

Biochemical evaluation in renal stone disease

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Summary

Renal stone disease may ensue from either derangements of urine biochemistries or anatomic abnormalities of kidneys and urinary tract.

Genetic, environmental and dietary factors may also cooperate in the pathophysiology of nephrolithiasis.

An adequate metabolic evaluation should focus on the urinary excretion of promoters and inhibitors of stone formation as well as on the occurrence of systemic diseases potentially related to secondary nephrolithiasis (i.e., endocrine disturbances, malabsorption, bone diseases). Moreover, metabolic investigations should provide reliable information on patient's dietary habits, guide towards the best therapeutic approach and enable the physician to verify patient's compliance to prescribed therapies.

An extensive metabolic evaluation is recommended in patients with active stone disease (namely, at least one new stone within the last two years), or in those having had a single stone episode occurred in peculiar conditions: familial history of disease, childhood, menopause, pregnancy, systemic diseases. Simplified protocols may be adequate in non-active nephrolithiasis or in patients with single stone and no relevant risk factors.

In our Stone Centre, a so-called "first level screening" is performed by routine, in order to assess urinary supersaturation with stone forming salts and evaluate the excretion of dietary-related metabolites in urine. Relative blood and urine determinations are reported below.

In venous blood: urea, creatinine, uric acid, Na, K, total and ionised Ca, Mg, P, Cl, alkaline phosphatase, gas analysis.

In 24-hr urine samples: urea, creatinine, uric acid, Na, K, Ca, Mg, P, Cl, oxalate, inorganic sulphate, citrate, pH, ammonia and titratable acidity.

In fasting urine samples: Ca, citrate, creatinine, hydroxyproline, Brand's test for cystinuria, urine sediment, urine culture.

If the first-level evaluation suggested an abnormal bone turnover, then further determinations are warranted, namely, calciotropic hormones (blood Vitamin D and PTH), markers of bone resorption (urine pyridinium crosslinks, serum cross-laps) and formation (serum osteocalcin) bone mineral density. Eventually, more sophisticated investigations are required to

improve the diagnosis of peculiar diseases: serum oxalate and glycolate, urine glycolate and L-glycerate, hepatic AGT activity (primary hyperoxalurias); genetic tests (hereditary nephrolithiasis); acidification tests (renal tubular acidosis).

KEY WORDS: *nephrolithiasis, urolithiasis, hypercalciuria, hyperoxaluria, hypocitraturia, urine supersaturation.*

Introduction

The rationale for the investigation on the urinary composition of stone forming patients comes from the assumption that derangements of urine biochemistries may play a pivotal role in the pathogenesis of nephrolithiasis. Also, anatomic abnormalities of kidneys and urinary tract, genetic, environmental and dietary factors may cooperate in the pathophysiology of renal stone disease (1-3).

The urinary excretions of many substances (i.e., water, electrolytes, nitrogen, ash-acid and alkali) depend on their glomerular filtration and the subsequent tubular handling, which, in turns, is usually modulated so as to keep their external balance in equilibrium.

In other cases (for example, fasting hypercalciuria syndromes, renal tubular acidosis and cystinuria) the tubular handling of promoters and inhibitors of stone formation, as well as their urinary pattern, can be strongly influenced by genetic factors (4-6).

Eventually, despite nephrolithiasis is a multifactorial disease, the study of the propensity towards the crystallization of stone forming salts in urine still remains the easiest strategy for the Nephrologist to estimate the propensity towards the relapses of stone disease in individual patients (7).

In addition to the assessment of the urinary pattern of promoters and inhibitors of stone formation, a suitable metabolic evaluation should also focus on the occurrence of systemic diseases potentially complicating with secondary nephrolithiasis, i.e., endocrine disturbances, intestinal malabsorption, bone diseases (8-10).

Moreover, metabolic investigations should also provide reliable information on patient's dietary regimen, in order to better define the pathophysiology of the disease and enable the physician to verify the compliance of the patient to the prescribed therapies.

In this paper, we described the pattern of biochemical investigations routinely used for the clinical management of stone-forming outpatients referring to the Renal Stone Centre of the Mauriziano Hospital in Torino (Italy).

Methods

The main clinical objectives of an extensive metabolic evaluation for stone-forming patients are reported in Table I.

In our Stone Centre, recurrent stone-forming patients are submitted by routine to a "first-level screening", aimed at both investigating on the main urinary risk factors for nephrolithiasis and getting information on dietary habits, namely, water, sodium, vegetables, calcium and protein intakes. Taken together,

Table I - Main clinical objectives of metabolic evaluation for stone-forming patients.

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- a) Estimation of the urinary propensity to the crystallisation of stone-forming salts.
 - b) Investigation on the pathophysiology of urine excretion of promoters and inhibitors of nephrolithiasis.
 - c) Search for underlying systemic diseases.
 - d) Information on dietary habits.
 - e) Background for therapy prescription.
 - f) Assessment of patients' compliance to therapy.
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serum ionised calcium, serum phosphate and tubular resorption of phosphate ($TmPO_4$) inform on the biological activity of PTH (Tables II, III).

Starting from the main urine biochemistries, by means of a dedicated computer-based method, urine supersaturations with calcium oxalate ($\beta CaOx$), brushite (βbsh) and uric acid (βUA) are calculated in each patient, to get an estimate of the propensity towards the crystallization of these stone-forming salts in urine (11).

Further investigation can be used for the differential diagnosis among severe hypercalciuria syndromes, or in case of hypercalcemia. To this purpose, our "second-level screening" is focused on the study of both the profile of calciotropic hormones and bone turnover (Table IV).

More sophisticated investigations are recommended in case of rare diseases, as follows.

If primary hyperoxaluria is suspected, Oxalate and Glycolate are measured in plasma and Glycolate and Glycerate in urine as well (12).

In selected cases of primary hyperparathyroidism, especially

Table II - "First-level screening" for nephrolithiasis.

Blood:	Urea, Creatinine, Uric acid, Na, K, Ca, Ca 2+, Mg, P, Cl, ALP, pH and bicarbonate.
24-hr urine:	Urea, Creatinine, Uric acid, Na, K, Ca, Mg, P, Cl, Oxalate, Inorganic sulphate, Citrate, pH, NH_4^+ , Bicarbonate and Titratable acid.
Fasting urine:	Ca, Citrate, Creatinine, OH-proline, Brand's test for cystinuria.
Urinalysis	
Urine culture	
Chemical analysis of available stone fragments	

Table III - "First-level screening": calculated parameters.

Index	Calculation	Related to
Creatinine clearance (ml/min)	Standard formula	Glomerular filtration rate
Total nitrogen excretion (mmol/24h)	Nitrogen from urea, creatinine, uric acid, ammonia, in 24 hr-urine	Whole protein intake
Net acid excretion (mmol/24h)	Ammonia + titratable acid – bicarbonate, in 24-hr urine	Animal protein intake
Sulphate excretion (mmol/24h)	Urinary sulphate	Animal protein intake
Na and Cl excretion (mmol/24h)	Urinary Na and Cl	NaCl intake
Calcium excretion (mg/24 h) (mg/24 h/kg bw) fasting Ca/Creatinine ratio	Urinary calcium	Dietary dependence on calcium excretion
Intestinal absorption of alkali (mmol/24h)	$(Na + K + Ca + Mg) - (Cl + P)$	Alkali and vegetables intake
$TmPO_4/GFR$	Nomogram	Tubular handling of phosphate
$\beta CaOx$	Computer method	Urine state of saturation with calcium oxalate
βbsh	Computer method	Urine state of saturation with brushite
βUA	Computer method	urine state of saturation with uric acid

bw: body weight.

Table IV - "Second-level screening".

Vitamin D metabolites:	Serum 25 OH Vitamin D; serum 1.25 (OH) ₂ Vitamin D.
Parathyroid hormone:	Intact PTH.
Markers of bone formation:	Serum osteocalcin (BGP); Alkaline phosphatase isoenzymes.
Markers of bone resorption:	Serum crosslaps, urine deoxypyridinoline (DPD).
Multiple myeloma:	Plasma protein electrophoresis, urine light chains.
Thyroid function:	TSH, thyroid hormones.
Bone mineral density:	Lumbar or femoral DEXA.
Acid-base balance:	Venous blood gasanalysis, fasting urine pH and ammonium.

when multiple endocrine neoplasia type I (MEN I) is suspected (13), DNA assay in circulating leukocytes can be used.

If an incomplete form of distal Renal Tubular Acidosis (RTA type I) is suspected, then the diagnosis can be confirmed by means of urine acidification tests (14, 15).

Discussion

An extensive metabolic evaluation, aimed at the calculation of urine supersaturation with stone-forming salts, requires a large number of biochemical analyses both in blood and urines. Consequently, it can be rather expensive and time-consuming. Urine must be carefully collected for twenty-four hours, by separating each sample into two separated bottles, one containing concentrated hydrochloric acid, the other chlorexidine as preservatives. The former is assayed for urea, creatinine, Na, K, Ca, P, Mg, oxalate, sulphate, citrate; the latter, for pH, Cl, titratable acid, ammonia.

Such extensive metabolic study must be performed in specialised laboratories, so it is recommended mainly for patients with active stone disease (i.e., those who have experienced at least one new stone within the last two years) or for those who have presented a single stone episode in peculiar conditions: familial history of disease, childhood, menopause, pregnancy, systemic diseases.

Conversely, a simplified protocol may be adequate for patients without either active stone disease or relevant risk factors for nephrolithiasis, referring after a single stone event. These patients should first refer to the General Practitioner who, after having excluded dangerous nephrolithiasis-related systemic disturbances (in particular, hypercalcemic syndromes), can give them suitable advice in order to reduce the risk for stone formation, namely, high water supply and low calories, salt and animal protein intakes. Thereafter, the Practitioner can survey the clinical course of these subjects and refer the frequent relapsers to a qualified Stone Centre.

Patients undergoing metabolic study for the first time must be reminded to keep on maintaining their dietary habits before the examinations, in order to obtain information on their biochemical profile *on steady state*. This will provide a reliable estimate of the actual propensity for stone formation and, during follow-up, enable to evaluate the effect of given therapies.

In this paper we referred on the pattern of laboratory determinations used for the assessment of the risk for urinary stone formation with patients referring to our Stone Centre.

The "first-level screening", as detailed in Table II, is aimed at measuring: first, renal function; second, urinary excretion of the main promoters (calcium, oxalate, phosphate, uric acid, cystine) and inhibitors (magnesium, citrate) of stone formation; third, urine supersaturation with stone forming salts. Further-

more, the same analyses inform on the usual dietary habits (Table III), as it will be summarised below.

In patients on steady state, who are not rapidly changing their body weight, total nitrogen excretion is closely related to their external balance of nitrogen and can be used to estimate the whole amount of dietary protein.

The greatest part of both sulphate and urinary acid excretion comes from the metabolism of sulphur-containing aminoacids. It follows that, in this subset, the measure of net acid and sulphate excretion helps in estimating the dietary intake of protein of animal origin, which are believed to play a pivotal role on calcium excretion (16, 17).

As far as vegetable and fruit intake are concerned, they can be considered as the main dietary source of alkali. It has been demonstrated that the intestinal absorption of alkali can be reliably estimated from the difference between urinary cations and anions (18).

There is a close relationship between sodium and calcium excretions. Sodium excretion depends on the intake of sodium-containing salts, mainly, sodium chloride. As each gram of NaCl contains 17 mmoles of both Na and Cl, the actual dietary intake of NaCl can be assessed from urinary Na and Cl excretions. For example, daily intake of 10 grams of NaCl will produce urinary excretion of about 170 mmoles of both Na and Cl per day.

The relationship between calcium excretion and calcium intake are complex; indeed, sodium and protein intake can affect calcium excretion even more than calcium intake itself (19). Therefore, for both diagnostic and therapeutic purposes, it appears to be more useful to get information on the *dietary*-dependence rather than on the *calcium*-dependence of hypercalciuria. By comparing daily urinary calcium excretion (either considered as calcium to creatinine ratio or calcium per kg of body weight) with fasting calcium excretion, fasting (dietary-independent) hypercalciuria can be distinguished from dietary-dependent hypercalciuria. The evaluation of calciotropic hormones and markers of bone turnover (second-level screening, Table IV) is usually unnecessary in calcium stone formers presenting with dietary-dependent hypercalciuria.

In the face of normal levels of calcium and phosphate in plasma, the assessment of tubular handling of phosphate (Tm-PO₄/GFR) can be taken as a suitable tool to exclude significant derangements in parathyroid hormone excretion.

In conclusion, our first-level biochemical approach, by providing a reliable assessment of metabolic profile with the stone-forming patients, can be sufficient for the Nephrologists to guide therapeutic prescriptions in the majority of cases.

On the other hand, the "second-level screening" (Table IV) is recommended if urine biochemistries or serum calcium levels are suggestive for complex derangements of physiological bone turnover, which can develop either as primary or sec-

ondary disease in stone-forming patients. In those cases, nephrolithiasis appears as a typical multidisciplinary disease, whose diagnostic and therapeutic approaches take the best advantages from the close collaboration among nephrologist, endocrinologist, oncologist, gynaecologist and general practitioner.

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