UIJ UroToday International Journal®

www.urotodayinternationaljournal.com
Volume 1 - October 2008

Study of Urinary Crystals for Type 1 Diabetics

Brahim Kacem*, Ahmed Benahmed Aissa†, Aissa Belouatek*, Ahmed Semmoud†, Ahmed Addou*, Noureddine Benderdouche*

*Laboratory (STEVA), Department of Biology, Faculty of Sciences, University of Mostaganem, Algeria

† Laboratory (LASIR), University of Lille, France.

Submitted on 7 June 2008 - Accepted for Publicaiton on 25 August 2008

ABSTRACT

INTRODUCTION: Diabetes mellitus is a common public health problem due to the seriousness of its complications. Because of the potential harm to the kidneys of diabetic patients, preventive and therapeutic measures should be taken against the various types of lithiasis.

MATERIALS AND METHODS: This survey included 116 type 1 diabetics. First morning urine samples were examined with a polarized light microscope for qualitative and quantitative analysis of crystalluria.

RESULTS: The calcium oxalates were more abundant in both genders compared to the other crystalline species, with a frequency of 76.7% at direct examination and 82.4% at +4°C. The total frequency of purine crystalluria was 22.0% at direct examination.

CONCLUSION: The crystalluria observed in type I diabetics showed the predominate prevalence of the oxalocalcic type (Weddellite) crystals with a frequency of 64.5%, followed by Whewellite with a frequency of 15.0%. The high percentage of the purine crystalluria in diabetics gives information about dangers to clinicians and experts.

KEYWORDS: Diabetics, Lithiasics, Crystalluria, Uric acid, Purine, Calcium oxalate

CORRESPONDENCE: Brahim Kacem, Department of Biology, Faculty of Sciences, University of Mostaganem, City 152 logts Bloc B 16, Mostaganem 27000, Algeria, kacem1b@voila.fr

INTRODUCTION

Diabetes is a disease resulting from either a lack of insulin or an incapacity to adequately use normal quantities of insulin. The number of diabetics in Algeria is estimated to be 1-1.5 million [1]. Due to its prevalence and because of the frequency and seriousness of its complications, diabetes mellitus constitutes a public health problem in many countries. Different organs, the kidneys in particular, may later undergo fatal consequences.

Urinary lithiasis, as well as diabetes, is a frequent pathology that affects approximately 10% of the population of industrialized countries [2]. Its prevalence has increased considerably during the last 50 years in France, and there are now two million lithiasics, 100,000 stone expulsions per annum, and a stone recurrence rate of more than 60% [3]. The nature of stones varies with the patient's sex and age and emphasizes the influence of risk factors dependent on gender, body weight, and other associated pathologies like diabetes.

One approach of the lithiasic pathology is the study of crystalluria, which consists of analyzing the crystals in urine. To obtain clinically interpretable information, it is recommended to make an exhaustive identification of the crystalline species present in the sample under examination. This assumes the knowledge of various morphologies under which the urinary crystals can be observed. Some crystals may have an unusual morphology, often a sign of particular pathological conditions, which may be a source of lithogenic risk or renal function complications. Crystalluria is the main factor that differentiates urine from healthy subjects and lithiasic patients. A multiparametric study of crystalluria (chemical nature and size of the crystalline species, global crystalline volume, pH, density, urinary cytological analysis, etc.) must be carried out in order to determine the risk of crystallogenesis. Biological exploration is necessary to identify the biological factors implied in the lithogenic process and specify the causes of anomalies.

The clinical symptoms of lithiasis are uniform and stereotyped. Its location, frequency and chemical nature have evolved significantly. Clinical interrogation and chemical or biological explorations well oriented by analytical results facilitate the knowledge of the lithogenic risk factors, determine the responsible pathology in most cases, and allow the proposition of suitable prophylactic measures. A reliable means to predict the risk in patients from clinical and biological data remains one of the preoccupations of all clinicians and researchers interested in urinary lithiasis. Studies referring to lithogenic risk of hormonal pathologies, like diabetes and thyroid dysfunction, are not numerous. Previous works clearly highlight epidemiological and biological links between uric lithiasis and certain pathologies such as metabolic syndrome, major obesity, diabetes, or gout [4-7].

It is necessary to study crystalluria in type I diabetics to help eliminate the risk of forming lithiasis and protect the kidneys from this danger associated with diabetes. We studied the crystalluria of 116 type 1 diabetic patients (insulin-dependent) with an aim of comparing their crystalluria with that of stone-formers and control groups. The study was carried out at ambient temperature and at +4°C, according to the current protocol. The purpose of this work was to detect possible lithiasics or risk factors stemming from certain types of crystals known to favor the precipitation of stones and thus have a diabetics' "cartography." This is based on the study for crystalluria that is the expression of an excessive supersaturation of urine. It can be used to detect certain genetic pathologies and assess lithogenic urinary anomalies in nephrolithiasic patients susceptible to lithiasis.

MATERIALS AND METHODS

348 first-voided urine samples from 116 type 1 diabetic patients (3 samples per patient) were collected from a nearby diabetes clinic in a hospital in Mostaganem, Algeria. These patients were divided according to their gender (83 women, 33 men), and the average age was 37 years (13 to 83 years). All the urine samples were collected in sterile tubes after the first morning micturition and subjected to direct examination in the two hours following voiding. The samples were refrigerated at +4°C for 48 hours and then re-examined to assess de novo crystallization. Homogenized urine was transferred with Pasteur pipettes into a Malassez cell. The urinary crystals were classified according to the nature and size of each crystalline species. Aggregates were examined with a (ZEISS) polarized-light microscope. The pH of each urine was measured in the laboratory immediately after collection. A comparison crystalluria study on 200 non-lithiasic subjects was also carried out.

RESULTS

Table 1 displays the nature and frequency of the crystalluria for all the analyzed samples at direct examination and at + 4°C. The total frequency of positive crystalluria was 21.0% at ambient temperature and 39.3% after cold conservation of urine at +4°C. For the non-diabetics, we noticed that the frequency of the positive crystalluria was 13.3%. Many crystalline species of metabolic origin were recorded in this work, such as weddellite, whewellite, complex amorphous urates, uric acid dihydrate, brushite and ACCP. The frequency of the pure crystals was 94.5%, and the frequency of the associated crystals was 5.5%. The analysis of 348 urine samples of type 1 diabetics

Table 1. Pure and associated crystals frequency (%) of type I diabetic patients at direct examination and at +4°C

doi: 10.3834/uij.1939-4810.2008.10.03.t1

Crystals		Direct Examination		+4°C	
		n	%	n	%
Positive Crystalluria		73	21.0	137	39.3
Pure Crystals	Wd*	43	58.6	62	45.2
	CAU†	11	15.0	20	14.5
	Wh‡	09	12.3	10	07.3
	UAD§	04	05.4	04	02.9
	Brushite‖	01	01.3	-	-
	Stuvite¶	01	01.3	-	-
Mixed Crystals	CAU+Wd	-	-	32	23.3
	Wd+Wh	02	02.7	05	03.6
	Wd+UAD	01	01.3	02	01.5
	Wd+Br	01	01.3	02	01.5
Total		73	100	137	100

*weddellite, †complex amorphous urates, ‡whewellite, §uric acid dihydrate, ‖dicalcium phosphate dihydrate, ¶magnesium ammonium phosphate hexahydrate

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original study
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showed a predominance of the calcium oxalate dihydrate (weddellite) crystals at direct examination with a frequency of 58.6%, followed by complex amorphous urates with a rate of 15.0% and whewellite with a rate of 12.3%. Calcium oxalate, being a majority component, attained a rate of 76.7% at direct examination and 82.4% at +4°C. Urates in addition to dihydrated uric acid, whether pure or mixed, had a frequency of 22% compared to other crystalline species observed during analysis.

For the distribution of crystalluria correlated with gender, we noticed a significant difference in the frequency of the crystalline species between men and women. As shown in Table 2, crystalluria was more frequent in women for nearly all the crystalline species observed in this study. In women, weddellite had a frequency of 30.1%, followed by complex amorphous urates (13.9%), whewellite (10.9%), uric acid dihydrate (2.7%), and struvite (1.3%). In men, the frequency for all the crystalline species examined was less than 3% except for weddellite, which had a frequency of 28.7%. Regarding the urine of the control group, the weddellite crystalline species was more frequent in men (12.4%) compared to women (5.9%). Additionally, whewellite had a greater frequency (6.5%) in control women than weddellite, but did not exceed 2.6% in control men. The urine acidity for diabetics was remarkable: more than two-thirds of the samples with positive crystalluria had an average pH lower than 6. The average pH values recorded are given in Table 3.

Table 2. Pure crystals frequency (%) of diabetic patients and non-diabetics as a function of gender at direct examination
doi: 10.3834/uij.1939-4810.2008.10.03.t2

Pure Crystals	Diabetic Subjects				Non-Diabetics			
	Men		Women		Men		Women	
	n	%	n	%	n	%	n	%
Wd	21	28.7	22	30.1	19	12.4	9	5.9
UAC	1	1.3	10	13.9	3	1.9	4	2.6
Wh	1	1.3	8	10.9	4	2.6	10	6.5
UAD	2	2.7	2	2.7	--	--	1	0.7
Str	--	--	1	1.3	--	--	2	1.3
Br	1	1.3	--	--	--	--	--	--
Total	26	35.6	43	58.9	26	17	26	17

DISCUSSION

Diabetes is a serious affection, and people suffering from this disease run a higher risk of morbidity and mortality than the general population [8]. Epidemiological studies carried out during the last ten years show an alarming increase of diabetes, which is the result of a pathological process commonly known as the metabolic syndrome [9,10]. This metabolic disorder is characterized by hyperglycemia caused by a decrease in insulin secretion. Diabetes mellitus is a disease that provokes serious late complications that will alter the sight, renal system, nervous system, and blood circulation [11,12].

Few works studied the relationship between diabetes and renal lithiasis. Liu *et al.* [13] studied the composition of the urine of lithiasic and non-lithiasic diabetic patients compared to healthy subjects and non-diabetic calcic lithiasics. He found that the lithogenic urinary metabolic anomalies were less pronounced and the probability of being lithiasic is lower in diabetics than in normal subjects, suggesting that being diabetic does not predispose one to urinary lithiasis per se. However, Abate *et al.* [14] reported that patients with recurrent uric acid stones exhibit clinical and metabolic abnormalities consistent with the metabolic syndrome. As the latter is often predictive of the development of type 2 diabetes mellitus, it may be assumed that diabetic patients may have a particular risk to develop uric acid renal lithiasis.

Meydan *et al.* [15] recently reported that 21% of diabetic patients were affected by urolithiasis, as compared with only 8% in the non-diabetic population, but the chemical type of stones was not examined. Pak *et al.* [16] reported that 33.9% of 59 stone-forming patients with type 2 diabetes had uric acid stones, as compared with only 6.2% in non-diabetic stone formers. Lastly, a recent study by Mbarki *et al.* [17] showed that diabetics are less exposed to the risk of forming crystals in urine than idiopathic lithiasics without diabetes.

In this study, it can be noted that the total positive crystalluria of type 1 diabetic patients reached 21%, but it did not exceed 13.3% for normal subjects without lithiasic antecedent. On the other hand, samples from calcic idiopathic stone formers contain 60-70% of the crystals [18,19]. In comparison with data gathered in other works devoted to non-diabetic stone formers' crystalluria (notably Wernes *et al.* [20]), it can be noticed that the nature of the urinary crystals identified is roughly the same in lithiasics and diabetics, with the same great diversity of crystalline species in both cases. However, the crystalluria frequency appears moderately increased in type 1 diabetics (almost double that of normal subjects), which indicates

Table 3. Correlation between major crystalline species and average pH
doi: 10.3834/uij.1939-4810.2008.10.03.t3

	Crystals	Average pH
Pure Crystals	Calcium oxalate dihydrate	5.75
	Calcium oxalate monohydrate	5.79
	Complex amorphous urates	5.70
	Uric acid dihydrate	5.74
	Struvite	7.02
	Brushite	6.06
Mixed Crystals	CAU + Wd	5.84
	Wd +Wh	5.68
	Wd + UAD	5.42
	Wd + Br	6.33

that diabetics are more likely to develop nephrolithiasis [17]. Supersaturation of the urinary environment is a fundamental factor of lithogenesis, which generates the primitive insoluble crystal phase and ensures its later growth in most cases.

From an analytical point of view, all first morning urine samples studied at direct examination had calcium oxalates in the majority of cases (76.7% total, 71.2% pure, 5.5% mixed). The crystalline species most frequently observed in diabetics was weddellite (dihydrated calcium oxalate) with a frequency of 58.9% compared to the control group rate of 18.3%. This anomaly can be expressed by the nutritional and metabolic difference among diabetics. On the other hand, the crystalline species of monohydrated calcium oxalate had a frequency of 12.3% for diabetics and 9.1% for healthy subjects. This indicates the natural behavior of crystalluria evolution between these various types of pathology and allows for differentiation of diabetics from healthy subjects. This finding may be a factor in the tracking of urinary lithiasis in diabetics.

Calcic phosphate crystals (brushite and ACCP) occurrence was particularly small in diabetics (2.3%) and healthy subjects (0%). Magnesium ammonium phosphate hexahydrate (struvite) had a frequency of 1.3% in healthy subjects and 0% in the urine of diabetics. This is due to the acidic urine recorded in diabetics, which does not favor struvite formation. Another analysis of these results indicates that diabetic women exhibit a positive crystalluria in 63% of all cases (compared to 36.9% in men) and a higher frequency for most of the identified crystalline species. Thus, oxalate frequency is 45.2% and purine frequency 16.5% in women, compared to 31.5% of oxalates and 5.5% of urates in men. This is completely the opposite in non-diabetic stone formers [21,22]. The crystalline species that presents the highest risks of lithiasis formation is whewellite. Its oxalate-dependent character indicates a significant hyperoxaluria found in most of female diabetics: 16.4% against 13% in non-diabetic stone formers and 6.5% in healthy subjects [23]. This would imply more monitoring and regular tracking to avoid any precipitation of stones.

The results of our work show that the average pH of the analyzed urine samples was acidic in almost 75% of cases. This was confirmed by the appearance of uratic crystals (complex amorphous urates and uric acid) with a frequency of 22% in diabetics and 5.2% in healthy subjects. This indicates that the acidity sometimes generated by certain metabolic dysfunctions in diabetics can be a lithogenic risk in the formation of purine lithiasis. In a study undertaken about diabetics' lithiasis risk factors, Pak *et al.* [24] showed that in addition to urine hyperacidity, the fractional excretion of uric acid was not decreased, contrary to what is observed in uric lithiasics with no metabolic syndrome. Several other authors have observed an increase in the fractional excretion of uric acid in insulin-dependent diabetics [25]. Recently, Daudon *et al.* [26] have shown that the proportion of uric acid stones was 2.2x higher in diabetic than in non-diabetic stone formers, but the difference was strikingly more marked in women (3.8x) than in men (1.7x). The same author indicated in another study that the increase of the body weight is accompanied by a significant decrease in the urinary pH [2]. The drop of urinary pH and the simultaneous maintenance of excretion favor the formation of an excessive quantity of non-dissociated uric acid, thus facilitating its crystallization in the urine [6]. The higher prevalence of crystalluria of uric acid in diabetics in general and women diabetics in particular is an argument for immediate patient care in order to avoid any serious complications. Indeed, a diabetic patient is always threatened by a fatal nephropathy that can cunningly destroy kidneys and lead towards renal insufficiency necessitating hemodialysis. Finally, the surveillance of crystalluria in these patients may allow doctors to evaluate the risk of forming stones and implement adapted preventive measures, such as alkaline diuresis, in patients threatened by lithiasis.

CONCLUSION

Our work shows that positive crystalluria in type 1 diabetic patients was more frequent in comparison with control groups, which indicates that these patients are more exposed to the risk of forming renal stones threatening their kidneys. The increased acidity of diabetics' urine, especially in women, indicates an excess in purine substances eliminated by the kidneys. This could be a very useful indicator of lithogenous risk factors of forming uric lithiasis or a prognostic factor of the risk of lithiasis recurrence. In diabetic patients, crystalluria monitoring might be necessary in certain conditions to detect risk and propose preventive measures at the same time.

REFERENCES

[1] Khiati M. Le diabète sucré chez l'enfant, édition OPU. Alger. 1993

[2] Daudon M. [Epidemiology of nephrolithiasis in France]. Ann Urol (Paris). 2005 Dec;39(6):209-31.

[3] Donsimoni R, Hennequin C, Fellahi S, Troupel S, Moël GL, Paris M, Lacour B, Daudon M. New aspect of urolithiasis in France. Eur Urol. 1997;31(1):17-23.

[4] Daudon M, Jungers P. Diabète et calculs. Feuillets de biologie. 2001;42:37-39.

[5] Grosse H. [Frequency, localization and associated disorders in urinary calculi. Analysis of 1671 autopsies in urolithiasis]. Z Urol Nephrol. 1990 Sep;83(9):469-74.

[6] Powell CR, Stoller ML, Schwartz BF, Kane C, Gentle DL, Bruce JE, Leslie SW. Impact of body weight on urinary electrolytes in urinary stone formers. Urology. 2000 Jun;55(6):825-30.

[7] Schröder HE, Lohse R, Böhm WD. [Incidence of metabolic disorders in patients with recurrent urinary calculi]. Z Urol Nephrol. 1981 Mar;74(3):235-41.

[8] Koda-Kimble MA, Carlisle BA. Diabetes mellitus. In: Koda-Kimble, MA. Young LY editors. Applied therapeutics: the clinical use of drugs. 7th ed. Philadelphia: Lippincott Williams & Wilkins. 2001;48-1-92.

[9] Beer-Borst S, Morabia A, Hercberg S, Vitek O, Bernstein MS, Galanb P, Galasso R, Giampaoli S, Houterman S, McCrum E, Panico S, Pannozzo F, Preziosi P, Ribas L, Serra-Majem L, Verschuren WMM, Yarnell J, Northridge ME. Obesity and other health determinants across Europe: The EURALIM Project. J Epidemiol Community Health 2000 June;54:424-430.

[10] Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the Third National Health and Nutrition Examination Survey. JAMA. 2002 Jan 16;287(3):356-9.

[11] Meltzer S, Leiter L, Daneman D, Gerstein HC, Lau D, Ludwig S. *et al*. 1998 clinical practice guidelines for the management of diabetes in Canada. Canadian Diabetes Association. CMAJ, 1998;159: Suppl 8:S1-29.

[12] Foster DW. Diabetes mellitus. In: Harrison's principles of internal medicine. 14th ed. New York: McGraw-Hill, 1998;2060-81.

[13] Liu JG, Hu M, He XQ. [Risk factors for the formation of urinary calcium-containing stones in diabetics]. Zhonghua Nei Ke Za Zhi. 1989 Nov;28(11):649-53, 699-700.

[14] Abate N, Chandalia M, Cabo-Chan AV, Moe OW. Sakhaee K. The metabolic syndrome and uric acid nephrolithiasis: novel features of renal manifestation of insulin resistance. Kidney Int. 2004 Feb;65(2):386-92.

doi:10.3834/uij.1939-4810.2008.10.03

ISSN 1944-5792 (print), ISSN1944-5784 (online)

[15] Meydan N, Barutca S, Caliskan S, Camsari T. Urinary stone disease in diabetes mellitus. Scand J Urol Nephrol. 2003;37(1):64-70.

[16] Pak CY, Sakhaee K, Moe O, Preminger GM, Poindexter JR, Peterson RD, Pietrow P, Ekeruo W. Biochemical profile of stone forming patients with diabetes mellitus. Urology. 2003 Mar;61(3):523-7.

[17] Mbarki M, Jabrane J, Ossama A, Daudon M. [Study of crystalluria in diabetic patients]. Prog Urol. 2005 Jun;15(3):420-5; discussion 425-6.

[18] Bader CA, Chevalier A, Hennequin C, Jungers P, Daudon M. Methodological aspects of spontaneous crystalluria studies in calcium stone formers. Scanning Microse. 1994;8(2):215-31; discussion 231-2.

[19] Caudarella R, Rizzoli E, Malavolta N, Severi B, Vast V, Biagini G. [Urinary crystalluria. A debatable problem]. Acta Urol Belg. 1986;54(1):49-56.

[20] Wernes PG, Bergert JH, Smith LH. Crystalluria. J Crystal Growth. 1981 May;53(1):166-81.

[21] Koide T, Oka T, Takaha M, Sonoda T. Urinary tract stone disease in modern Japan. Stone incidence, composition and possible cause on Osaka district. Eur Urol. 1986;12(6):403-7.

[22] Daudon M, Donsimoni R, Hennequin C, Fellahi S, Moël GL, Paris M, Troupel S, Lacour B. Sex and age related composition of 10617 calculi analyzed by infrared spectroscopy. Urol Res. 1995;23(5):319-26.

[23] Kacem B, Kaid-Omar Z, Daudon M, Semmoud A, Lacour B, Bougeard D, Addou A. Comparison of crystalluria in patients drinking either free or controlled water intake. Biosciences Biotech Research Asia. 2004;2:93-98.

[24] Pak CY, Sakhaee K, Peterson RD, Poindexter JR, Frawley WH. Biochemical profile of idiopathic uric acid nephrolithiasis. Kidney Int. 2001 Aug;60(2):757-61.

[25] Erdberg A, Boner G, van Dyk DJ, Carel R. Urine uric acid excretion in patients with insulin-dependent diabetes mellitus. Nephron. 1992;60(2):134-7.

[26] Daudon M, Lacour B, Jungers P. High prevalence of uric acid calculi in diabetic stone formers. Nephrol Dial Transplant. 2005 Feb;20(2):468-9.

TO CITE THIS ARTICLE: Kacem B, Aissa AB, Belouatek A, Semmoud A, Addou A, Benderdouche N. Study of Urinary Crystals for Type I Diabetics. UIJ. 2008 Oct.1(4). doi: 10.3834/uij.1939-4810.2008.10.03