Urol Res (2012) 40:41–46
DOI 10.1007/s00240-011-0389-3

ORIGINAL PAPER

Urinary pH and renal lithiasis

F. Grases · A. Costa-Bauzá · I. Gomila · M. Ramis · A. García-Raja · R. M. Prieto

Received: 9 February 2011 / Accepted: 6 May 2011 / Published online: 19 May 2011
© Springer-Verlag 2011

Abstract Formation of calcium oxalate crystals, either as monohydrate or dihydrate, is apparently unrelated to urinary pH because the solubilities of these salts are practically unaltered at physiologic urinary pH values. However, a urinary pH <5.5 or >6.0 may induce uric acid or calcium phosphate crystals formation, respectively, which under appropriate conditions may induce the development of the calcium oxalate calculi. We assessed the relationship between the urinary pH and the formation of different types of calculi. A retrospective study in 1,478 patients was done. We determined the composition, macrostructure, and microstructure of the calculi and the urinary pH, 50.9% of calcium oxalate monohydrate unattached calculi were present in patients with urinary pH <5.5. We found that 34.1 and 41.5% of calcium oxalate dihydrate calculi were present in patients with urinary pH <5.5 and >6.0, respectively. Infectious calculi were found primarily in patients with urinary pH >6.0 (50.7%). Only calcium oxalate monohydrate papillary calculi were associated with urinary pH between 5.5 and 6.0 (43.1%). Urine of pH <5.5 shows an increased capacity to develop uric acid crystals, which can act as a heterogeneous nuclei of calcium oxalate crystals. In contrast, urine of pH >6.0 has an increased capacity to develop calcium phosphate crystals, which can act as a heterogeneous nuclei of calcium oxalate crystals. Oxalate monohydrate papillary calculi were associated to pH between 5.5 and 6.0 because the injured papilla acts as a heterogeneous nucleant. Consequently, measurement of urinary pH may be used to evaluate the lithogen risk of given urine.

Keywords Urinary pH · Calcium oxalate · Uric acid · Phosphate · Heterogeneous nucleation · Renal lithiasis

F. Grases (✉) · A. Costa-Bauzá · I. Gomila · M. Ramis · A. García-Raja · R. M. Prieto
Laboratory of Renal Lithiasis Research, Universitary Institute of Health Sciences Research (IUNICS), University of Balearic Islands, 07122 Palma de Mallorca, Spain
e-mail: fgrases@uib.es

Introduction

Renal lithiasis is a consequence of crystallization of diverse substances in the urinary tract. Because the uric acid crystallizes at urinary pH <5.5 due to the formation of insoluble undissociated uric acid crystals, uric acid renal stones typically form in individuals with low urinary pH but a normal concentration of uric acid [14]. In contrast, calcium phosphate crystallizes as hydroxyapatite at urinary pH >6.0, together with other factors such as deficit of crystallization inhibitors [8].

Formation of calcium oxalate crystals, either as monohydrate or dihydrate, is apparently unrelated to urinary pH because the solubilities of these salts are practically unaltered at physiologic urinary pH values. Nevertheless, due to the concentrations of calcium and oxalate in urine, the direct formation of crystals does not occur through the homogeneous nucleation [4]. Hence, the presence of heterogeneous nuclei is necessary to induce calcium oxalate crystal development. Crystals of uric acid and hydroxyapatite have demonstrated to act as the heterogeneous nuclei of calcium oxalate [5, 11, 16]. Thus, minute amounts of these crystals may be sufficient to induce the generation of calcium oxalate crystals, resulting in calcium oxalate renal stones, without forming uric or calcium phosphate stones. Therefore a urinary pH <5.5 or >6.0 may induce the formation of uric acid or calcium phosphate crystals,

respectively, which, under appropriate conditions, may induce the development of calcium oxalate renal stones. For this reason, low or high urinary pH may be indirectly related to calcium oxalate stone formation. We, therefore assessed the relationship between the urinary pH and the formation of different types of renal stones. Study of a large series of calculi allows clear correlations to be established between stone composition and internal morphology, on the one hand, and etiopathogenic factors on the other, some of which are related to 24-h urine parameters, thus, for example, hyperoxaluria can be related to calcium oxalate monohydrate calculi formation and hypercalciuria to calcium oxalate dihydrate stones [1, 2, 8].

Materials and methods

Patients

Our study included 1,478 patients over a period of 10 years. We determined the composition, macrostructure, and microstructure of the 1,478 formed calculi as well as the urinary pH of all patients.

Assessments of urinary calculi

Renal calculi were dried, stored in sterile containers, and immediately analyzed by macroscopic and microscopic techniques (Optomic stereoscopic microscopy), along with IR spectrometry (Bruker IFS 66 infrared instrument) and scanning electron microscopy coupled with X-ray microanalysis (Hitachi S-530 in association with an Oxford Link Isis X-ray microanalysis system) [10]. The external aspects of each calculus were first assessed by stereoscopic microscopy. Each calculus was sectioned into two parts along a plane as near as possible to the geometric center, and its internal structure was examined. When a calculus is analyzed after surgical fragmentation, all fragments were examined by stereoscopic microscopy to determine the form of the original calculus and its internal structure. These approaches usually indicate what further techniques should be applied, including IR spectrometry of one or several fragments. If the fragments of a calculus differed in appearance, it was necessary to perform IR analysis on representative fragments, usually by the KBr method, which requires less than 1 mg of sample. If necessary, the fine inner structure of a calculus was examined by scanning electron microscopy coupled with X-ray microanalysis to detect and identify the microcomponents. The presence of a substance in minute quantities, not identifiable by conventional IR spectrometry, may be decisive in establishing the etiology of calculus formation. Thus, to determine the importance of any given microcomponent, an accurate knowledge of calculus fine structure is essential so that the initial zone of calculus development can be identified. This zone is one of the keys to calculus formation. This methodology involved the fixing of calculus fragments on a microscope slide using a silver salt. The sample was gold-spattered (with a film 300 Å in thickness) and examined at 30–20,000× magnification. The combination of this technique with X-ray microanalysis was of great assistance in identifying the particular microcomponents. Because of the small size and hardness of papillary calculi, most were obtained as spontaneously passed stones. In fact, all papillary calculi included in the present study were non-fragmented. Portions of several calculi of diameter greater than 1 cm were obtained after surgical fragmentation, as indicated above. However, when only a few fragments were available, it was not possible to establish the original structure of the calculus; hence, data on such stones are not included in the present study.

Assessments of calcium oxalate monohydrate (COM) calculi (either papillary or unattached) began with the direct examination of their external aspects by stereoscopic microscopy. Each calculus was subsequently sectioned into two parts along a plane as near as possible to the geometric center, to establish its internal structure and identify its core.

A typical papillary COM stone consists of an eccentric core located near the concave region, where it is attached to a papilla, and a radially striated convex peripheral layer [10, 13]. Scanning electron microscopic analysis allows the detection of microcomponents present in the core and confirms the papillary origin of the calculus by the examination of concave external cavity. Thus, the presence of abundant organic matter and tubular apical cells identify a point of attachment to a renal papilla. A typical unattached COM calculus consists of a symmetrical round stone with a central core surrounded by columnar COM crystals emerging from the core, and by the complete absence of any site of epithelial stone attachment.

Our methodology allowed us to distinguish the authentic COM calculi (reflecting the initially formed crystalline phase) from the COM calculi derived from crystalline transformation of an initially formed calcium oxalate dihydrate (COD) calculus. Thus, the SEM clearly showed that the latter type of calculus exhibits a typically disorganized noncompact structure with clearly identifiable COD phantoms [13].

The methodology described above allowed the classification of renal calculi into 11 main types (Table 1) [8]. All calculi contain several components; however, in most instances, all except a single component are minor constituents (less than 10% of total by weight). Our classification [8] considers a calculus to be of mixed composition when at least 10% of the entire calculus is composed of one or more components.

Table 1 Type, composition, quantity and percentage of calculi generated in 1,478 patients over a 10-year period

Type	Composition	*N*	%
1	Calcium oxalate monohydrate papillary (COM-p)	137	9.27
2	Calcium oxalate monohydrate unattached (COM-u)	224	15.16
3	Calcium oxalate dihydrate (COD)	511	34.57
4	Calcium oxalate dihydrate/ hydroxyapatite mixed (COD/HAP)	198	13.40
5	Hydroxyapatite (HAP)	118	7.98
6	Magnesium ammonium phosphate or struvite (STR)	97	6.56
7	Calcium hydrogen phosphate dihydrate or brushite (BRU)	6	0.41
8	Uric acid (UA)	134	9.07
9	Calcium oxalate/uric acid mixed (COM/UA)	53	3.59
10	Cystine	0	0
11	Other	0	0
	Total	1,478	100

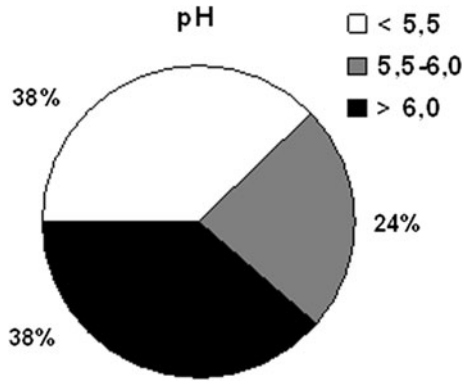

Fig. 1 Percentage of the 1,478 calculi associated with urinary pH <5.5, 5.5–6.0 and >6.0

Analysis of urine samples

All subjects were on unrestricted diets at the time of urine collection and none was receiving pharmacological treatment of any kind. After overnight fasting, 2-h urine samples were collected, and the pH of each was immediately measured using a daily calibrated glass electrode (pH-meter, Crison S.L. Barcelona. Spain). Only 2-h urine samples were used for pH measurements because urinary pH can change owing to precipitation of calcium salts over 24 h of storage. Moreover, overnight fasting 2-h urine samples better represent urinary basal pH because they are minimally affected by dietary factors [8]. Urine samples were also collected 1–2 months after stone passage/removal.

Statistics

The percentage of each type of renal calculus at urinary pH >6.0, 5.5–6.0 and <5.5 was compared using non-parametric χ^2 tests. A p value <0.001 was considered significant.

Results

The percentages of each type of calculus are shown in Table 1. Of the 1,478 renal calculi examined, 34.57% were COD calculi, the largest percentage. In contrast, only 0.41% was brushite (BRU) calculi (type 7) and there were no cystine (type 10) or other (type 11) calculi. We therefore omitted these three types from further consideration.

As shown in Fig. 1, 38% of renal calculi were in patients with urinary pH >6.0, 37.8% were in patients with urinary pH <5.5, and only 23.7% were in patients with urinary pH between 5.5 and 6.0.

The distribution and the percentage of each type of renal calculus associated with each urinary pH interval (pH >6.0, 5.5–6.0 and <5.5) are shown in Fig. 2. As expected, 53% of COD/hydroxyapatite (HAP) mixed calculi and 66.1% of HAP calculi were in individuals with urinary pH >6.0. In contrast, 64.9% of uric acid calculi (UA) and 60% of COM/UA mixed calculi were in individuals with urinary pH <5.5. Interestingly, 50.9% of calcium oxalate monohydrate unattached calculi (COM-u) were present in patients with urinary pH <5.5. We found that 34.1 and 41.5% of COD calculi were present in patients with urinary pH <5.5 and >6.0, respectively. Infectious calculi or struvite calculi (STR) were found primarily in patients with urinary pH >6.0. Only calcium oxalate monohydrate papillary calculi (COM-p) were associated with urinary pH between 5.5 and 6.0 (43.1%).

Table 2 shows the comparisons of the percentage distribution of each type of calculus among the three urinary pH intervals (see Fig. 2). A p value <0.001 indicated a significant difference in the distribution of each type of calculus among the three urinary pH intervals. The distribution of COM-p calculi among the three pH intervals differed from that of the other types of renal calculi. The distribution of COM-u, UA and COM/UA calculi were similar among the three urinary pH intervals. We found that 50.9% of COM-u calculi, 64.9% of UA calculi, and 60.4% of COM/UA calculi were in patients with urinary pH <5.5 (Fig. 2). The distributions of COD, COD/HAP and STR calculi were similar; 66.5% of HAP calculi, 53% of COD/HAP calculi, and 50.7% of STR calculi were present in patients with urinary pH >6.0 (Fig. 2).

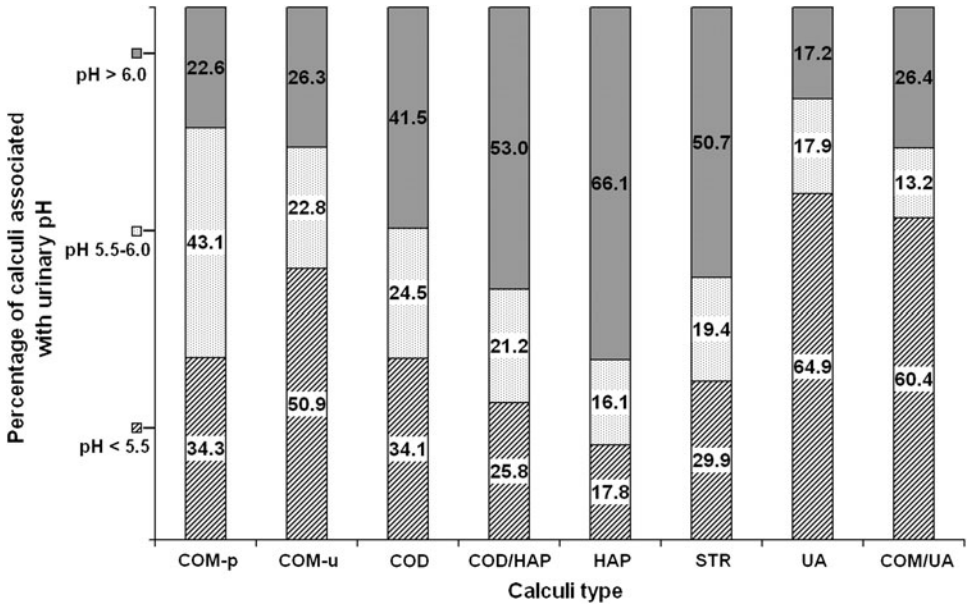

Fig. 2 Percentage of distribution of each type of calculus in patients with urinary pH <5.5, 5.5–6.0 and >6.0. COM-p or type 1, COM-u or type 2, COD or type 3, COD/HAP mixed or type 4, HAP or type 5, STR or type 6, UA or type 8, COM/UA mixed or type 9. $N = 1,478$

Table 2 Distribution of each type of calculus relative to the three urinary pH intervals, as determined using χ^2 test

χ^2	Type of calculi							
p	1	2	3	4	5	6	8	9
Type	COM-p	COM-u	COD	COD/HAP	HAP	STR	UA	COM/UA
1		17.21	23.42	33.29	49.58	18.58	27.85	16.29
COM-p		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2	17.21		21.15	36.36	53.63	14.84	6.97	2.60
COM-u	<0.001		<0.001	<0.001	<0.001	<0.001	ns	ns
3	23.42	21.15		7.99	23.69	2.14	44.22	14.43
COD	<0.001	<0.001		ns	<0.001	ns	<0.001	<0.001
4	33.29	36.36	7.99		5.24	0.44	56.60	22.77
COD/HAP	<0.001	<0.001	ns		ns	ns	<0.001	<0.001
5	49.58	53.63	23.69	5.24		4.74	70.13	32.3
HAP	<0.001	<0.001	<0.001	ns		ns	<0.001	<0.001
6	18.58	14.84	2.14	0.44	4.74		28.14	11.42
STR	<0.001	<0.001	ns	ns	ns		<0.001	ns
8	27.85	6.97	44.22	56.60	70.13	28.14		2.27
AU	<0.001	ns	<0.001	<0.001	<0.001	<0.001		ns
9	16.29	2.60	14.43	22.77	32.3	11.42	2.27	
COM/UA	<0.001	ns	<0.001	<0.001	<0.001	ns	ns	

Discussion

The development of calcium oxalate crystals in the urine takes place through a heterogeneous nucleation process [4]. Thus, the presence of an adequate number of solid preformed microparticles is an indispensable condition for the formation of the corresponding stones. At urinary pH <5.5, UA becomes insoluble and forms crystals of anhydrous or dihydrate UA, depending on the concentrations [9]. If the amounts of crystallized UA are considerable, UA or COM/UA stones will form. As expected, these types of stones were more prevalent in patients with urinary pH <5.5 than in those with higher urinary pH. If the amount of formed uric crystals is small, it can be eliminated as asymptomatic crystaluria. However, due to the ability of anhydrous UA crystals to act as a heterogeneous nucleant of COM, the development of these types of calculi may be favored under appropriate conditions of calcium oxalate supersaturation and a deficiency of crystallization inhibitors [5, 8].

Indeed, the capacity of UA to serve as a heterogeneous nucleant of COM crystals is superior to that of mucin (a glycoprotein) or cellular detritus, but inferior to that of some calcium salts [5]. Moreover, UA was detected, as a minor component, in the core of unattached COM renal calculi, indicating that the core was principally formed by COM crystals, UA, and organic matter [7]. In such calculi, UA probably played an important role as a heterogeneous nucleant of COM crystals, which in turn formed the core of the calculus. Thus, UA was ultimately responsible for calculus formation. Hence, it is not surprising that the majority of COM-u renal stones developed in patients with urinary pH <5.5.

At urinary pH >6.0, calcium phosphates (hydroxyapatite and brushite) form insoluble crystals. If large amounts are present, HAP or BRU stones will develop as shown here. Indeed, we found that there were high percentage of these stones in patients with urinary pH >6.0. If smaller amounts of these crystals are present, they cannot form these types of stones. However, due to their high capacity to induce COD heterogeneous nucleation [16], they may favor the formation of corresponding calculi. Indeed, we found that COD calculi were present primarily in patients with urinary pH >6.0.

We also found that STR (infectious) calculi were present mainly in patients with urinary pH >6.0. Although these types of calculi are clearly related to the infected urine, bacterial colonization is more prevalent at high pH. Thus, indirectly, these high pH values also favor the development of these types of renal stones, with acidification being a prophylactic measure against bacterial infection and the formation of these stones.

Papillary calculi (COM-p) were the only type associated with the urinary pH between 5.5 and 6.0. These pH values can be considered as a safe zone, at which no heterogeneous nuclei of UA or calcium phosphate crystals can appear. The injured papillary epithelium acts, through Randall's plaques or other alterations, as a heterogeneous nucleant of COM [3, 6]; thus not requiring the presence of preformed solid particles in the urine.

We found that similar percentages of renal calculi were associated with urinary pH <5.5 and >6.0 (37.8 and 38.4%, respectively), with a smaller percentage (23.7%) associated with urinary pH between 5.5 and 6.0. Dietary modifications, including the consumption of foods that lower the urinary pH such as meat, fish, bananas, whole rice, oatmeal, seafood, eggs, ammonium chloride and arginine, or foods that increase the urinary pH such as citrus fruits, kiwifruit, fruit juices, vegetables, potatoes, carbonated beverages and sodium bicarbonate, may therefore adjust the urinary pH values and reduce the stone formation in each case [12, 15].

Human urine of pH <5.5 shows an increased capacity to develop the UA crystals, which can act as heterogeneous nuclei of calcium oxalate crystals. This has an interesting clinical implications. A significant number of COM-u calculi appear to be *pure,* because UA is undetectable in the core (as a result of limitations of the analytical methods routinely employed). Nevertheless, UA may make an important contribution to the formation of such calculi when urinary pH values are less than pH 5.5. In contrast, human urine of pH >6.0 has an increased tendency to develop the calcium phosphate crystals, which can act also as heterogeneous nuclei of calcium oxalate crystals. In urine of pH 5.5–6, UA or calcium phosphate cannot act as heterogeneous nuclei for calcium oxalate crystallization, and it is thus necessary to search for other heterogeneous nucleants, principally when considering COM-u calculi. Such nucleants may be organic matter or insoluble drugs. The presented results emphasizes the important role of pH as a determinant not only for UA and apatite stone formation, but also indirectly, through heterogeneous nucleation processes, for different calcium oxalate stone types. Consequently, accurate and precise measurement of the urinary pH may be used to evaluate the lithogen risk of given urine, even in calcium oxalate stone-formers.

Acknowledgments This work was supported by Project Grant CTQ 2010-18271 from the *Ministerio de ciencia e innovación del Gobierno de España*. I.G. expresses her appreciation to the *Conselleria d'Innovació i Energia del Govern de les Illes Balears* (Spain) for a fellowship supporting her work.

References

1. Daudon M, Bouzidi H, Bazin D (2010) Composition and morphology of phosphate stones and their relation with etiology. Urol Res 38:459–467
2. Daudon M, Bader CA, Jungers P (1993) Urinary calculi: review of classification methods and correlations with etiology. Scan Microsc 7:1081–1104 (discussion 1104–1106)
3. Evan AP, Coe FL, Lingeman JE, Shao Y, Sommer AJ, Bledsoe SB, Anderson JC, Worcester EM (2007) Mechanism of formation of human calcium oxalate renal stones on Randall's plaque. Anat Rec (Hoboken) 290:1315–1323
4. Finlayson B (1978) Physicochemical aspects of urolithiasis. Kidney Int 13:344–360
5. Grases F, Sanchis P, Isern B, Perello J, Costa-Bauza A (2007) Uric acid as inducer of calcium oxalate crystal development. Scand J Urol Nephrol 41:26–31
6. Grases F, Costa-Bauza A, Gomila I, Conte A (2010) Origin and types of calcium oxalate monohydrate papillary renal calculi. Urology 76:1339–1345
7. Grases F, Sanchis P, Perello J, Costa-Bauza A (2006) Role of uric acid in different types of calcium oxalate renal calculi. Int J Urol 13:252–256
8. Grases F, Costa-Bauza A, Ramis M, Montesinos V, Conte A (2002) Simple classification of renal calculi closely related to their micromorphology and etiology. Clin Chim Acta 322:29–36
9. Grases F, Villacampa AI, Costa-Bauza A, Sohnel O (2000) Uric acid calculi: types, etiology and mechanisms of formation. Clin Chim Acta 302:89–104
10. Grases F, Garcia-Ferragut L, Costa-Bauza A (1998) Analytical study of renal calculi: a new insight. Recent Res Dev Pure Appl Anal Chem 1:187–206

11. Grases F, Kroupa M, Costa-Bauza A (1994) Studies on calcium oxalate monohydrate crystallization: influence of Inhibitors. Urol Res 22:39–43
12. Grases F, Costa-Bauza A, Prieto R (2006) Renal lithiasis and nutrition. Nutrition J 5:23
13. Iwata H, Iio S, Nishio S, Takeuchi M (1992) Architecture of mixed calcium oxalate dihydrate and monohydrate stones. Scan Microsc 6:231–237 (discussion 237–238)
14. Liebman SE, Taylor JG, Bushinsky DA (2007) Uric acid nephrolithiasis. Curr Rheumatol Rep 9:251–257
15. Remer T, Manz F (1995) Potential renal acid load of foods and its influence on urine pH. J Am Diet Assoc 95:791–797
16. Wu W, Gerard DE, Nancollas GH (1999) Nucleation at surfaces: the importance of interfacial energy. J Am Soc Nephrol 10(Suppl 14):S355–S358