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ORIGINAL PAPER

Preservation of urine samples for metabolic evaluation of stone-forming patients

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Abstract Metabolic evaluation of stone-forming (SF) patients is based on the determination of calcium, oxalate, citrate, uric acid and other parameters in 24-h urine samples under a random diet. A reliable measurement of urinary oxalate requires the collection of urine in a receptacle containing acid preservative. However, urinary uric acid cannot be determined in the same sample under this condition. Therefore, we tested the hypothesis that the addition of preservatives (acid or alkali) after urine collection would not modify the results of those lithogenic parameters. Thirty-four healthy subjects (HS) were submitted to two non-consecutive collections of 24-h urine. The first sample was collected in a receptacle containing hydrochloric acid (HCl 6 N) and the second in a dry plastic container, with HCl being added as soon as the urine sample was received at the laboratory. Additionally, 34 HS and 34 SF patients collected a spot urine sample that was divided into four aliquots, one containing HCl, another containing sodium bicarbonate ($NaHCO_3$ 5 g/l), and two others in which these two preservative agents were added 24 h later. Urinary oxalate, calcium, magnesium, citrate, creatinine and uric acid were determined. Urinary parameters were also evaluated in the presence of calcium oxalate or uric acid crystals. Mean values of all urinary parameters obtained from previously acidified 24-h urine samples did not differ from those where acid was added after urine collection. The same was true for spot urine samples, with the exception of urinary citrate that presented a slight albeit significant change of 5.9% between samples in HS and 3.1% in SF. Uric acid was also not different between pre- and post-alkalinized spot urine samples. The presence of crystals did not alter these results. We concluded that post-delivery acidification or alkalinization of urine samples does not modify the measured levels of urinary oxalate, calcium, magnesium, creatinine and uric acid, and that the change on citrate was not relevant, hence allowing all parameters to be determined in a single urine sample, thus avoiding the inconvenience and cost of multiple 24-h urine sample collections.

Keywords Urinary preservatives · Metabolic evaluation · Oxalate · Calcium · Kidney stones

Introduction

The routine laboratory investigation among urolithiasis patients includes the determination of urinary parameters involved in stone formation such as urinary calcium (uCa), oxalate (uOx), magnesium (uMg), citrate (uCit), uric acid (uUrAc), sodium (uNa), potassium (uK) and creatinine (uCreat) [1, 2].

It has been suggested that for a more reliable uOx measurement, urine must be collected in a receptacle containing hydrochloric acid (HCl) as a preservative, in order to ensure the complete dissolution of calcium oxalate crystals [3], to prevent calcium- and magnesium phosphate salt precipitation, and to counteract oxidation of ascorbate to oxalate [4–6]. In acidified samples, determinations of uCa, uMg, uCit and uCreat

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can also be performed. However, when acid is added to the plastic container before urine collection, another important parameter, such as uUrAc, cannot be determined in the same urine sample, since previous alkalinization with sodium bicarbonate would be necessary for determination of the latter. In addition, when utilizing ion-selective electrode method, uNa and uK also cannot be determined in acidified or alkalinized samples, unless the samples are previously diluted in a buffer.

A complete urinary metabolic profile to guide prophylaxis of stone recurrence has lately been advocated to be time-consuming and expensive [7]. According to Pak et al. [8], a simple metabolic evaluation consisting of a single 24-h urine collection for analysis of all urinary stone risk factors is sufficient and cost-effective [9] for a medical evaluation of urolithiasis, although Parks et al. [10] and others [11] reported that only one specimen may lead to misdiagnosis of common metabolic disturbances.

The aim of the present study was to test whether the addition of acid or alkali preservatives when the urine specimen is delivered at the laboratory would interfere with the results of urinary parameters compared to previously acidified or alkalinized urine samples.

Methods

Thirty-four (34) healthy subjects (HS) (14 M/20 F, 32 ± 11 years old), participated in the study. They were instructed to abstain from vitamin C supplements to avoid interference on urinary oxalate results. Two 24-h urine samples were obtained on two non-consecutive occasions for determination of uCa, uOx, uMg and uCit. The first sample was collected in a vessel containing acid preservative, hydrochloric acid (HCl 6 N, 20 ml/l). This amount of acid has been reported to be sufficient to decrease urinary pH to values between 1.0 and 2.0 [12–16]. The second sample was collected a week later in a dry plastic container, with HCl being added as soon as the urine sample was delivered to the laboratory. Medical treatment was withdrawn 72 h before urine collection.

Additionally, 34 HS (15 M/19 F, 31 ± 8 years old) and 34 stone-forming (SF) patients (19 M/15 F, 43 ± 11 years old) collected a single spot urine sample that was divided into four aliquots of 10 ml each: one containing 200 µl of HCl, another containing 200 µl of sodium bicarbonate, $NaHCO_3$ (5 g/l), and two others in which the same preservative agents were added 24 h after the collection. Data concerning the definition of distinct metabolic diagnosis in SF patients (hypercalciuria, hyperuricosuria, hypocitraturia and hyperoxaluria) were obtained from the results of 24-h urine samples contained in the patients' medical records. Hypercalciuria was defined as uCa $\geq$ 4 mg/kg of body weight in 24 h, hyperuricosuria as uUrAc > 750 or 800 mg/24 h (for female or male, respectively), hypocitraturia as uCit < 320 mg/24 h, and hyperoxaluria as uOx > 45 mg/24 h. Normal ranges were based on literature data as previously described [17]. The study was approved by the Ethics Committee of Universidade Federal de São Paulo.

Crystallization experiments

In order to demonstrate if post-acidified urine samples would differ from pre-acidified ones in the presence of CaOx crystals with respect to uOx, uCit, uCa and uCreat values, two sets of crystallization experiments were undertaken. In the first, male healthy subjects ($n = 20$, 31 ± 9 years old) collected a single spot urine sample in which CaOx exogenous precipitation was induced by addition of 200 µl of NaOx solution (0.1 M) in 10 ml of urine. In the second, urinalysis ($n = 10$, 7 M/3 F, 43 ± 13 years old) in which endogenous CaOx crystals had been evidenced in our laboratory was selected for crystal counting. The initial amount of crystals (basal) was quantified in a Neubauer chamber and visualized in an Olympus BX-60 microscope (200×). Soon after counting, the samples were divided into two aliquots: one in which 200 µl of HCl was added immediately and the other in which the same preservative agent was added 24 h later. A new crystal counting was performed after 24 h in these two aliquots. Urinary levels of oxalate, calcium, citrate and creatinine were determined in both samples.

Finally, in order to demonstrate if post-alkalinized urine samples would differ from pre-alkalinized ones in the presence of uric acid crystals with respect to the uUrAc determination, a third set of crystallization experiments was performed. Uric acid crystals were prepared as described elsewhere [18]. Briefly, a uric acid solution was prepared by dissolving 100 mg of uric acid (Sigma, St Louis, MO) in 250 ml of hot distilled water. A 250-ml volume of ethanol was added and the solution was allowed to cool to room temperature with stirring overnight. The resulting crystal suspension was filtered, washed twice with ethanol, twice with acetone, and finally air-dried. Uric acid exogenous precipitation was induced by addition of 200 µl of a 0.1 M solution (in which previously formed crystals had been re-suspended) in 10 ml of urine obtained from male healthy subjects ($n = 10$, 30 ± 8 years old). The initial amount of crystals (basal) was quantified and the samples were

divided into two aliquots in which 200 µl of $NaHCO_3$ was added immediately or 24 h later. A new crystal counting was performed after 24 h in the two aliquots and uUrAc and uCreat were determined in both samples.

Calcium and magnesium were determined by atomic absorption spectrophotometry (Perkin Elmer Atomic Spectrophotometer 3110, Norwalk, CT, USA); oxalate by an enzymatic method [19] using the Sigma Oxalate Diagnostic Kit (Sigma, St Louis, MO); and citrate by an enzymatic assay using citrate lyase [20]. Creatinine was determined by Jaffe's method [21], and uric acid by the uricase method [22]. The variation coefficient (CV) of our laboratory was: 1.5% for uOx, 2.5% for uCa, 6.9% for uMg, 8.4% for uCreat, 6.8% for uUrAc, and 13.4% for uCit.

Table 1 Mean values of urinary parameters in 24-h urine samples previously acidified or not

Urinary parameters (mg/24 h)	Healthy subjects ($n = 34$)	
	HCl before	HCl after
Oxalate	27 ± 12	29 ± 13
Calcium	158 ± 84	150 ± 83
Citrate	510 ± 251	461 ± 94
Magnesium	71 ± 29	66 ± 38

Data are presented as mean ± SD

Statistical analysis

The values of all urinary parameters were submitted to a normality test (Kolmogorov–Smirnov). As most of the parameters did not present a normal curve distribution (with exception of uCa and uCreat in 24-h urine samples), nonparametric tests were then chosen. The Wilcoxon test was used for comparison between samples with or without previous addition of HCl or $NaHCO_3$. The Friedman test was used to compare the results of crystallization experiments. Results are reported as mean ± SD. The null-hypothesis of no difference in the values obtained by the two forms of urine preservation was proposed. All statistical tests were performed at a significance level of $P < 0.05$ and the 95% confidence level was determined where appropriate. Finally, Bland–Altman graphical analysis was utilized to further compare the two methods by plotting the differences between previous versus later addition of preservatives against the average of the two values.

Results

Table 1 shows the mean urinary parameters in 24-h urine samples. No significant differences were detected between the values obtained under either previous acidification or not. In spot urine samples (Table 2),

Table 2 Mean values of urinary parameters in spot urine samples previously acidified/alkalinized or not

Urinary parameters	Healthy subjects ($n = 34$)		Stone-forming patients ($n = 34$)	
	HCl before	HCl after	HCl before	HCl after
Oxalate				
mg/l	21 ± 10	20 ± 9	22 ± 10	21 ± 10
mg/mg creat	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.01
Calcium				
mg/l	122 ± 77	122 ± 77	105 ± 61	106 ± 62
mg/mg creat	0.15 ± 0.09	0.15 ± 0.08	0.14 ± 0.08	0.14 ± 0.09
Citrate				
mg/l	362 ± 201	333 ± 188*	275 ± 173	262 ± 166 *
mg/mg creat	0.42 ± 0.22	0.39 ± 0.23*	0.33 ± 0.21	0.32 ± 0.19 *
Magnesium				
mg/l	57 ± 28	56 ± 28	49 ± 26	49 ± 27
mg/mg creat	0.07 ± 0.02	0.07 ± 0.02	0.06 ± 0.03	0.06 ± 0.03
Creatinine				
mg/l	924 ± 494	935 ± 514	874 ± 431	881 ± 445
	$NaHCO_3$ before	$NaHCO_3$ after	$NaHCO_3$ before	$NaHCO_3$ after
Uric acid				
mg/l	453 ± 229	445 ± 65	398 ± 170	404 ± 185
mg/mg creat	0.50 ± 0.11	0.49 ± 0.09	0.63 ± 0.79	0.63 ± 0.80

Data are presented as mean ± SD; *$P < 0.05$ versus HCl before

the mean values of uOx, uCa, uMg, uCreat and uUrAc from both HS and SF patients also did not differ if the preservatives had been added previously or later. A very slight but significantly lower mean value was observed for uCit determined in spot urine samples when HCl was added 24 h later either in HS (0.39 ± 0.23 vs. 0.42 ± 0.22 mg/mg uCreat) or SF groups (0.32 ± 0.19 vs. 0.33 ± 0.21 mg/mg uCreat).

Figure 1 shows the individual values of all these urinary parameters. As shown in Table 3, when the SF patients were analyzed according to their metabolic disturbances, no alterations were observed in the results of oxalate among the hyperoxaluric patients, of uric acid among the hyperuricosuric group, and of calcium among the hypercalciuric group. In the hypocitraturic group, a very mild but significant lower mean

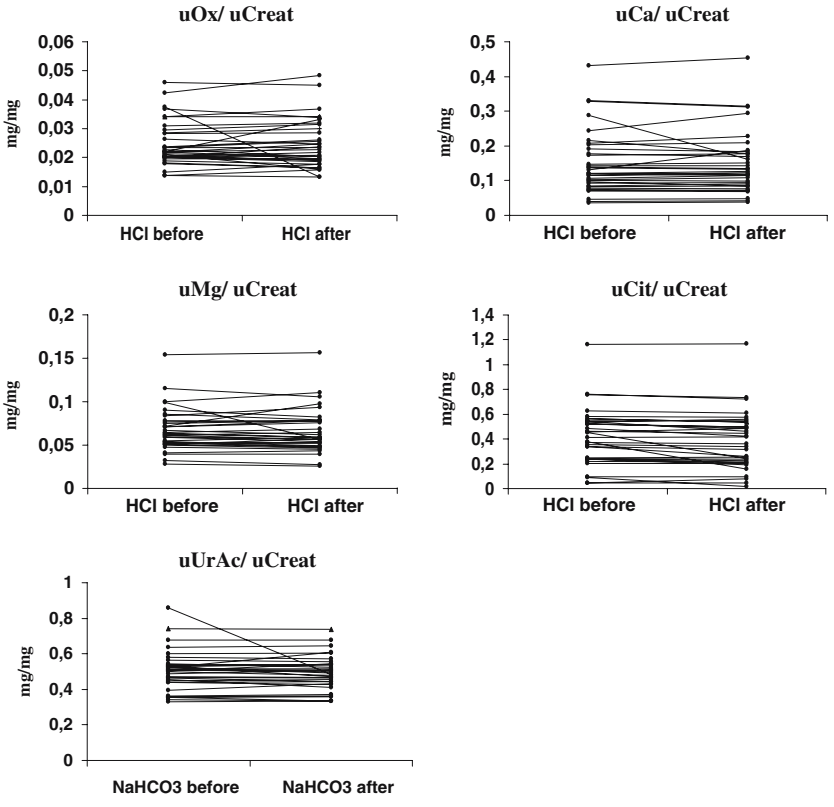

Fig. 1 Individual values of urinary oxalate, calcium, magnesium, citrate and uric acid in spot urine samples of SF patients when $HCl/NaHCO_3$ were added before or 24 h after the collection

Table 3 Mean urinary parameters in spot urine samples with or without previous addition of HCl or $NaHCO_3$ in SF patients according to their metabolic disturbances

	Oxalate		Calcium		Citrate		Magnesium		Uric Acid	
	Before	After	Before	After	Before	After	Before	After	Before	After
Hypercalciuria (n=10)										
mg/l	17 ± 10	17 ± 10	80 ± 41	81 ± 43	245 ± 173	238 ± 170 *	40 ± 25	38 ± 24	424 ± 163	437 ± 196
mg/mg creat	0.03 ± 0.01	0.03 ± 0.01	0.14 ± 0.09	0.14 ± 0.09	0.30 ± 0.17	0.28 ± 0.16	0.05 ± 0.02	0.05 ± 0.02	1.26 ± 1.53	1.26 ± 1.54
Hypocitraturia (n=10)										
mg/l	22 ± 7	23 ± 8	122 ± 74	124 ± 78	380 ± 154	348 ± 163 *	49 ± 22	49 ± 21	420 ± 131	425 ± 135
mg/mg creat	0.03 ± 0.01	0.03 ± 0.01	0.12 ± 0.07	0.13 ± 0.08	0.48 ± 0.29	0.43 ± 0.28 *	0.05 ± 0.02	0.05 ± 0.02	0.47 ± 0.11	0.47 ± 0.11 *
Hyperuricosuria (n=8)										
mg/l	23 ± 7	20 ± 6 *	99 ± 66	101 ± 67	186 ± 89	182 ± 95	49 ± 23	47 ± 22	316 ± 177	312 ± 166
mg/mg creat	0.03 ± 0.01	0.03 ± 0.01 *	0.14 ± 0.07	0.14 ± 0.08	0.28 ± 0.17	0.28 ± 0.18	0.07 ± 0.03	0.07 ± 0.03	0.42 ± 0.23	0.42 ± 0.22
Hyperoxaluria (n=3)										
mg/l	34 ± 19	32 ± 20	104 ± 47	107 ± 46	290 ± 180	302 ± 192	66 ± 52	75 ± 59	490 ± 144	447 ± 90
mg/mg creat	0.03 ± 0.02	0.03 ± 0.02	0.11 ± 0.06	0.12 ± 0.06	0.31 ± 0.19	0.34 ± 0.21	0.07 ± 0.05	0.08 ± 0.06	0.50 ± 0.13	0.49 ± 0.06

Data are presented as mean ± SD; *$P < 0.05$ versus $HCl/NaHCO_3$ before

value of uCit was again observed when HCl was added 24 h later (0.48 ± 0.29 vs. 0.43 ± 0.28 mg/mg uCreat). The method-related differences in the results of the investigated parameters were listed against the respective CV for each urinary parameter in our laboratory in Table 4. The Bland–Altman plots (Fig. 2) showed a proportional bias between the two methods. The mean bias ± 1.96 standard deviations ranged between −5.2 and 6.1 mg/l for uOx, −13.2 and 10.2 mg/l for uCa, −7.8 and 8.1 mg/l for uMg, −44.6 and 71.0 mg/l for uCit, −101.7 and 88.4 mg/l for uCreat, and −83.1 and 72.7 mg/l for uUrAc.

As shown in Fig. 3, mean values of endogenous CaOx crystal counting did not differ between the prompt or (24 h) later HCl addition but had significantly decreased for both in comparison with the basal counting (2.72 ± 1.98 and 3.61 ± 1.97 vs. 5.10 ± 0.56, $\log_{10}$ crystals/ml, respectively). The same has been observed for exogenous CaOx crystals (3.28 ± 0.86 and 3.16 vs. 0.84 vs. 4.04 ± 0.38, $\log_{10}$ crystals/ml). However, it is noteworthy that complete dissolution of crystals did not take place in these crystallization experiments. Since we did not measure the final urine pH in the latter experiment, and the lack of complete dissolution of crystals might have been ascribed to inadequacies on the achieved urinary pH, an ancillary experiment aimed to reduce pH at different levels and to count crystals was performed. A single spot urine sample was obtained from a male normal volunteer and a further one divided into ten aliquots of 10 ml each in which the exogenous crystals were added according to the procedures previously described in the Methods section. Increasing amounts of HCl were added to the aliquots (0, 25, 50, 100, 200, 400, 500, 1,000, 2,000 and 4,000 µl) and the pH values as well as the amount of crystals were then determined in each of these 10 aliquots. The obtained results are shown in Fig 4. This figure clearly shows that even when pH reached values as low as 0.4, using huge amounts of HCl (4,000 µl), the amount of crystals did not decrease significantly. This ancillary experiment also confirmed that the amount we had previously tested (200 µl, HCl 6 N, 20 ml/l) is able to reduce urinary pH to 1.2.

In the uric acid crystallization experiments, mean values of exogenous uric acid crystal counting did not differ between the prompt or (24 h) later addition of $NaHCO_3$, neither in comparison with basal values (4.15 ± 0.20 and 4.32 ± 0.20 vs. 4.33 ± 0.21, $\log_{10}$ crystals/ml). Table 5 shows that no differences had occurred in urinary parameters evaluated in pre- or post-acidified/alkalinized urine samples under any crystallization conditions. The extremely high levels of uOx observed in both pre- and post-acidified samples in the presence of exogenous CaOx crystals, as opposed to endogenous crystals, was consequent to the addition of NaOx solution to these samples, necessary to induce crystallization.

Table 4 Mean values of method-related percentual differences for each urinary parameter and the respective variation coefficient (CV) of the laboratory

Urinary parameters	Healthy subjects	Stone-forming patients	Variation coefficient (CV)
Oxalate	0.1	1.4	1.5
Calcium	0.3	1.3	2.5
Citrate	5.9	3.1	13.4
Magnesium	2.0	4.2	6.9
Creatinine	1.2	0.7	8.4
Uric Acid	1.4	2.0	6.8

Discussion

The addition of stabilizers and the conditions under which urine is collected and stored are important aspects to consider in the determination of 24-h urinary parameters excretion. Some authors reported that the addition of acid reducing urinary pH to values around 1.0 or 2.0 prior to urine collection is required for oxalate measurement in order to avoid the formation of calcium oxalate crystals [3, 12–16, 23, 24], and to counteract oxidation of ascorbate to oxalate [4–6].

Considering that metabolic disturbances such as hyperoxaluria, hypercalciuria, and hypocitraturia may present variation according to diet and other environmental factors, sometimes two or three 24-h urine samples must be obtained for the determination of abnormalities of importance for stone formation [6, 10, 11], despite the fact that some authors suggest that one single sample is enough [7, 8]. Anyway, due to the methodological aspects concerning the need of acidification for some parameters, alkalinization for others, the number of necessary samples may increase, raising the cost and causing the inconvenience of multiple collections.

In the present study, we did not observe statistically different results or clinical relevant differences between urinary parameters obtained from 24-h urine samples collected under previous acidification or not. In addition, the lack of difference occurred when the same subject collected two different samples and also when two aliquots of the same individual were either promptly submitted to analysis or analyzed only after addition of acid or alkali 24 h later. The analysis accuracy performed comparing the present method (later

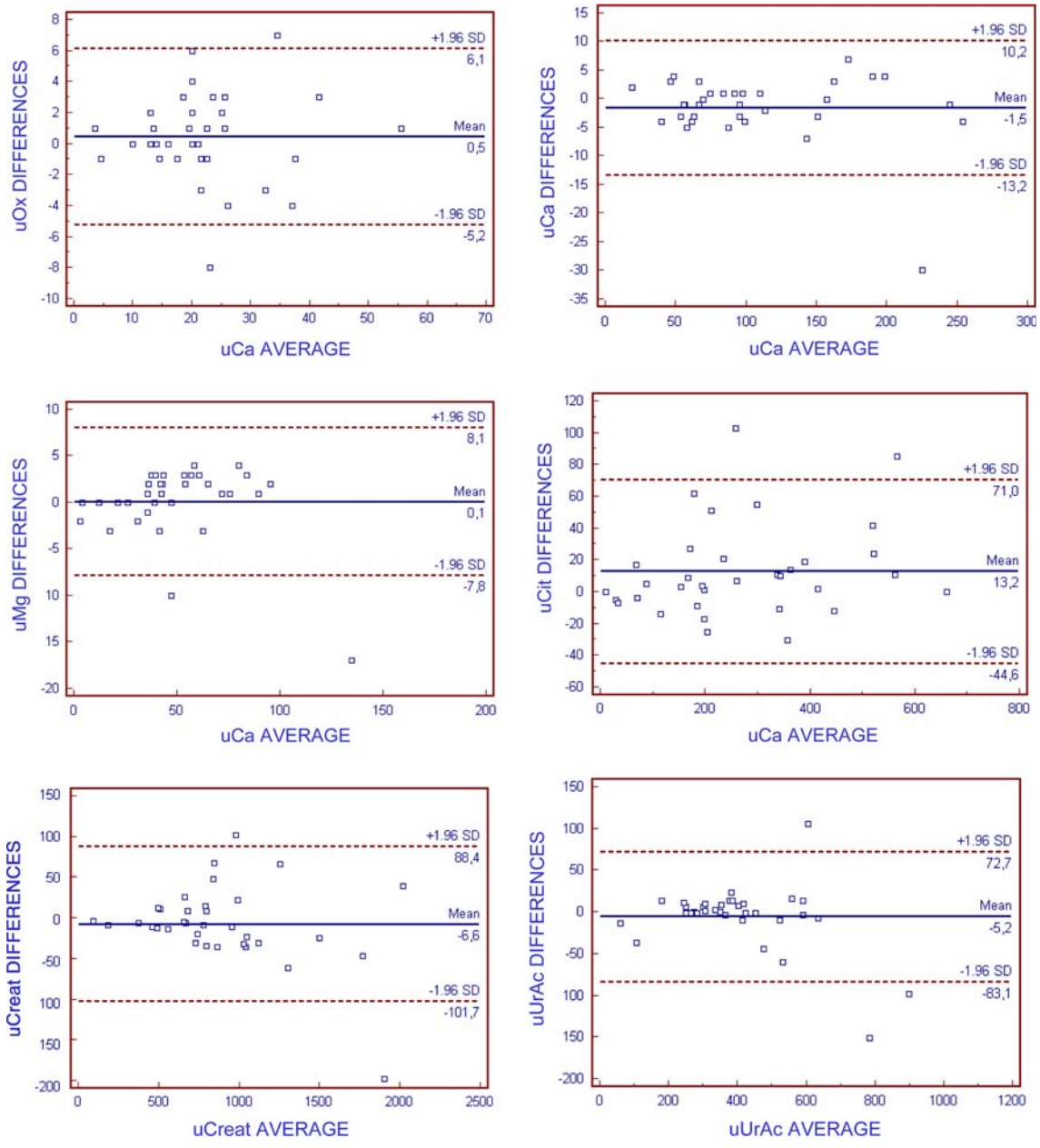

Fig. 2 Differences between the two methods of preservative addition were plotted against the average of both values with respect to uOx, uCa, uMg, uCit and uUrAc determination by Bland–Altman analysis

addition of preservatives) to the classic one using previous addition of preservatives, through a Bland–Altman analysis, further showed a close agreement between the two assays.

Our data regarding oxalate determination are in accordance with those of Braiotta et al. [24], who showed no differences between urinary oxalate measured in spot urine samples collected under previous acid addition or not. In our study, a further evaluation utilizing 24-h urine samples, the method of choice to evaluate urinary parameters involved in stone formation showed the same results for oxalate and also for other urinary parameters. However, as the 24-h urine samples were obtained on non-consecutive occasions, we opted for a further analysis in the same spot urine sample divided into aliquots. Again, the results of the parameters were not different, except for citrate.

The slight but significant decrease that occurred in uCit values of spot urine samples both in HS (5.9%) and SF (3.1%) was not clinically relevant, since it remained within the variation coefficient of the method in our laboratory (13.4%). When patients were divided according to their metabolic disturbance, the urinary parameter related to the correspondent metabolic

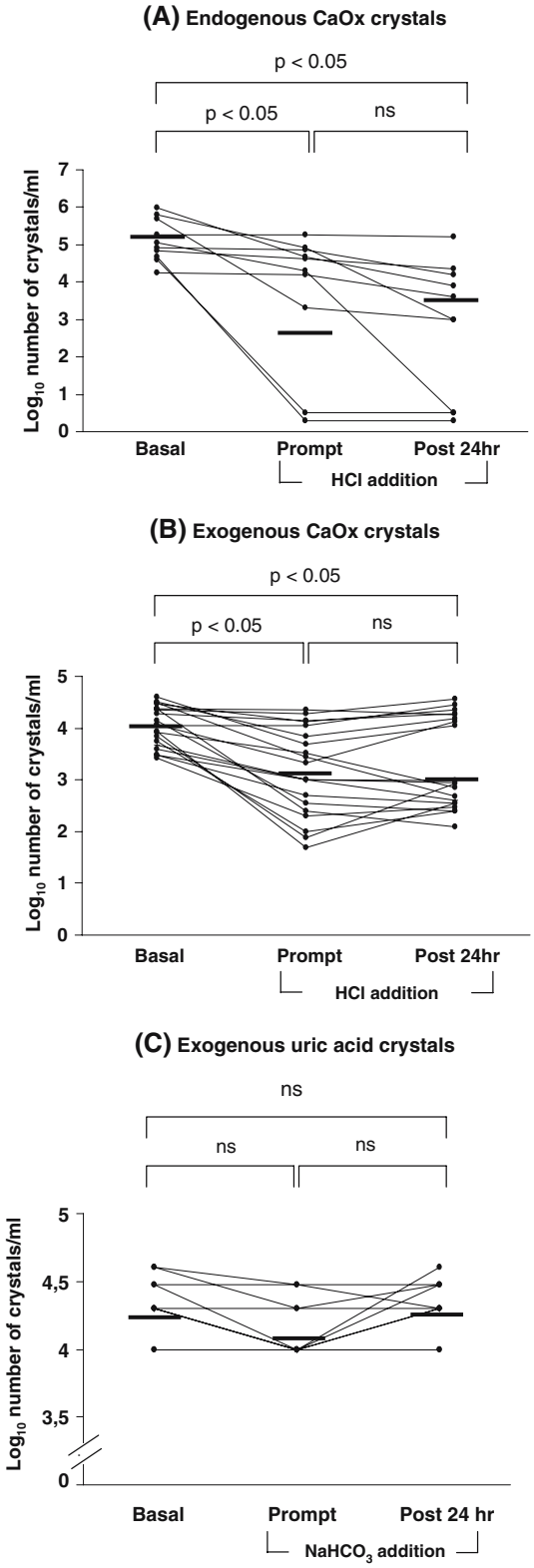

Fig. 3 Crystal counting (log_{10} number of crystals/ml) of endogenous (**a**) or exogenous (**b**) CaOx crystals and exogenous uric acid crystals (**c**) in basal conditions compared to prompt or 24 h later HCl or $NaHCO_3$ addition. Mean values are indicated by *horizontal bars*

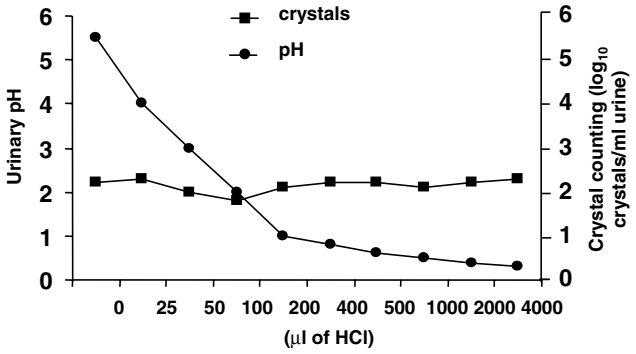

Fig. 4 Urinary pH versus crystal counting according to the amount of HCl (µl) added to the samples

disturbance was not affected, except for the hypocitraturics. Nevertheless, as the hypocitraturic patients tended to have a slight decrease and not increase in the level of urinary citrate, we believe that this would not jeopardize the diagnosis of the hypocitraturia in a 24-h urine sample. In addition, the modest reduction of citrate levels in spot urine samples occurred only in two or three samples (Fig. 1), not representing a general trend. The possibility that bacterial overgrowth was responsible for such observation cannot be ruled out. Therefore, an antibacterial agent could have been added to the collection vessel to avoid this problem, as performed by many laboratories. Although we did not measure urine pH in the present study, it must be remembered that another possible benefit of collecting urine without alkali or acid as a preservative is that the urinary pH can be measured in the same sample.

In the presence of endogenous or exogenous CaOx crystals, the prompt or later addition of HCl to urine samples was able to significantly reduce the number of crystals compared to baseline counting in a similar magnitude. In addition, determination of urinary analytes did not differ under both conditions. However, according to the data provided in both Figs. 3a, b, 4, the complete dissolution of crystals after the addition of preservatives did not occur. Plenty of them remained in the final counting, even when urinary pH decreased to values as low as 0.4. Despite the wide range of variation in the reported amounts of HCl added to urine samples in order to measure different analytes obtained from stone formers, the amount that we had used in the present study, to show that timing of acidification makes no difference to final values, was 20 ml/l 6 N, in agreement with the literature data [6, 15, 25–27]. On the other hand, the highest amount of HCl that we tested (Fig. 4) corresponded to 40% of urine volume (4,000 µl, or 4 ml in 10 ml of urine), and was still not able to dissolve crystals. Considering that urinary parameters are often determined in 24-h urine

Table 5 Urinary analytes in the presence of exogenous (CaOx and uric acid) and endogenous (CaOx) crystals in spot urine samples

Urinary analytes	Exogenous crystals		Endogenous crystals	
	HCl	HCl 24-h after	HCl	HCl 24-h after
Oxalate				
mg/l	421 ± 253	426 ± 248	47 ± 15	49 ± 15
mg/mg creat	0.49 ± 0.51	0.48 ± 0.48	0.03 ± 0.01	0.03 ± 0.00
Calcium				
mg/l	112 ± 72	121 ± 85	205 ± 75	196 ± 61
mg/mg creat	0.09 ± 0.06	0.10 ± 0.07	0.15 ± 0.05	0.13 ± 0.04
Citrate				
mg/l	388 ± 234	392 ± 238	229 ± 211	204 ± 213
mg/mg creat	0.33 ± 0.20	0.34 ± 0.20	0.16 ± 0.12	0.13 ± 0.12
Creatinine				
mg/l	1291 ± 624	1289 ± 629	1382 ± 174	1445 ± 213
	$NaHCO_3$	$NaHCO_3$ 24 h after		
Uric acid				
mg/l	640 ± 260	610 ± 280		
mg/mg creat	0.50 ± 0.26	0.48 ± 0.27		
Creatinine				
mg/l	1360 ± 390	1370 ± 400		

Data are presented as mean ± SD

samples (in volumes ranging from 1,000 to 2,000 ml), even if the complete dissolution of crystals had been achieved, the amount of acid needed to decrease urinary pH to the required levels would be extremely high (as large as 400 ml in 1,000 ml of urine), being impracticable on a routine basis.

The reasons why the complete dissolution of CaOx crystals did not occur even in conditions of very low pH levels remain uncertain. One possibility is that the amounts of calcium salts present were too great to be accommodated in the available volume of urine. Further experiments employing diluted crystallized urine samples must be performed to clarify these findings.

The 24-h urine collection in dry plastic containers with later addition of preservatives is largely employed in the diagnosis of stone formation [14, 24, 28, 29]. In the majority of American Stone Research centers, samples are collected at a wide range of clinical sites and sent to a central laboratory via mail or courier [29].

Nevertheless, in the few studies in which urinalysis or crystal counting and oxalate measurement had been performed in the urine of the same subject, the researchers divided the samples into two different aliquots and each experiment was undertaken separately [29, 30], not proving that the preformed crystals were completely dissolved before the oxalate measurement.

With respect to uric acid crystals, we did not observe a significant decrease in crystal counting following immediate or later $NaHCO_3$ addition if compared to basal counting. The possibility that such results might have been ascribed to methodological difficulties cannot be discounted, especially because most of the in vitro uric acid crystallization studies are usually performed in cells [18, 31, 32]. The reason for not performing experiments with endogenous uric acid crystals relied on the rarity in which these crystals are naturally found in urinalysis. However, in the presence of a similar amount of crystals, compared to the CaOx crystallization experiments, the lack of decrease in uric acid crystal counting might have been clinically irrelevant since the determination of uUrAc in those samples did not change.

We concluded that post-delivery acidification or alkalinization of urine samples does not modify the levels of urinary oxalate, calcium, magnesium, creatinine and uric acid, and that the change in citrate was not relevant, hence allowing all of these parameters (and also Na and K) to be determined in the same sample, preventing the discomfort and the cost of multiple 24-h urine sample collection. In practical terms, when a 24-h urine sample arrives in the laboratory, urinary volume, pH and uNa/uK can be measured, and then half or part of it can be acidified for uOx, uCa, uMg and uCit determinations, while the remaining amount should be alkalinized for uUrAc determination.

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