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ORIGINAL

Urine sediment crystals in Bitola, North Macedonia: results from 3200 urines made with LabUMat 2 & Urised 3 Pro

Cristales de sedimento urinario en Bitola, Macedonia del Norte: resultados de 3.200 orinas realizadas con LabUMat 2 & Urised 3 Pro

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Summary

Introduction: Urine sediment testing is a diagnostic medicine that remains a long-standing practice in clinical laboratories. Urine analysis, which is an important test in clinical medicine, is used in screening, diagnosing and monitoring diseases of the urinary system, and diseases that are detected through the urinary system. We use the performance of a new model of UriSed (also sediMAX) automated microscope called UriSed 3 PRO. Thus, this paper presents a case of interest for clinical laboratory practice, as it demonstrates the utility of urine sediment examination as a diagnostic tool in the evaluation kidney disease. Therefore, we summarized the data from 3200 urinalysis and our aim was to evaluate the distribution of urinary crystal components and different crystal forms according to the age and gender of the patients.
Methods: The study was performed using 3200 urine samples collected into the clean tubes without preservatives, which have been obtained from the patients applied to Hospital for routine visit. The patients left the urine samples and we pipette 10 mL into test tubes. The samples were analyzed every day in small batches for six months in 2023.The urine sample were analyzed in an hour at the latest.
Results: Of the 3200 urines analyzed, 2000 females and 1200 males. 2079 of them were free of crystals and 1121 urines were with crystals and were included in the analysis. 67% were mostly calcium oxalate dihydrate crystals, followed by calcium oxalate monohydrate 22%, followed by 11% triple phosphate crystals and 0.01% uric acid crystals. The majority of crystals were detected in males (37%) versus females (33%). Calcium oxalate dihydrate crystals (26% vs.22%) was the most common major component in both men and women, followed by crystals of calcium oxalate monohydrate (8% vs.7%).
Conclusion: The most abundant crystals in the urine in our study were found to be calcium oxalate crystals, followed by tripel phosphate and uric acid. The highest incidence of crystals is in patients aged 61 to 70 years. The male gender is more affected compared to the female gender. The distinction between different crystal forms can provide clues to the activity and mechanisms of the lithogenic process. Knowledge of the mechanisms of crystal and stone formation is necessary in order to provide appropriate individualized treatment to each patient and to prevent their recurrence.

Key words: Urine sediment testing, Calcium oxalate monohydrate crystals, Calcium oxalate dihydrate crystals, Triple phosphate crystals, Uric acid crystals.

Resumen

Introducción: El análisis del sedimento urinario es una prueba diagnóstica que sigue siendo una práctica arraigada en los laboratorios clínicos. El análisis de orina, que es una prueba importante en la medicina clínica, se utiliza en el cribado, diagnóstico y seguimiento de enfermedades del aparato urinario y de enfermedades que se detectan a través del aparato urinario. Utilizamos el rendimiento de un nuevo modelo de microscopio automatizado UriSed (también sediMAX) denominado UriSed 3 PRO. Así, este trabajo presenta un caso de interés para la práctica del laboratorio clínico, ya que demuestra la utilidad del examen del sedimento urinario como herramienta diagnóstica en la evaluación de la enfermedad renal. Por lo tanto, resumimos los datos de 3200 análisis de orina y nuestro objetivo fue evaluar la distribución de los componentes de los cristales urinarios y las diferentes formas de cristales según la edad y el sexo de los pacientes.
Métodos: El estudio se realizó utilizando 3200 muestras de orina recogidas en tubos limpios sin conservantes, obtenidas de pacientes que acudieron al hospital para una visita rutinaria. Los pacientes dejaron las muestras de orina y pipeteamos 10 mL en tubos de ensayo. Las muestras se analizaron todos los días en pequeños lotes durante seis meses en 2023.La muestra de orina se analizaron en una hora a más tardar.
Resultados: De las 3200 orinas analizadas, 2000 eran de mujeres y 1200 de hombres. 2079 de ellas estaban libres de cristales y 1121 orinas estaban con cristales y se incluyeron en el análisis. El 67% eran en su mayoría cristales de oxalato cálcico dihidratado, seguido de oxalato cálcico monohidratado 22%, seguido de un 11% de cristales de fosfato triple y un 0,01% de cristales de ácido úrico. La mayoría de los cristales se detectaron en varones (37%) frente a mujeres (33%). Los cristales de oxalato cálcico dihidratado (26% frente a 22%) fueron el componente principal más frecuente tanto en hombres como en mujeres, seguidos de los cristales de oxalato cálcico monohidratado (8% frente a 7%).
Conclusión: Los cristales más abundantes en la orina de nuestro estudio fueron los de oxalato cálcico, seguidos de los de tripel fosfato y ácido úrico. La mayor incidencia de cristales se da en pacientes de 61 a 70 años. El sexo masculino está más afectado que el femenino. La distinción entre las diferentes formas cristalinas puede proporcionar pistas sobre la actividad y los mecanismos del proceso litogénico. El conocimiento de los mecanismos de formación de cristales y cálculos es necesario para proporcionar un tratamiento individualizado adecuado a cada paciente y prevenir su recurrencia.

Palabras clave: Análisis de sedimento urinario, Cristales de oxalato cálcico monohidratado, Cristales de oxalato cálcico dihidratado, Cristales de fosfato triple, Cristales de ácido úrico.

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Introduction

Urine sediment testing is a diagnostic medicine that remains a long-standing practice in clinical laboratories. Urine analysis, which is an important test in clinical medicine, is used in screening, diagnosing and monitoring diseases of the urinary system, and diseases that are detected through the urinary system[1]. The use of automated urine analyzers has become a clinical standard in medium and large laboratories; they count cells using flow cytometry or several types of image analyzers. Urinalysis is used routinely in daily practice to detect hematuria and proteinuria, which are initial signs of kidney disease, but its importance is invaluable in assessing the morphology of urinary erythrocytes and in distinguishing glomerular disease from non-glomerular disease[2].

We use the performance of a new model of UriSed (also sediMAX) automated microscope called UriSed 3 PRO (77 Elektronika, Budapest, Hungary, represented by Mediq Ltd in Finland) launched in 2018, that takes phase-contrast images on urine particles, by using urine samples from our routine of mixed patient populations. Our practice has recently introduced the UriSed2 analyzer which captures images of urine centrifuged in a disposable tube with a digital camera attached to a bright field microscope at 400× magnification. The sediment is processed with image processing software. The images are then rechecked by a technician, when necessary. The analyzer software detects red blood cells (RBC), white blood cells (WBC), squamous epithelial cells (EPI), crystals, and cylinders[3,4].

We analyzed the end products of metabolism are found highly concentrated in the urine and can precipitate in the form of crystals. The presence of crystals does not always indicate pathological conditions, but several types of crystals are associated with certain diseases and with the risk of stones. Thus, this paper presents a case of interest for clinical laboratory practice, as it demonstrates the utility of urine sediment examination as a diagnostic tool in the evaluation kidney disease. Therefore, we summarized the data from 3200 urinalysis and our aim was to evaluate the distribution of urinary crystal components and different crystal forms according to the age and gender of the patients.

Materials and methods

The study was performed using 3200 urine samples collected into the clean tubes without preservatives, which have been obtained from the patients applied to Hospital for routine visit. The patients left the urine samples and we pipette 10 mL into test tubes. The samples were analyzed every day in small batches for six months in 2023.The urine sample were analyzed in an hour at the latest.

Statistical analyses

Categorical variables are presented as percentages. The effect of age and gender on different crystals in urine types was assessed by the Chi-squared test. For analysis we used Fisher's exact test and it was used if the Chi-square test was not applicable. The significance level was considered as $p<0.05$. Statistical analysis was performed using SPSS for Windows, version 27.

Results

Crystal composition in urines

Of the 3200 urines analyzed, 2000 females and 1200 males. 2079 of them were free of crystals and 1121 urines were with crystals and were included in the analysis. 67% were mostly calcium oxalate dihydrate crystals, followed by calcium oxalate monohydrate 22%, followed by 11% triple phosphate crystals and 0.01% uric acid crystals presented in **table I**.

Gender

The majority of crystals were detected in males (37%) versus females (33%). Calcium oxalate dihydrate crystals (26% vs.22%) was the most common major component in both men and women, followed by crystals of calcium oxalate monohydrate (8% vs.7%). Crystals of calcium oxalate monohydrate and calcium oxalate dihydrate were more often obtained from men than women ($p < 0.001$). Crystals of triple phosphate crystals were more often obtained from women than in males.

Age

The peak incidence of crystals in both women and men were between the ages of 61 and 70 years. Age trends in crystals distribution were similar in both genders for most crystals types. Calcium oxalate dihydrate was the

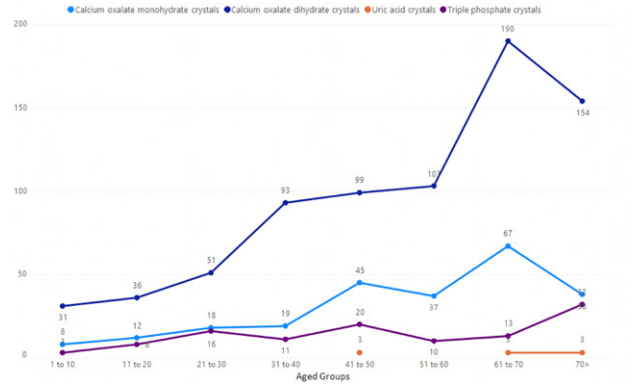
Table I: Distribution of urine crystals ($n=1121$).

Main components	Urine without crystals					Urine with crystals				
	All	Females	%	Males	%	All	Females	%	Males	%
Calcium oxalate monohydrate crystals	2958	1859	93	1099	92	242	141	7	101	8
Calcium oxalate dihydrate crystals	2443	1555	78	888	74	757	445	22	312	26
Triple phosphate crystals	3087	1922	96	1165	97	113	78	4	35	3
Uric acid crystals	3191	1997	99	1194	99	9	3	0	6	0

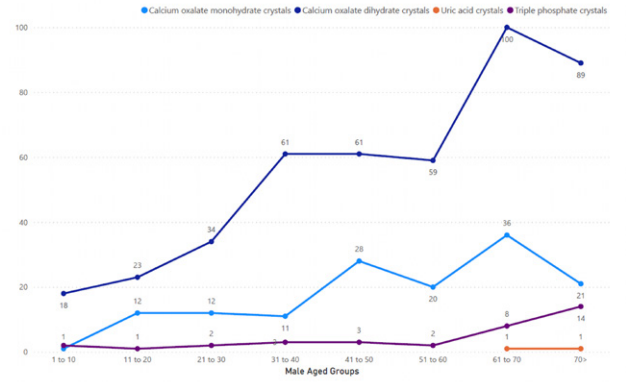
most common main stone component in both genders and all age groups. While crystals of calcium oxalate monohydrate was second most frequently obtained from patients 61- 70 years old. The occurrence of triple phosphate crystals increased strongly in men and women ≥ 60 years old (**Figure 1**).

Figure 1: Association of gender and age with stone type.

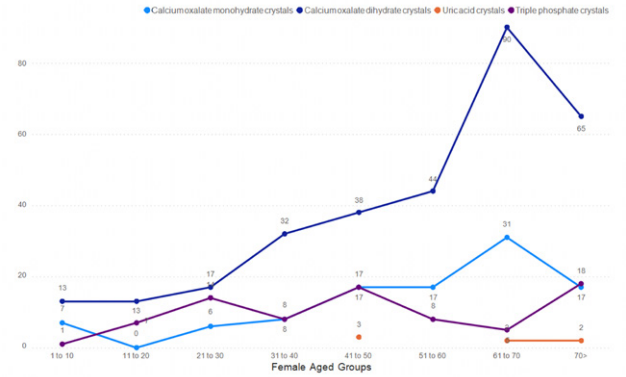
a: Total



b: Males



c: Females



Discussion

Urine sediment analysis with the advent of automated urine analyzers is performed more quickly, reducing potential analysis variations and increasing precision and accuracy. However, manual microscopy remains the "gold standard" despite methodological problems and shortcomings[5,6]. Manual microscopy is labor-intensive, time-consuming, large inter-technician variation has been observed, and has low reproducibility[7]. At the moment, automated urine analyzers are used in the world, which allow us better productivity, increased reproducibility and reduced time and effort required for processing urine samples[8,9]. The analysis of urine sediment and specifically crystals in urine allows us to get to know the frequency of different forms of hydrates and how demographic factors affect them, and thus we would have a more accurate idea of the etiology, therapy and prevention of the recurrence of stone disease in the kidneys. The current study presents the latest data on the characteristics of urine sediment and specifically urine crystals in patients in Bitola, Macedonia.

Previous studies have analyzed the distribution of urinary stone types in different countries and found that calcium oxalate is the most commonly diagnosed stone, followed by carbonate apatite and uric acid stones.Calcium oxalate was the most common major urinary crystal in both sexes in our study, accounting for 89% of all crystals submitted. The incidence of calcium oxalate crystals is higher in men. Numerous studies confirm the high percentage of calcium oxalate stones especially in male respondents[10-12]. In our study, the majority of calcium oxalate crystals were calcium oxalate dihydrate crystals compared to calcium oxalate monohydrate crystals with a ratio of approximately 3:1 in both sexes. The tendency to develop a calcium oxalate crystal is associated with specific urinary risk factors. Recent studies have suggested that hyperoxaluria may contribute to the formation of calcium oxalate monohydrate crystals[13,14], while hypercalciuria leads to the formation of calcium oxalate dihydrate crystals and stones[13-15]. Osteopontin, which is an inhibitor of calcium oxalate stone synthesis, modifies the kinetics of calcium oxalate crystallization and prevents the formation of calcium oxalate dihydrate [16]. With advancing age are decreasing levels of osteopontin in the blood leading to higher proportions of calcium oxalate dihydrate with increasing age[17]. This was confirmed in our study, as the most frequently affected age group in which crystals were detected in urine are patients over 60 years of age.

Triple phosphate crystals are the second most common crystals found in urine samples in our study, they are composed of magnesium ammonium phosphate. Their mechanism of occurrence has been explained in numerous studies and it has been proven that in conditions of bacterial infection of the urinary tract caused by *Proteus mirabilis*, *K. pneumoniae* or *Corynebacterium*, urease is created, it breaks down urea in the urine to carbon dioxide and ammonia. Ammonia together with water creates ammonium hydroxide, it increases the pH in the urine, it becomes alkaline. In our study, we observed triple phosphate crystals to be more common in women, and this is confirmed by

other studies that have linked the presence of triple phosphate crystals in urine to patients at high risk of developing urinary tract infections, such as those with neurogenic bladder, urinary diversion, or indwelling Foley catheter[18,19].

Uric acid urine crystals are the rarest urinary crystals in our study. They are slightly more common in men than in women. According to the scientific literature, they are found in 10 to 11% of all kidney stones[20] and this percentage increases with advancing age[21]. The appearance of these crystals is associated with low urine pH, followed by hyperuricosuria and low diuresis[22-24], but there is not a small number of patients in whom their appearance is idiopathic and not associated with hyperuricosuria or low diuresis[25].

Conclusion

The most abundant crystals in the urine in our study were found to be calcium oxalate crystals, followed by tripel phosphate and uric acid. The highest incidence of crystals is in patients aged 61 to 70 years. The male gender is more affected compared to the female gender. The distinction between different crystal forms can provide clues to the activity and mechanisms of the lithogenic process. Knowledge of the mechanisms of crystal and stone formation is necessary in order to provide appropriate individualized treatment to each patient and to prevent their recurrence.

Conflicts of interest

Authors have no conflicts of interest to disclose.

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