International Journal of KIU

Journal home page : https://www.kiu.lk/ijkiu/
DOI:10.37966/ijkiu2021021010

Original Article

Association between urinary crystals and bacterial flora in urinary tract infection suspected patients

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Article history:
Received 08 December 2020
Received in revised form
18 February 2021
Accepted 20 February 2021

Cite as:

Abeysekara, W. E. C. M., Kehelpannala, S. A. K., Weerawansa, K. R., Priyadarshani, A. M. B., Arachchi, A. M. P., (2021). Association between urinary crystals and bacterial flora in urinary tract infection suspected patient. International Journal of KIU. 2 (1). 27-32. https://doi.org/10.37966/ijkiu2021021010

Abstract

Urinary stones are an increasing clinical problem in Sri Lanka. It has been reported that 1/10 individuals experience urinary stones, yet the mechanisms responsible remain unknown. Bacteria have long been recognized to contribute to urinary crystals. However, the role of bacteria in development of urinary crystals has not been extensively investigated. This study was carried out to investigate the association between urinary bacteria flora and urinary crystals in patients with symptoms of urinary tract infections (UTI). Forty five UTI suspected patients were enrolled in the study. Mid-stream urine specimens were collected and cultured on Hi-Chrome UTI culture media. Culture plates were incubated at 37 ºC for 18-24 hours. Colony appearance and colony count were recorded and this was followed by urinary crystal count by crystal concentration technique. The Fisher's exact test was carried out to determine the association between urine crystals and presence of urinary bacterial flora. Out of 45 subjects, 29 were positive for urinary crystals, where 14 were females and 15 were males. Nineteen patients were culture positive while 18 were positive for both crystals and culture. The patients between 30-40 years showed a higher possibility to have urinary crystals and organisms. Predominant crystal type and organism detected were calcium oxalate and coliforms, respectively. Male patients in 30-40 years presented with a higher crystal concentration compared to other males. All female patients, between 30-40 years, who were positive for coliforms and presented with higher crystal concentration compared to other females in the study population. A significant association ($p < 0.001$) was observed between presence of urinary crystals and bacterial flora in UTI suspected patients.

Key words: Urinary crystals, Calcium oxalate, Coliforms, Urinary tract infections, Urine culture

Introduction

Presence of crystals in urine is known as "Crystalluria". Analysis of crystals is carried out by microscopic examination of urine. The stone formation is considered to be a medical challenge due to its multi-factorial etiology (Schwaderer and Wolfe, 2017). The etiology of urinary crystal formation is included chronic dehydration, urinary tract malformations, obstructed uropathy, metabolic diseases such as hyperparathyroidism, gout, and obesity, foreign bodies found in urinary tract and infections (Straub and Hautmann, 2005). Bacteria have been recognized as contributors in urinary crystal formation. However, the exact role of bacteria in crystal formation has not been extensively investigated though there are several findings to indicate the association between urinary crystals and bacteria (Schwaderer and Wolfe, 2017; Rahman et al., 2003; Torzewska et al., 2014). It has been noted that an elevated urinary crystal concentration, especially calcium oxalate had been recorded while performing urine full report (UFR) in routine urinalysis in Sri Lanka (Chandrajith et al., 2006; Chandrajith et al., 2019). The objective of the present study was to investigate the association between urinary crystals and bacterial flora in the urinary tract infection suspected patients.

Methods

This study is a descriptive cross-sectional study carried out among 45 patients aged 18-50 years. Patients who were enrolled at 3 private clinics with symptoms of urinary tract infections such as backache, dysuria and burning sensation while passing urine. Known UTI patients, patients with chronic diseases, antenatal mothers and urine specimens that were not delivered to the laboratory within 30 minutes of specimen collection was excluded from the study.

The study was conducted after obtaining ethical approval from the Ethical Review Committee of KIU, Battaramulla, Sri Lanka (KIU/ERC/18/049).

Data collection method

The demographic and other related data were recorded using a self-administered questionnaire.

Urine specimen collection

Urine specimens were collected from each patients who enrolled to private clinics according to the guidelines given in Laboratory Manual in Microbiology (Laboratory Manual in Microbiology Sri Lanka, 2011). Prior to collection of urine specimens, volunteers were provided with guideline which included all the necessary instructions regarding collection of clean-catch urine/mid-stream urine specimen. Volunteers were then provided with disposable, sterile, screw capped urine containers to collect urine specimens.

Urine specimen processing

The urine analysis was carried out within 30 minutes of specimen collection. Urine specimens were well mixed and inoculated on HiChrome UTI culture medium by using calibrated 0.001mL (1µL) wire loop. Inoculation was carried out according to inverted cone shaped manner and incubated at 37 °C for 24 hours. All the laboratory techniques were carried out under sterile conditions.

Reading and recording of results from culture plates growth of the colonies was observed and number of colonies were counted. Interpretation was done according to the Table 1 (Pezzlo, 1998).

Table 1: Interpretation of colony count in HiChrome UTI culture medium

Colony count	Interpretations
<10	Insignificant bacterial growth
10 – 99	Pure growth of 10^4 - 10^5 CFU/mL
More than 100	Pure growth of $\geq 10^5$ CFU/mL

Colony colour and appearance were compared with the instruction chart provided with culture medium as given in Table 2.

Table 2: Colony colour and appearance in Hichrome UTI culture medium

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Enterococcus faecalis ATCC 29212	50-100	Luxuriant	>=70%	Blue, small
Escherichia coli ATCC 25922	50-100	Luxuriant	>=70%	Pink-purple
Klebsiella pneumoniae ATCC 13883	50-100	Luxuriant	>=70%	Blue to purple, mucoid
Pseudomonas aeruginosa ATCC 27853	50-100	Luxuriant	>=70%	Colourless (greenish) Pigment may be observed)
Proteus mirabilis ATCC 12453	50-100	Luxuriant	>=70%	Light brown
Staphylococcus aureus ATCC 25923	50-100	Luxuriant	>=70%	Golden yellow

When there was a significant growth, it was recorded with the type of organisms present and colony count.

Gram stain was then performed in order to identify the gram characteristics of the organisms.

Microscopic examination of urine sediment

Each urine specimen was poured into labeled, clean 12 mL centrifuged tubes and centrifuged at 2000 rpm for 5 minutes. The supernatant was decanted and the remaining sediment was re-suspended by adding 5 drops (0.5 mL) of supernatant onto the sediment. Type of crystals were counted in 10 high power fields and calculated in to crystal count per microliter and per milliliter of urine according to the European Urinalysis Guidelines (European Urinalysis Guidelines, 2000).

RESULTS

Demographic characteristics of study population

Gender and age distribution within the study population is given in Table 3. Majority (62.2%) of the enrolled patient population was females. Most (60.0%) of the patients were 30-39 years of age.

Table 3: Demographic chracteristics of the study population

Demographic chracteristics		Number of patients	Percentage (%)
Gender	Male	17	37.8
	Female	28	62.2
Age (in years)	20 - 29	8	17.8
	30 - 39	27	60.0
	40 - 49	10	22.2

Distribution of urinary crystals in the study population

Out of 45 subjects, 29 individuals were positive for urinary crystals whereas 16 were negative for urinary crystals. Among crystal positive patients, 15 were males and 14 were females. The types of urinary crystals present in the specimens were calcium oxalate, triple phosphate and uric acid. The number of patients with each crystal type is given in Table 4. There was no significant association ($p = 0.519$) between the age of the patients and urinary crystals (Table 5).

Table 4: Number of patients with each crystal type

Crystal type	Number of patients	Percentage (%)
Calcium oxalate	26	89.7
Triple phosphate	2	6.9
Uric acid	1	3.5

Table 5: Association between age and urinary crystals

Age groups	Patients with crystals	Patients without crystals	X^2	p value
20 - 29	4	4		
30 - 39	19	8	1.23	0.519
40 - 49	6	4		

Analysis of Hichrome UTI culture medium results

In the study population, only 19 patients had given positive results for culture; among them, 10 were

females and 9 were males. Microorganisms present were coliforms, Pseudomanas spp Staphylococcus spp. Distribution of microorganisms in culture positive cases are given in Table 6.

Table 6: Distribution of microorganisms in culture positive cases

Organism type	Male	Female	Total
Coliform spp	4	10	14
Pseudomanas spp	2	0	2
Staphylococcus spp	3	0	3

Association between urinary crystals and urine culture

Only 1 male patient with coagulase negative Staphylococcus spp was negative for urinary crystals. Other 18 patients were positive for both urinary crystals and organisms.

Eleven patients who were crystal positive, which included 4 females and 7 males, were negative for urine culture. In the study population, 15 patients including 14 females and 1 male were negative for both crystals and culture. These results have been summarized in Table 7.

Table 7: Association between urinary crystals and urine culture

	Crystal positive specimens			Crystal negative specimens		
	Female	Male	Total	Female	Male	Total
Positive cultures	10	8	18	0	1	01
Negative cultures	4	7	11	14	1	15

Both males and females in age 30-40 year group showed a higher possibility to have urinary crystals and organisms. Male patients in age 30-40 year category had a higher crystal concentration compared to others. Female patients between 30-40 years who were positive for coliforms presented with higher crystal concentration compared to other females.

According to the Fisher's exact test, there was a significant association ($p < 0.001$) between presence of urinary crystals and presence of urinary bacterial floras in UTI suspected patients as given in Table 8.

Table 8: Association between urinary crystals and urinary bacterial flora

	Crystal positive	Crystal negative	X^2	p value
Positive cultures	18	01	13.17	<0.001
Negative cultures	11	15		

Discussion

Urinary stone formation is a common disease with an increasing incidence and prevalence worldwide. This disease appears even more pronounced in industrialized countries (Daudon et al., 2004). Such observations seem to underscore the impact of lifestyle and dietary choices. According to a previous study calcium oxalate and uric acid stones are more common compared to other types (Grifth and Osborne, 1987).

In this study, 64.4% (29/45) of the UTI patients were positive for urinary crystals. Other studies have reported the prevalence of crystal formation to be about 1–5% in Asia, 5–9% in Europe, 13% in North America and 20% in Saudi Arabia (Amato, Lusini, and Nellie, 2004). In addition, the recurrence rate has been increased and exceeded 50% over a 5 to 10 year period (Bouatia et al., 2015). These reports indicate that there is a significant incidence of urine crystal formation in different populations and prevention of stone formation is of great importance to the health of general population.

According to Bouatia et al., urinary stone formation and frequency is higher in men than in women (Bouatia et al., 2015). However, an association between gender and urinary stone formation could not be found in this study as the number of males and females with urinary crystals were similar in the patient population.
Further, the same study has highlighted that the knowledge regarding mineralogical composition of urinary stones is important as it is needed to the

scientific community to explain the chemistry and the causative factors of the calculi in the urinary system (Bouatia et al., 2015). Majority of the urine specimens collected from the study population contained calcium oxalate crystals, which is similar to several reports published by other researchers (Chandrajith et al., 2019; Chandradith et al., 2006; Grifth and Osborne, 1987).

Other studies have also reported that renal stone formation and the predominant chemicals composition are age and gender dependent (Daudon et al., 2008). Most stones are formed in older patients. However, clinical observations have indicated not only a changing frequency and composition of urinary calculi but also a shift in gender and age-related incidences (Strope, Wolf and Hollenbeck, 2010). Studies have reported that children as well as adults, with factors implicated in the metabolic syndrome such as obesity now are at a higher risk for urinary stone formation (Sarica et al., 2009). However, the results of the current study found no significant association between the crystal formation and age, even though, majority of the patients with urinary crystals were between 30 – 39 years of age.

In this study, the prominent organism type was coliforms. This finding is similar to a report published by a previous study where UTI was identified to be mainly caused by E. coli (90 %), Proteus spp and Klebsiella spp (Bochud and Calandra, 2003).

The results of this study showed a significant association between urinary bacterial flora and urinary crystals. The association between bacteria and urinary crystals can be explained in different ways; bacteria may adhere to crystals leading to increased number of crystal aggregations or may bind to the tubular epithelium resulting in expression of stone matrix proteins (Schwaderer and Wolfe, 2017; Rahman et al., 2003; Torzewska et al., 2014).

Conclusion

In the present study, the main crystal type found was calcium oxalate and the predominant organism type was coliforms. There was a significant association between presence of urinary crystals and urinary bacterial flora in UTI suspected patients.

Acknowledgement

We thankful to Dr. M.M. Abeykoon, Mr. M.G.A.N.Meedeniya, Mr. G.A.N.S. Sirimanna, Mr. J.D.W.P.K. Ranasingha and all the volunteers participated in the study.

Conflict of interest

There are no conflicts of interest.

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