JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2014. Vol. **8**(3), p. 2259-2263

Study of the Relationship between Urinary Infection and Crystalluria in Lithiasic and Non-lithiasic Patients

Sbahi Kheyra[1], Kacem Brahim[1], Semmoud Ahmed[2], Addou Ahmed[1] and Chibani Abdelwahab[1]

[1]Research Unit "stones", Department of Biology, Faculty of Natural Sciences and Life, University Abd elhamid Ibn Badis of Mostaganem, Algeria.
[2]LASIR Laboratory, University of Science and Technology of Lille 1 France.

(Received: 15 February 2014; accepted: 26 March 2014)

The urolithiasis results of pathology or metabolic abnormalities varied with urinary infection. It is characterized by the presence of stones in the urinary tract. These consist of substances which crystallize in the urine under certain conditions. A multi-parametric study of crystalluria and cyto-bacteriology of urine was performed to determine the risk crystalliferous and involvement of urinary infection in the lithogenic process. 617 first morning urine from 306 patients were collected and analyzed. 59 of these patients were nephrolithiasic and 247 suffering from renal pain (without any calculi formation i.e. non lithiasic NL). Meanwhile, 202 urine samples from 100 healthy subjects were also analyzed and considered as control. The results obtained clearly showed the crystalliferous role of urinary infection. The frequencies of positive crystalluria in nephrolithiasic and NL patients with urinary infection were 87.5% and 37.4% respectively (18.3% in controls). The study of the relationship "crystalluria-germs identified" has shown that the most frequent crystalluria was found in urine infected with *Proteus* sp with a frequency of 83.3% for both nephrolithiasic and NL subjects. The presence of urinary crystals is not pathological in itself, except in special cases. However, in a nephrolithiasic patient, or in certain pathological contexts such as urinary tract infection, crystalluria can be a very useful indicator of the observed pathology.

Keywords: Crystalluria, Urolithiasis, urinary tract infection, struvite.

Urinary stones are composed of substances that crystallize in the urine under certain conditions. Identification of urinary crystals is shown to be as a means for early detection of crystalliferous pathologies before they cause renal complications. The involvement of the urinary tract infection in the formation of lithiasis and the crystalliferous role of certain micro-organisms have been noted by many authors (Dussol *et al*, 1993; Tuma *et al*, 2001). Therefore, we propose a monitoring crystalluria associated with a study of the influence of urinary infection on the formation of crystals in three groups. The first one was composed of nephrolithiasic patients, the second consisted of patients who were not lithiasic but had other uro-nephrological diseases and the third group represented healthy subjects.

* To whom all correspondence should be addressed.
Dr. KACEM Brahim
Cité 152 Logts Bloc B N° 16 Mostaganem, 27000, Algeria
Tel: 00213773893120; E.mail: kacem1b@voila.fr

MATERIALS AND METHODS

Samples collection

617 first morning urine from 306 patients including 59 with nephrolithiasis and 247 non lithiasic patients (NL) but had other problems in the urinary tract were collected under sterile conditions in hospital laboratories of Ain Tadelesse, Mazouna and some private uro-nephrologists clinics (West Algeria). Meanwhile, 202 samples from 100 healthy subjects were

analyzed to compare the results with those of patients. For each subject, one of the samples taken was used to perform a urinary cytobacteriological examination (UCBE).

Procedure

Just after reception, the urine is homogenized by inversion, a cytobacteriologic examination, study of crystalluria were performed. The pH was determined using a urinary dipstick. The diagnosis of urinary tack infection was based on UCBE.

For each sample, urine was taken with a Pasteur pipette and placed on a Malassez slide for examination by a polarized light microscopy (Zeiss). The study of crystals comprises a search and identification of all crystal species. The crystals were identified by their morphology and characteristics in polarized light according to the criteria described by Daudon *et al* , 2003 and Daudon *et al*, 2004).

RESULTS

Frequency of infection

The cytobacteriologic study showed that urinary infection was present in 16.6% of lithiasic patients and 22.7% NL patients judged on the leucocyturia. The results of this study are shown in Table 1.

Table 1. Frequency of urinary tract infection in patients with and without lithiasis (NL)

Urinary infection	Lithiasic patients	Non Lithiasic (NL)
Presence of infection: Number (%)	24 (16.6%)	107 (22.7%)
Absence of infection: Number (%)	121 (83.5%)	365 (77.3%)
Total	145	472

Correlation between urinary infection and crystalluria

Two types of crystalluria can be considered: positive and negative crystalluria. Positive crystalluria is an indication of lithogenic risk factors roughly asserted by the presence of inducers such as crystals of calcium oxalate, phosphates and cystine (Daudon, 1987).

Table 2 shows the crystalliferous role of infection. The frequencies of positive crystalluria in nephrolithiasic patients and NL with urinary infection were 87.5% and 37.4% respectively. The frequency was 18.3% in controls.

Table 3 shows that the most frequently encountered crystalline species in lithiasic patients with a urinary tract infection were struvite, whewellite (C1) and weddellite (C2) present in the urine with the same frequency (33,3%) followed by PACC with a frequency of 25%. In non lithiasic NL patients, struvite dominated with 14.9% and PACC with 12.2%, whewellite and weddellite both with the same frequency of 11.2%.

Relationship germs, pH and different crystalline forms

The results of the study of the relationship between the three factors crystalline species, urinary pH and type of germs identified in nephrolithiasic patients and non lithiasic (NL) subjects with urinary tract infection showed that the most frequent crystalluria was found in urine infected with *Proteus* sp (83.3%), followed by those infected with *Enterobacter* sp (62.5%), *Kleibsiella* sp, *E coli* and *Serratia* sp (50%), *Staphylococcus* sp (46.4%) and *Pseudomonas* sp (33.3%).

Crystalline species identified in urine infected by *Proteus* sp were likely whewellite in 35.3% and weddellite in 37.5% of cases with a mean pH of 6.9. Struvite was present in 40.9% of cases in the urine infected by *Staphylococcus* sp, followed by brushite with a frequency of 50% and PACC

Table 2. Frequency of positive and negative crystalluria in lithiasic and non lithiasic (NL) patients, compared with controls.

Cristalluria	Lithiasic Patients	Non Lithiasic NL patients	Controls
Positive : Number (%)	21 (87.5%)	40 (37.4%)	37 (18.3%)
Negative: Number (%)	3 (12.5%)	67 (62.6%)	165 (81.7%)
Total	24	107	202

Table 3. Type and frequency of crystalline species detected in infected urine in lithiasic and non lithiasic patients.

Type of crystals*	Lithiasic patients (%)	Non Lithiasic patients NL
Struvite	33.3	14.9
C1	33.3	11.2
C2	33.3	11.2
PACC	25	12.1
AUD	16.7	0.9
Brushite	12.5	1.9
UAC	8.3	6.5

* C2: Weddellite (Cacium oxalate dihydrate), C1: Whewellite (calcium oxalate monohydrate) PACC (amorphous calcium carbonate phosphates), Struvite (magnesium phosphate hexahydrate amoniaco), brushite (calcium phosphate dihydrate), UAC (complex amorphous urates) AUD: uric acid dihydrate

with 43.8%. The mean pH was roughly acid in contaminated urine with various germs (Table 4).

DISCUSSION

Many studies have shown that crystalluria is common in normal subjects as well as in lithiasic patients, although its frequency and abundance were often higher in the latter (Werness *et al*, 1981, Fan *et al*, 1999; Bader *et al*, 1994).

Our cytobacteriologic study showed that urinary infection was present in nephrolithiasic in 16.6% of cases. This frequency is less important compared to the results found by several researchers Daudon, 1990 and Belhadji *et al*, 2004 where the infection frequency reached 30%. In NL patients 22.7% of the cases had urinary infection judged upon leucocyturia, results of direct examination and identification of bacteria.

Table 4. Average pH and frequency of crystalline species in the urine of lithiasic and non lithiasic patients with an infection by different germs.

Germs	average pH	positive Cristalluria (%)	Frequencies (%)						
			C1	C2	Struvite	Brushite	PACC	AUD	UAC
Kleibsiella sp	6.02	50	17.7	31.3	9.1	25	12.5	50	20
Staphylococcus sp	6.78	46.4	17.7	-	40.9	50	43.8	50	-
E.coli	5.97	50	17.7	31.3	-	-	6.3	-	60
Proteus sp	6.92	83.3	35.3	37.5	27.3	-	6.3	-	20
Enterobacter sp	6.75	62.5	11.8	-	22.8	25	18.8	-	-
Pseudomonas sp	6.8	33.3	-	-	-	-	6.3	-	-
Streptococcus sp	5.95	-	-	-	-	-	-	-	-
Serratia sp	6.15	50	-	-	-	-	6.3	-	-

In lithiasic patients with urinary tract infection, the frequency of crystalluria was 87.5%. This result is greater than that found in other studies where the frequency varied between 50 and 70% in idiopathic calcium nephrolithiasis (Werness *et al*, 1981; Bader *et al*, 1994; Kacem *et al*, 2004; Caudarella *et al*, 1986). This shows that urinary infection is a risk factor for lithogenesis. These results confirm those obtained by other studies (Netelenbos *et al*, 2005).

This lithogenic risk of urinary tract infection was confirmed in NL patients where the frequency of crystalluria was positive (37.4%). This frequency, although less than that observed in our lithiasic patients, is six fold greater than the 6.4 % reported from 5956 urine samples from hospitalized NL patients having no uro-nephrological pathologies (Daudon *et al*, 1983).

The most frequently crystalline species encountered in lithiasic suffering from a urinary tract infection are struvite, whewellite and weddellite present with the same frequency (33.3%). During direct examination, struvite was in 40.91 % of cases associated with the presence of *Staphylococcus* sp with an average pH of 6.78 and 27.3% of cases associated with the presence of *Proteus* sp with average pH of 6.92. These results indicate that these bacteria have a strong ability to

alkalize the urine and synthesize correspondingly struvite.

If the lithogenic role of urine germs is well known (Grihith *et al*, 1987) that of *E. coli* and other bacterial species known to be devoid of urease is less clear. Some strains of *E. coli* are capable of inducing crystallization of struvite, although this organism does not produce urease. This could be due to a slow production of urease by some strains of *Escherichia coli*. Indeed, it has been shown that different bacteria isolated from human urine (including *E. coli*) incubated on a medium amended with urea as a source of nitrogen, are capable of transiently synthesize urease to meet their metabolic needs (Sabinski. *et al*, 1996).

CONCLUSION

Crystalluria reflects a super-saturation of urine with one or more substances eliminated by the kidney. The presence of urinary crystals is not, except in special cases, pathological in itself. However, in lithisic persons, or in certain pathological contexts crystalluria can be a very useful indicator of the pathology observed.

In our study we found that the role of the crystalliferous urinary infection has been verified in lithiasic and NL patients. The correlation between urinary infection and the presence of crystals showed that the most frequently crystalline species encountered in lithiasic patients are struvite, followed by whewellite , weddellite and PACC . The nature of crystalline species precipitated showed that precipitation of whewellite and weddellite was favored in urine contaminated *Proteus* sp. These bacteria had the largest alkalizing nature compared to other identified microorganisms. Struvite, brushite and PACC formed mainly in urine infected by *Staphylococcus* sp. So we can conclude that although the frequency of crystalluria in uro-nephrological diseases was lower than that of nephrolithiasis, it remained high and showed that these patients are at risk of crystal formation more than normal and therefore a risk of formation of urinary calculus. This confirms that the study of crystalluria is an effective examination which can help to diagnose lithiasis disease and urinary tract infection. So more importance should be given to crystalluria in urinalysis.

REFERENCES

1. Bader C.A., Chevalier A., Hennequin C., Jungers P., Daudon M.: Methodological aspects of spontaneous crystalluria studies in calcium stone formers. *Scanning Microsc*., 1994; **8**: 215-232.
2. Belhadji. A, kacem.B, Kaid-Omar.Z, Daudon.M, Addou.A. Eude des relations entre infection urinaire et lithiase rénale dans l'Ouest Algérien. *Eurobiologiste*, N° 269, Janvier-Février 2004.
3. Caudarella R, Rizzoli E, Malavolta N, Severi B, Vasi V, Biagini G. Cristallurie urinaire. Un problème à débattre. *Act Urol Belg*, 1986 ; **54**: 49-56.
4. Daudon M., Protatm.F.,Réveillaud R.J., Rouchon M. : Étude de la cristallurie spontanée par spectroscopie infrarouge. Recherche de corrélations entre les cristaux, les calculs, les germes et le sexe des malades. *Ann. Biol. Clin*, 1983 ; **41**: 199-207.
5. Daudon.M. Cristallurie. Aspects pratiques et interprétations / I. *Aspects pratiques Feuillets de biologie*, 1987 : **28**: 25-40.
6. Daudon.M. Lithiase urinaire et infection. *L'eurobiologiste*, 1990; **24**: 257-264.
7. Daudon M, Cohen Solal F, Lacour. Etude de la cristallurie : réalisation pratique et signification clinique. *Feuillets de biologie*, 2003 ; **43:** 31-53.
8. Daudon M. P. Jungers, B. Lacour. Intérêt clinique de l'étude de la cristallurie. *Ann Biol Clin*, 2004 ; **62***:* 379-393.
9. Dussol B., Lechevalliere E. et Berland Y. Particularités des lithiases en déhors des lithiases calciques: Lithiase infectieuse. *Encycl Méd Chir, Néphrologie-Urologie*, 1993.
10. Fan J, Chandhoke PS. Examination of crystalluria in freshly voided urines of recurrent calcium stone formers and normal individuals using a new filter technique. *J Urol*, 1999; **161**: 1685-8.
11. Grihith.DP, Osborne.CA. Infection (urease) stones. *Miner Electrolytr Metab*, 1987; **13** :278-285.
12. Harrache D., Mesri Z., Addou A., Semmoud A., Lacour B., Daudon M. (a) : Analyse des calculs urinaires de l'adulte dans l'ouest algérien par spectroscopie infrarouge à transformée de Fourrier. *L'Eurobiologiste*, 1997; **31**: 69-74.
13. Kacem B., Z.Kaid-Omar, M.Daudon, A. Semmoud, B. Lacour, D. Bougeard, A.Addou. Comparaison of crystalluria in patients drinking either free or controles water intake. *Biosciences & Biotechnology Research Asia*, 2004; **2**(2); 93-98.
14. Netelenbos JC, Zwijnenburg PJ, Wee PM. Risk

factors determining active urinary stone formation in patients with urolithiasis. *Clin Nephrol.* 2005; **63**: 188-92.

15. Nguyen HV, Daudon M, Réveillaud RJ, Jungers P. Study of spontaneous crystalluria in patients with calcium oxalate calculi. *Nephrologie,* 1987; **8** (2): 65-9.
16. Sabinski.F, Leusmann.DB. Potential contribution of optional urease-positive bacteria to idiopathic urinary calcium stone formers. I. expression of urease activity in bacteria from the urinary tract that are commonly classified as urease-negative. *Urol Res,* 1996 ;24 :51-54.17.
17. Tuma.J, Hess.B. Néphrolithiase. Epidémiologie, physiopathologie, colique néphrétique, imagerie diagnostique. *Forum Med Suisse,* **41**, 2001.
18. Werness P.G., Bergertj. H., Smith l. H: Crystalluria. *J. Crystal Growth,* 1981; **53**: 166-181.