium urate 20
	14	Urate	Urate 65.19 and Calcium Oxalate Monohydrate 28.69	Uric acid 80 and Whewellite 20
	47	Urate	Urate 73.33	Uric acid 50 and Ammonium urate 50
	89	Urate	Urate 69.69 and Calcium Oxalate Monohydrate 16.72	Uric acid 50 and Ammonium urate 50
Typing**	30	Oxalate	Calcium Oxalate Monohydrate 60.16	Whewellite 80, Ammonium carbonate 10 and Ammonium urate 10
	45	Oxalate	Calcium Oxalate Monohydrate 63.62 / Calcium Oxalate Dihydrate 71.48	Whewellite and Weddellite 80 Carbonate apatite 10 and Ammonium urate 10
	56	Oxalate	Calcium Oxalate Monohydrate 63.72 and Hydroxy apatite 11.06	Whewellite 80, Carbonate apatite 10 and Ammonium urate 10
	57	Oxalate	Calcium Oxalate Monohydrate 61.52 / Calcium Oxalate Dihydrate 69.11	Whewellite and Weddellite 80 Carbonate apatite 10 and Ammonium urate 10
	68	Oxalate	Calcium Oxalate Monohydrate 60.41 / Calcium Oxalate Dihydrate 67.86	Whewellite and Weddellite 80 and Newberylite 20
	81	Oxalate	Calcium Oxalate Dihydrate 60.9 and Hydroxy apatite 8.63	Weddellite 50 and Uric acid 50
	69	Phosphate	Calcium Oxalate Monohydrate 23.50 and Hydroxy apatite 40.47	Whewellite 30, Carbonate apatite 50 and Ammonium urate 20
	100	Phosphate	Calcium Oxalate Dihydrate 38.56, Hydroxy apatite 40.95 and Magnesium Ammonium Phosphate Hexahydrate (Struvite) 6.12	Weddellite 40, Hydroxy apatite 50 and Struvite 10
	86	Urate	Calcium Oxalate Dihydrate 40.12 and Urate 15.14	Weddellite 50 and Ammonium urate 50
	90	Urate	Calcium Oxalate Dihydrate 42.32 and Urate 16.43	Weddellite 50 and Ammonium urate 50

Whewellite- Calcium Oxalate Monohydrate
Weddellite - Calcium Oxalate Dihydrate
Struvite - Magnesium Ammonium Phosphate Hexahydrate
Newberylite- Magnesium Hydrogenphosphate Trihydrate

Further the amounts of compounds such as Calcium oxalate monohydrate ($CaC_2O_4 . H_2O$), Magnesium Ammonium Phosphate Hexahydrate [$Mg(NH_4)PO_4 . 6H_2O$] and Hydroxy apatite [$Ca_{10}(PO_4)_6(OH)_2$] were calculated[6] using the ionic compositions of Oxalate, Magnesium, Inorganic Phosphate and Calcium estimated by wet chemical method.

The calculated ionic compositions of the 20 stones analysed by the wet chemical method[21-25] and by the FTIR method[16] fitted well (Table 4).

When the 20 stones were analysed by the FTIR method, majority of the stones contained Whewellite and Weddellite (CaC_2O_4), Carbonate apatite $Ca_5(PO_4,CO_3)_3(OH)$, Ammonium urate ($C_5H_7N_5O_3$) and Struvite ($NH_4MgPO_4 \cdot 6H_2O$) (Table 4).

The urinary stone No. 21, which was typed as Oxalate stone based on the ionic composition obtained by wet chemical method contained Calcium Oxalate Monohydrate / Calcium Oxalate Dihydrate and when it was analysed by FTIR method the results showed that the stone contained Whewellite and Weddellite 80 and Ammonium urate 20 (Table 4). Similar observations were also made with Phosphate, Magnesium and Urate stones.

The results indicated that the new alternative method proposed in this paper is in good agreement with that described by Abdel-Halim, *et al.*[17] to type the urinary stones and with the results obtained with the FTIR. Hence the method proposed in this paper is useful for future typing of the stones especially those which are analysed by wet chemical method and do not have the anion concentrations to comply with Abdel-Halim *et al.*[17]

DISCUSSION

Main objective of this study was to type the 100 urinary stones obtained from the patients who underwent surgical interventions at Genitourinary Surgical Unit of Teaching Hospital, Jaffna using a method described by Abdel-Halim, *et al*[17] and finding an easy alternative method to type the stones which do not comply with the said classification method. As there are no relevant literature available to suggest the stone typing other than those described by Abdel-Halim, *et al.*[17] an attempt was made to study the typing in accordance with their typing while suggesting an alternative calculation. Furthermore the other methods used for the stone typing are expensive and need sophisticated equipment, which are difficult for the third world country researchers to purchase.

The method used by Abdel-Halim *et al*,[17] has considered the concentration of characterising and indicating ions as shown in Table 1. But if the ion concentrations of the stones do not comply with the values, there are no alternative methods to classify the stones. Hence to type the Oxalate stones, Oxalate to Urate ratio between 16.8:1 and 67.7:1; Urate stones, Urate to Oxalate ratio between 0.7:1 and 101.7:1 and Non- infection Phosphate stones, Phosphate to Oxalate ratio between 0.4:1 and 24.4:1 were considered. Furthermore, this newly proposed method for the typing of urinary stones also gave the compositions of the stones similar to the results obtained by the FTIR method.

Based on both the methods of stone typing, of the total 100 stones, 54 stones were Oxalate type, 25 stones were Uric acid / Urate type, 16 stones were Non-infectious Phosphate stones and 05 were Infectious stones.

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