Selective screening for distal renal tubular acidosis in recurrent kidney stone formers: initial experience and comparison of the simultaneous furosemide and fludrocortisone with the short ammonium chloride test

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Abstract

INTRODUCTION: Distal renal tubular acidosis (dRTA) is associated with renal stone disease and it often needs to be considered and excluded in some recurrent calcium kidney stone formers (KSF). However, a diagnosis of dRTA, especially when 'incomplete', can be missed and needs to be confirmed by a urinary acidification test. The 'gold standard' reference test is still the short ammonium chloride (NH_4Cl) test, but it is limited by gastrointestinal side effects and occasionally failure to ingest sufficient NH_4Cl. For this reason, the furosemide plus fludrocortisone (F+F) test has been proposed as an easier and better-tolerated screening test. The aim of the present study was to assess the usefulness of the F+F test as a clinical screening tool for dRTA in a renal stone clinic.

METHODS: We studied 124 patients retrospectively in whom incomplete dRTA was suspected: 71 had kidney stones only, 9 had nephrocalcinosis only, and 44 had both. A total of 158 urinary acidification tests were performed: 124 F+F and 34 NH_4Cl; both tests were completed in 34 patients.

RESULTS: Mean age was 45.4 ± 15 years and 49% of patients were male. The prevalence of complete and incomplete dRTA was 7% and 13.7 %, respectively. Of 34 patients tested using both tests, 17 (50%) were abnormal and 4 (12%) were normal. Thirteen (39%) patients were abnormal by F+F, but normal by NH_4Cl (sensitivity 100% [95% CI 80% to 100%], specificity 24% [95% CI 7% to 50%]; positive predictive value 57% [95% CI 37% to 75%], negative predictive value 100% [95% CI 40% to 100%]).

CONCLUSIONS: The F+F test is characterised by an excellent sensitivity and negative predictive value, and the diagnosis of incomplete dRTA can be excluded reliably in a patient who acidifies their urine normally with this test. However, its lack of specificity is a drawback, and if there is any doubt, an abnormal F+F test may need to be confirmed by a follow-up NH_4Cl test. Ideally, a prospective blinded study in unselected KSF is needed to accurately assess the reliability of the F+F test in diagnosing, rather than excluding, dRTA.

Introduction

Urinary pH is an important factor in determining the risk of forming particular types of kidney stone: an acid pH favours uric acid, mixed uric acid and oxalate, and cystine stones; an alkaline pH favours calcium phosphate stones. The latter stone type, especially when almost 100%, can suggest a defect in urinary acidification (UA) and an underlying diagnosis of renal tubular acidosis (RTA). (1). Since an alkaline pH favors calcium phosphate precipitation, especially when there is also increased urinary calcium excretion and hypocitraturia, normal UA helps prevent calcium phosphate stone formation. (2-4)

One of the commonest causes of abnormal UA is RTA, most commonly in its classic type I or distal form (dRTA), which is characterized by a hyperchloremic, normal anion gap metabolic acidosis, with reduced net acid excretion, and inability to lower urine pH below 5.3. The clinical features of dRTA can range from an asymptomatic UA defect without systemic acidosis (so-called 'incomplete' dRTA), but with stones or radiological nephrocalcinosis, to major signs and symptoms in childhood due to metabolic acidosis causing growth retardation and rickets, and extensive medullary nephrocalcinosis with stones, leading rarely to renal failure (5).

Several studies have reported a high prevalence of dRTA in recurrent KSF; moreover, incomplete dRTA has been shown to be associated with a more clinically severe form of stone disease characterised by early onset, multiple recurrences, and increased need for surgery (6-9). In patients with a metabolic acidosis and near-normal glomerular filtration rate, a diagnosis of dRTA can be made when urine pH is consistently >5.3, but if there is no systemic acidosis, a test of urinary acidification is required.

In 1959, Wrong and Davies (10) described the short ammonium chloride (NH_4Cl) test, which involved the oral ingestion of ammonium chloride to generate an acid load

to lower systemic pH, and has been adopted as the 'gold standard' diagnostic test for dRTA. However, this test suffers from the drawback of frequent gastric irritation, with nausea and sometimes vomiting, from the ingested acid load. Subsequently, several authors described the use of oral furosemide to augment distal nephron sodium delivery and increase the lumen-negative electrical gradient favoring increased proton (H^+) secretion (11-14). This effect has been shown to be enhanced by concomitant administration of fludrocortisone, which stimulates the sodium pump and increases epithelial sodium channel-mediated sodium reabsorption in principal cells, as well as directly stimulating H^+ pump activity in α-intercalated cells (5,14). In 2007, we described the 'F+F test', in which the administration of furosemide and with fludrocortisone was shown to provide a sufficient and more consistent stimulus to acid excretion than furosemide alone in healthy subjects, and in patients known to have dRTA (5). In that study, we evaluated 11 control subjects and 10 patients with known dRTA by giving oral NH_4Cl or F+F in random order, and we found that the simultaneous administration of furosemide and fludrocortisone provided an easy, effective, and well-tolerated alternative to the standard short NH_4Cl test described by Wrong and Davies (10). Since our report, we have continued to use the F+F test in our routine clinical practice to screen patients with suspected dRTA, and we have studied many patients with recurrent calcium nephrolithiasis. The aim of the present study is to report our experience to date in using the F+F test in a large cohort of selected recurrent calcium KSF, and to compare the F+F and NH_4Cl test findings.

Methods

Study population

This is a retrospective study that evaluated adult patients attending the metabolic stone metabolic of the Royal Free Hospital, London. Patients who completed simultaneous F+F and/or NH_4Cl tests, and had a confirmed diagnosis of recurrent calcium kidney stone disease and/or nephrocalcinosis were investigated. All patients had one of the following clinical criteria suggestive of underlying dRTA: repeated clinic dipsticks urine pH of 6 or greater, relatively low (normal) 24-h urinary citrate excretion, or a predominantly calcium phosphate stone in the absence of infection.

Most patients included in this study had normal kidney function, with mean serum creatinine concentrations of 100 ± 61.6 µmol/L in the whole group. However, 17 patients had CKD IIIa and IIIb (creatinine ranged between 125 to 267µmol/L), and one patient had CKD V (creatinine 586µmol/L).

Patients without nephrolithiasis or nephrocalcinosis or non-calcium kidney stone disease (uric acid stones, cystine stones, infection-related stones, also primary hyperoxaluria, and known dRTA or drug-related stones) were excluded from the analysis.

Study variables

Demographic and clinical characteristics were collected retrospectively by reviewing the patient's computerized records and included age, sex, duration of stone disease, family history of kidney stones, and history of urinary tract infections. Patients underwent a fasting blood sample for the determination of urea, creatinine, electrolytes, calcium (total and corrected for serum albumin), bicarbonate, and a 24-h

urine collection for measurement of urinary pH (u.pH), citrate (u.Cit), and creatinine excretions. For patients that spontaneously passed stones or that underwent surgery for kidney stones, biochemical stone analysis was performed.

F+F and NH_4Cl tests

All patients underwent a simultaneous F+F test. Patients with a normal test result (trough pH <5.3) were considered not have dRTA. All patients with an abnormal F+F test (a failed test: trough pH>5.3) went on to have a short NH_4Cl test (Figure 1). This was done to confirm the diagnosis of dRTA and to evaluate the reliability of the F+F test in detecting dRTA compared with the reference NH_4Cl test. Tests were performed on separate days, without fasting, and with at least 1 week between tests.

The F+F test involved taking a baseline urine sample, followed by oral administration of furosemide (40 mg) and fludrocortisone (1 mg). Fluid intake was *ad libitum.* Urine was collected hourly for 6 h after the baseline sample.

The NH_4Cl test involved taking a baseline urine sample, followed by oral NH_4Cl at a dose of 100 mg/kg body weight given as 500 mg gelatine capsules. This dose was taken over 1 h with water to minimize gastric irritation. Urine was then collected every hour, starting 2 h after dosing began, until 8 h after the baseline sample.

Patients were instructed to note the time at which they had passed urine before the baseline urine sample to allow baseline values to be determined. Urine pH was measured immediately with an electrode pH meter (Hannah piccolo^{TM}).

University College London Hospital Ethics Committee had approved the test protocols. Written consent was not obtained for reporting this study from the

anonymised individual patients, because the study was based on data collected as part of routine clinical care.

Statistical analysis was by Student's *t*-test for unpaired data or by analysis of variance followed by Student's *t*-test for paired data, as appropriate. Nominal and categorical variables were compared using the chi-square likelihood ratio or Fisher's exact test. Values are given as means±SD; $P<0.05$ was taken to denote a significant difference. Sensitivity, specificity and predictive values of F+F test were also calculated.

Definitions of complete dRTA and incomplete dRTA

Complete distal RTA was diagnosed if a patient had metabolic acidosis, urine pH >6 and abnormal F+F and/or NH_4Cl test. A patient was diagnosed with incomplete dRTA if he or she had either an abnormal F+F or NH_4Cl test. In patients who had both tests, a diagnosis of dRTA was made if both tests were abnormal in the case of concordant results; in the case of a disagreement between the tests, the diagnosis of dRTA was based on the results of NH_4Cl test, which was considered to be the 'gold standard' reference test.

Results

During the period from 2007 to 2013 a total of 217 UA tests were performed in a cohort of 169 patients. Thirty-six patients (52 urinary acidification tests) were excluded as they had a diagnosis other than recurrent kidney stones and/or nephrocalcinosis. Nine patients were excluded as they had only NH_4Cl tests done (in two of them the test was not completed).

The remaining 124 patients suffered from recurrent kidney stones and/or had nephrocalcinosis, and were included in the analysis. Of these 124 patients, 71 had only kidney stones, 9 had only nephrocalcinosis, and 44 had both. A total of 158 urinary acidification tests were performed: 124 F+F and 34 NH_4Cl tests. Thirty-four patients had both tests. One patient had two F+F tests.

The demographic and clinical characteristics of the study patients are shown in **Table 1**. Mean age was 45.4 ± 15 years and 49% of the patients were male. The majority of KSF (99 patients, 79%) had stones in both kidneys and the remainder had unilateral disease. Nine patients (7.2%) had staghorn calculi, and two were diagnosed with medullary sponge kidney (MSK). Fifteen patients had a history of recurrent UTIs and 11 had a positive family history of kidney stones. Eighteen patients had a diagnosis of an autoimmune disorder; seven of these had Sjogren's syndrome.

Data on stone composition were available in 77 of the 124 patients (62%). More than 60% of samples were stones composed of more than one constituent. The prevalence of calcium phosphate stones was 35% (44 patients).

Table 2 shows laboratory characteristics of the study patients and prevalence of biochemical blood and urinary abnormalities. The majority of patients had normal serum potassium, calcium, bicarbonate, and creatinine levels. The prevalence of hypokalemia, hyperkalemia, hyperchloremia, and metabolic acidosis was 2.4, 4, 15

and 7%, respectively. Baseline urine pH > 6 and hypocitraturia were detected in 55 and 27% of the patients, respectively.

Prevalence of complete and incomplete dRTA as detected by F+F and/or NH_4Cl tests

Of 158 tests, 67 tests (42%) were abnormal (an inability to acidify urine below pH 5.3) and 91 were normal.

Demographic, clinical and laboratory characteristics of 50 patients with abnormal F+F test and/or NH4Cl were compared with 74 patients who had normal tests (**Table 3**). Patients with abnormal UA tests were significantly younger and, as expected, had a higher prevalence of nephrocalcinosis, lower serum potassium and bicarbonate levels, higher serum creatinine, lower urinary citrate, and more alkaline urine. There were no statistically significant differences in prevalence of bilateral kidney stones, autoimmune disease, recurrent UTIs, hyperchloremia or serum calcium level.

To assess the ability of the F+F test in detecting dRTA as compared with the NH_4Cl test, patients tested by using both tests were analyzed. Overall, 34 patients were tested by both methods, of which 17 (50%) were abnormal and 4 (12%) were normal by both methods. The remaining 13 (39%) patients were abnormal by F+F test, but normal by NH_4Cl test. Thus, the F+F test had a sensitivity of 100% (95% CI 80% to 100%), a specificity of 24% (95% CI 7% to 50%), a positive predictive value of 57% (95% CI 37% to 75%), and a negative predictive value of 100% (95% CI 40% to 100%). No demographic or clinical variables that predicted false positivity of the F+F test could be detected.

In Table 4 (see supplementary data) we have summarized the demographic, clinical and laboratory characteristics of patients stratified into 8 subgroups according to their results (normal or abnormal) of either NH_4Cl or F+F test as follow: NH4Cl only – normal; NH4Cl only – abnormal; F+F only – normal; F+F only – abnormal; NH4Cl abnormal, F+F abnormal (no patients); NH4Cl abnormal, F+F normal; NH4Cl normal, F+F abnormal; NH4Cl normal , F+F normal (Table 4, supplementary data).

Urinary acidification was more rapid after the F+F test than after NH_4Cl test, as noted previously. All patients acidified their urine to pH<5.3 by 3–4 h after the F+F test, whereas this occurred 4–5 h after the ingestion of NH_4Cl.

To access the ability of clinical parameters as hypocitraturia and alkaline urine to diagnose dRTA and probably substitute F+F test, we analyzed sensitivity and specificity of combination of 24 hour u. citrate < 1.52mmol/day and u. ph > 6 as compared with F+F test. We found sensitivity of 52% (CI 0.36-0.68); specificity 86% (CI 0.74-0.93), positive predictive value 71%, and negative predictive value 74%. Although high specificity of these combinations provides the ability of the test to correctly identify those patients without dRTA, a low sensitivity led to a high rate of false negative results.

However, because the F+F test has high sensitivity/low specificity, and hypocitraturia and alkaline urine have low sensitivity/high specificity, a suitable alternative is to subject patients initially to a F+F test with high sensitivity, and then evaluate urinary pH and citrate that have high specificity. In this way, nearly all the false positives reported by the F+F test may be correctly identified if 24 hour urinary citrate excretion and urine pH are also taken into account. That is if a patient has an abnormal F+F test, and 24 hour urinary citrate excretion is < 1.52 mmol/day and urine

pH > 6, a diagnosis of dRTA is very likely, and confirmation by a follow-up NH_4Cl test may not be required.

Discussion

Our study evaluated the prevalence of distal urinary acidification (UA) defects in a selected cohort of patients with recurrent KSF or nephrocalcinosis using the simultaneous F+F test, and has addressed its efficacy and reliability compared with the well-established short NH_4Cl test. Distal UA defects were common in our patients: up to 14% had incomplete, and an additional 7% had complete, dRTA. This finding can be explained by the clinical characteristics of our patients in whom a high prevalence of severe bilateral kidney stone disease, nephrocalcinosis, alkaline urine, and calcium phosphate stone type all suggested the possibility of a UA abnormality. As expected, lower serum potassium and bicarbonate, hypocitraturia and more alkaline urine were associated with incomplete dRTA. Interestingly, these patients had higher serum creatinine levels, suggesting more clinically severe stone disease that had resulted in some renal impairment, possibly due to complications of obstruction, infection, and the need for repeated surgical interventions.

Several previous studies have addressed the prevalence and clinical significance of dRTA in KSF and found that 19 -31% of recurrent KSF suffer from UA defects (6-9); indeed, an even higher prevalence was detected in patients with bilateral and severe stone disease. On the other hand, dRTA was less prevalent (6.7%) in a recent retrospective analysis of 150 consecutive male idiopathic recurrent calcium stone formers in which the NH_4Cl test was carried out in only 12 patients with a baseline urinary pH 5.8 or greater (15). In this study, incomplete dRTA was found in 1 in 15 male KSF, while in our more selective study it is in 1 in 8 patients. This difference can be explained by the differences in the populations studied, since incomplete dRTA is more common in female stone formers and is found more often in patients with bilateral stone disease (8,9). Moreover, selection of patients on the basis of

urinary pH alone can be misleading, for example, in the presence of infection or other cause of increased ammonium content; but can also result in underestimation of acidification defects, since a small proportion of our patients with incomplete dRTA (4%) had baseline urinary pH values of less than 5.8 (ranged 5.6 – 5.71). Regardless of these discrepancies, our results confirm that distal nephron acidification defects are common in patients with severe forms of calcium stone disease and/or nephrocalcinosis, and a defect in UA should be sought in all recurrent KSF, especially given the possibility of more targeted therapeutic and prophylactic interventions (16). Of importance, most of our patients had incomplete acidification defects that can be unmasked only if UA tests are performed.

The more novel aspect of our study is the assessment of the F+F test compared with the short NH_4Cl test in KSF. We found that the F+F test has high sensitivity and negative predictive value. Thus, the diagnosis of dRTA can be reliably excluded in patients who acidify urine normally with the F+F test. However, the test's lack of specificity in these circumstances is an important limitation, because an abnormal test may need to be confirmed by a follow-up NH_4Cl test. However, we could not identify any demographic or clinical variables that predict the likelihood of a falsely abnormal F+F test.

Approximately 10% of our patients suffered from CKD III. The impact of CKD on urinary pH and the potential effect of reduced GFR on performance of both ammonium chloride and F+F test are important (10). Metabolic acidosis is a frequent finding in CKD and is due to reduced excretion of ammonium, the major and adaptive buffer for renal acid excretion (and bicarbonate synthesis), with a preserved ability to lower urinary pH (17). The defective molecular pathways in metabolic acidosis in CKD have been investigated recently in an experimental study by Bürki and co-

workers (18). They found a significant decrease in the abundance and activity of key enzymes and transporters for proximal tubular ammoniagenesis (phosphate-dependent glutaminase, PEPCK and SNAT3) and bicarbonate transport (NBCe1) in animals with CKD compared with controls. However, final ammonium excretion and urinary acidification also depend on distal nephron function (19): the two rhesus proteins RhBG and RhCG mediate distal secretion of ammonia/ammonium, although the driving force for ammonia/ammonium secretion depends on the degree of urinary acidification by Type A intercalated cells. In the study of Bürki and colleagues, urinary pH was more acidic in CKD rats under baseline conditions and was acidified further on an HCl diet, demonstrating an intact capacity to generate and maintain a steep proton gradient from the interstitium to the collecting duct lumen (18). AE1 mRNA expression and protein abundance were decreased in CKD animals, whereas the B1H^+-ATPase subunit was unaffected. Although the capacity of the collecting duct to maximally acidify urine might be affected, excretion of a more acidic urine, and ability to reduce further urine pH with an acid load seems to be preserved in CKD, as reported originally by Wrong & Davies (10). Therefore, it seems likely that the results of UA testing should still be reliable in patients with CKD.

The present study confirms our previous finding that the simultaneous F+F test takes less time to complete, with any differences between normal and impaired UA evident by 3–4 h from the start of the test; whereas with the NH_4Cl test takes 4-5 h, and urine pH can remain low for up to 8 h. Thus, the convenience, low side-effect profile and shorter duration (lasting a maximum of 4 h with hourly urine pH measurements being made starting immediately before dosing) of the F+F test are useful advantages for a more routine screening tool to exclude a possible diagnosis of dRTA.

However, our study found a high incidence of false positives for incomplete dRTA with the F+F test when compared with NH_4Cl test. Although the reason for this is not completely clear, we suspect that the different physiological mechanisms of urine acidification underlying these two tests may explain the inconsistency. Direct NH_4Cl loading has an effect on systemic acid-base and plasma pH, and is a powerful stimulus to urine acidification. In contrast, the F+F test works essentially by increasing distal tubular delivery of sodium to the collecting duct principal cells and stimulates sodium reabsorption with its effect on the transepithelial voltage favoring intercalated cell H^+ secretion, both effects primed by co-administration of fludrocortisone; however, this may still prove to be a less potent and consistent stimulus to acid excretion and may therefore 'over-diagnose' an acidification defect. A similar problem can occur with NH_4Cl loading, if the full oral dose is not taken or tolerated because of nausea and vomiting - the main limitation of this test - in addition to the time it takes to complete.

Viljoen et al. have used both F+F and NH_4Cl tests in 10 adult patients with clinical suspicion of dRTA and recurrent nephrolithiasis and 10 healthy volunteers (20). While 10 patients acidified urine normally with NH_4Cl, with F+F only 3 of 10 reduced their urine pH to <5.3. Thus, similar to our study, the negative predictive value of the F+F for disease was 100%, but the positive predictive value was low, which, despite this, we still believe makes the modified F+F test a more convenient screening tool for a mixed population of KSFs. An important additional finding of our study is that the combination of abnormal F+F test with hypocitraturia and an alkaline urine increases its ability to correctly diagnose dRTA, and may avoid an NH_4Cl test.

Our study has limitations. First, demographic and clinical variables were collected retrospectively by reviewing patients' computerized records and we cannot exclude

all potential confounding factors. However, data collection was uniform in all patients and the same research physicians have completed all datasheets. Second, our patients represent a selected subgroup of KSF in whom a renal acidification defect was suspected on the basis of clinical characteristics. Thus, the high prevalence of incomplete dRTA cannot be extrapolated to lower risk and non-calcium KSF. A high pre-test probability of dRTA in our patients positively affects the predictive value of the F+F test and allows us to reach a scientifically reliable conclusion. Third, the incidence of side effects during either simultaneous F+F or NH_4Cl tests was not recorded consistently. However, our previous study showed a high safety profile of the F+F test, whereas adverse gastrointestinal side effects are frequently reported with the NH_4Cl test (10). Despite these limitations, we were able to demonstrate a high prevalence and clinical significance of distal urinary acidification defects in patients with recurrent and severe calcium KSF, and to provide evidence for the efficacy and reliability of simultaneous F+F testing in excluding dRTA.

In summary, we evaluated a selected cohort of recurrent calcium KSF and focused specifically on the diagnosis of incomplete dRTA using the previously reported simultaneous F+F test. We found that this test has a high sensitivity, but low specificity, and it can reliably exclude a diagnosis of dRTA. However, in combination with clinical measures such as hypocitraturia and alkaline urine, which are routinely recorded in many KSF, the F+F test is reliable and can substitute for the NH_4Cl test. This finding is important, because it can change the clinical approach to the patient and simplify the diagnosis of dRTA.

Administration of alkali is a widespread therapy in almost all patients with idiopathic calcium stone disease, but the diagnosis of dRTA in its incomplete form has an important additional implication for bone health; moreover, giving a potassium salt is

also more beneficial, because of the associated potassium loss in dRTA, and therefore the dose may need to be higher, especially in younger RTA patients. Finally, the ability to diagnose dRTA promptly and conveniently does have implications for treatment and long-term management, especially in relation to growth in children and young adolescents, and bone health in adults (21,22). A future prospective randomized blinded study designed to compare the efficacy and reliability of simultaneous F+F with the short NH_4Cl test in diagnosing dRTA in unselected recurrent calcium KSF would be an important and worthwhile next step.

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RJU is currently also Chief Scientist to iMed CVMD, AstraZeneca R&D, Mölndal, Sweden.

Conflict of interests statement

We declare that the manuscript is original research, has not been previously published and has not been submitted for publication elsewhere in whole or part, except in abstract format. It has been seen and approved by all authors. We haven't any conflicts of interest. No financial support was required.

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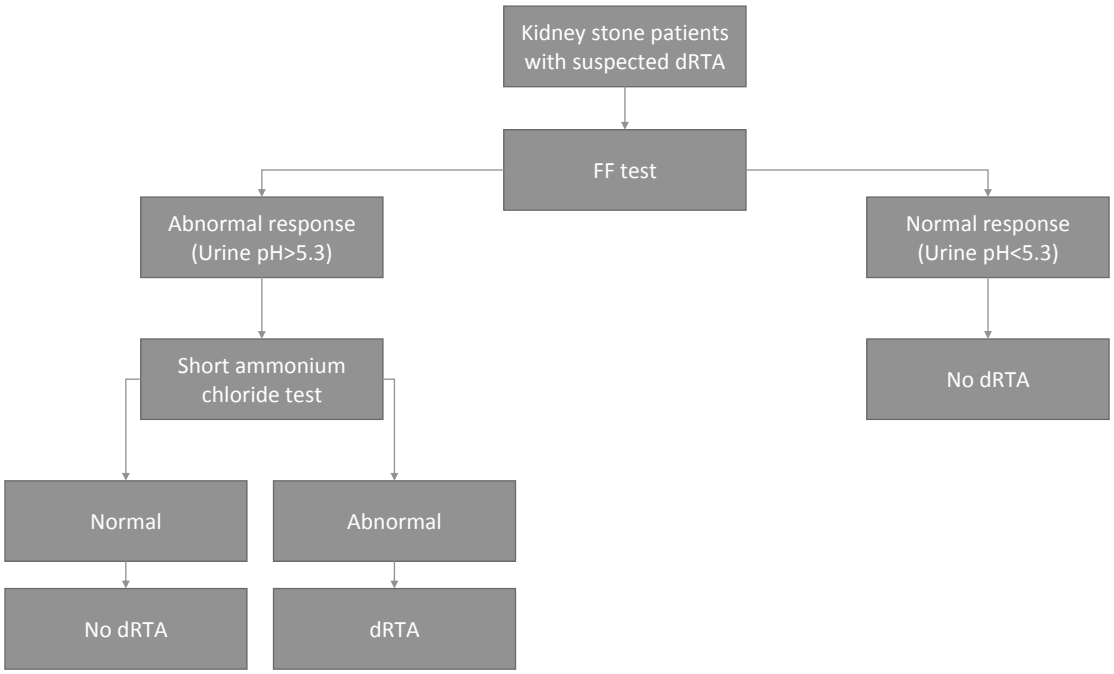
Figure 1 Patient screening, enrollment and study flow



All patients underwent a simultaneous F+F test. Patients with normal test were considered not have dRTA. All patients with abnormal F+F test (test was considered abnormal (confirming a diagnosis of dRTA) if the urine pH did not fall to <5.3) went on to have a short NH_4Cl test. This was done in order to confirm diagnosis of dRTA and to evaluate the ability of the F+F test in detecting dRTA as compared with the reference NH_4Cl test.

Table 1. Demographic and clinical characteristics of the study patients

All Patients	**N= 124**
Age, years	45.4 ± 15
Male, n (%)	61 (49)
Kidney Stone Disease	
Stones, n (%)	71 (57)
Nephrocalcinosis, n (%)	9 (7.2)
Stones and Nephrocalcinosis, n (%)	44 (35)
Bilateral stones, n (%)	99 (79)
Staghorn calculi, n (%)	9 (7.2)
Recurrent UTI, n (%)	15 (12)
Positive family history of stone disease, n (%)	11 (8.8)
Sjogren syndrome, n (%)	7 (5.6)
Other autoimmune diseases, n (%)*	11 (8.8)

Sarcoidosis, n (%)	2 (1.6)
MSK, n (%)	2 (1.6)
Patients with known stone composition, n (%)	77 (62)
Calcium phosphate stones, n (%)	44 (35)

* 4 patients had SLE, 2 – necrotizing vasculitis, 1 – rheumatoid arthritis, 1 – myasthenia gravis, 2- autoimmune thyroiditis, 1- APLA.

Table 2. Laboratory characteristics of the study patients and prevalence of biochemical blood and urinary abnormalities

All Patients	**N= 124**
Blood biochemistry	
Potassium, mmol/L	4.3 ± 0.47
Calcium, mmol/L	2.33 ± 0.13
Calcium corrected to albumin, mmol/L	2.32 ± 0.16
Creatinine, µmol/L	98 ± 46
Bicarbonate, mmol/L	26 ± 5.3
Chloride, mmol/L	104 ± 8.5
Hypokalemia, n (%)	3 (2.4)
Hyperkalemia, n (%)	5 (4)
Hyperchloremia, n (%)	25 (15)
Metabolic acidosis, n (%)	9 (7)
Urine biochemistry	
Creatinine, mmol/L	5.5 ± 4.2
Creatinine, mmol /day	11.6 ± 4.2
Baseline pH	6.3 ± 1.1

Citrate, mmol /day	0.91 ± 0.7
Baseline pH > 6, n%	70 (55)
Hypocitraturia, n%	34 (27)

Hyperchloremia was defined as serum chloride more than 106 mmol/L; Metabolic acidosis was defined as venous blood bicarbonate less than 22 mmol/L; Hypo- and hyperkalemia were defined as serum K less than 3.5 mmol/L or more than 5 mmol/L, respectively ; Hypocitraturia was defined as urine citrate excretion < 1.52 mmol/day.

Table 3. Demographic, clinical and laboratory characteristics of patients with abnormal F+F test and/or NH4Cl were compared to patients with normal tests

Variables	Patients with abnormal F+F test and/or NH4Cl N=50	Patients with normal F+F test and/or NH4Cl N=74	p
Age, years	41 ± 16	47.3 ± 13	0.05
Male, n (%)	20 (40)	59 (79)	0.2
Kidney Stone Disease			
Stones, n (%)	36 (72)	59 (78)	0.7
Nephrocalcinosis, n (%)	26 (52)	16 (22)	0.006
Bilateral stones, n (%)	34 (68)	50 (67)	0.8
Recurrent UTI, n (%)	10 (20)	5 (6.7)	0.5
Positive family history of stone disease, n (%)	5 (10)	6 (8)	0.5
Autoimmune diseases, n (%)*	9 (18)	9 (12)	0.4
Calcium phosphate stones, n (%)	16 (32)	26 (35)	0.9
Blood biochemistry			
Potassium, mmol/L	4.2 ± 0.4	4.4 ± 0.47	0.001

Calcium, mmol/L	2.3 ± 0.1	2.3 ± 0.1	0.2
Calcium corrected to albumin, mmol/L	2.3 ± 0.15	2.3 ± 0.2	0.9
Creatinine, µmol/L	121 ± 87	86 ± 21	0.02
Bicarbonate, mmol/L	24.3 ± 3.5	27 ± 7.3	0.001
Chloride, mmol/L	104 ± 3.5	104 ± 7.4	0.6
Urine biochemistry			
Creatinine, mmol/L	4.3 ± 2.4	5.6 ± 3	0.02
Creatinine, mmol /day	10.8 ± 3.9	12 ± 4.2	0.11
Baseline pH	6.67 ± 0.4	6.1 ± 0.4	< 0.0001
Citrate, mmol /day	1.4 ± 1	2.5 ± 1.4	< 0.0001
Baseline pH > 6, n%	46 (92)	25 (33)	0.002

* 7 patients had Sjogren Syndrome, 4 patients had SLE, 2 – necrotizing vasculitis, 1 – rheumatoid arthritis, 1 – myasthenia gravis, 2- autoimmune thyroiditis, 1- APLA.

Supplementary data

Table 4. Demographic and laboratory characteristics of the patients stratified according to results (normal or abnormal) of either NH_4Cl or F+F test as test as follow: NH4Cl only – normal; NH4Cl only – abnormal; F+F only – normal; F+F only – abnormal; NH4Cl abnormal, F+F abnormal (no patients); NH4Cl abnormal , F+F normal; NH4Cl normal , F+F abnormal; NH4Cl normal , F+F normal

	NH_4Cl Normal	NH_4Cl Abnormal	F+F Normal	F+F Abnormal	NH_4Cl abnormal/ F+F abnormal	NH_4Cl normal/ F+F abnormal	NH_4Cl normal/ F+F normal
Patients N	17	17	91	33	17	13	4
Age, years	46 ± 14	39 ± 16	48 ± 13	41 ± 16	39 ± 16	45 ± 18	47 ± 6
Blood biochemistry							
Potassium, mmol/L	4.3 ± 0.4	4± 0.5	4.5 ± 0.4	4.2 ± 0.4	4± 0.5	4.3 ± 0.3	4.4 ± 0.4
Calcium, mmol/L	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.09	2.3 ± 0.2
Creatinine, µmol/L	80 ± 21	131 ± 82	89 ± 32	116 ± 88	131 ± 82	90 ± 46	78 ± 19
Bicarbonate, mmol/L	26 ± 1.9	23 ± 3.5	26 ± 2.4	24 ± 3.5	23 ± 3.5	26 ± 2.6	25.7 ± 2.8
Chloride, mmol/L	104 ± 2.1	104 ± 2.5	105 ± 7	104 ± 3.5	104 ± 2.5	105 ± 2.7	104 ± 1.2
Urine biochemistry							
Baseline pH	6.3 ± 0.6	6.6 ± 0.6	6 ± 0.7	6.6 ± 0.3	6.6 ± 0.6	6.5 ± 0.5	6 ± 0.4
Citrate, mmol /day	2.7 ± 1.5	1.3 ± 1.2	2.4 ± 1	2.4 ± 1.3	1.3 ± 1.2	3 ± 1.4	2.3 ± 0.4