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Research Article

Prevalence of crystalluria and its association with *Escherichia coli* urinary tract infections

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ABSTRACT

Background: Some studies highlighted that there was increase in the incidence of urinary tract problems related to frequent Urinary Tract Infections (UTIs) and crystalluria. This induces us to understand the prevalence of crystals in urine samples and its association with bacterial urinary tract infections. For determining the different types of urinary crystals, the role of microscopic examination for identification are routine and identical.
Methods: The main objective of this study is to find out the predominant types of crystals in urine, age and sex incidence value, urine pH correlated with crystals and bacteriological examination of urine samples associated with UTIs.
Results: Among 88 urine samples included, 49 (55.6%) were males and 39 (44.3%) were females of different age groups. By urine sediment analysis, among the normal crystals, calcium oxalate and amorphous urates were found predominant followed by cholesterol found among abnormal crystals. The results highlighted the presence of different types of crystals in the urine samples and strongly supported the pH ranges. The variations in the pH range from 3 to 7. The correlation of the results of crystal formation with bacterial culture showed predominance of *Escherichia coli* (19.3%) and further it gets proved with the theory and reference interpretation.
Conclusion: By this study, the authors have a mystery whether the crystal formation leads to the bacterial infection or the infection leads to crystal formation. In the case of positive urine crystal analysis, the clinicians may consider the microbiological investigations to find out the real picture.

Keywords: Crystalluria, Prevalence, *Escherichia coli*, Calcium oxalate, Amorphous urates

INTRODUCTION

In the routine urine examination, crystalluria seems to be the most predominant sediments in the urine. In most of the cases, the crystals were formed due to transient super saturation of the urine, frequent usage and misuse of antimicrobials, ingestion of foods that precipitate crystal, changes of urine temperature and pH which occurs upon standing after micturition.[1] In every urine stone diseases, the formation of urine crystals is the necessary initial step of urolithogenesis**.** The formation of stones is always preceded by crystalluria, although crystalluria may occur without resulting in stone formation, but some studies proposed crystalluria is an index of stone disease;[2] therefore, one could hypothesize that the presence of persistent crystalluria reflects a propensity for stone formation and may constitute a marker of stone disease of potential clinical relevance in association with acidity and alkalinity nature of the urine.

The most common types of urine crystals may include calcium oxalate, cystine, uric acid, struvite etc. However, eventhough urine is supersaturated with calcium and oxalate ions in normal subjects, stones do not form because of the presence of inhibitors and other unknown mechanisms.[3] Calcium oxalate crystals form in acidic to neutral urine. They are also colourless and have an envelope or dumb bell shape. Therefore, the clinical relevance of crystalluria in kidney stone formers remains largely debated.

Urinary calculi formed as a result of bacterial infections comprise only 10-15% of all stones appearing within the urinary tract, but they pose a severe health problem. Infection has a twin role; urea splitting organism promotes precipitation of phosphates and oxalates in alkaline media.[4] Secondly, the sharp edges of oxalate calculi damage the urinary tract epithelium and support the growth of organisms by forming the nidus to the infectious agents. Persistent urinary tract infection with urea splitting or non- splitting bacteria may be the initial factors in the synthesis of infection renal stones.[5]

In metabolic stones bacterial superimposition may be responsible for the recurrent urinary tract infections. Half of the cases with recurrent illness lead to the loss of kidneys or even mortality occur if not properly treated. The development of infectious urolithiasis may be caused by several factors and despite long-term clinical and experimental investigation; some of the specific mechanisms responsible for urinary calculi formation remain a major mystery.[6] Thus this present study highlighted the importance of correlation among urinary tract bacterial infections, pH and crystal formation in the urine samples received in the clinical laboratory of tertiary care rural teaching hospital at Tiruchirappalli.

METHODS

Generally, Urine is a sterile body fluid within the bladder, but the crystals may form due to various reasons and also can pick up commensals and pathogens when egress through the urethra. The urine samples that are received in the Clinical laboratory for microbiological and pathological investigations were included for the study. The samples included were anonymously analyzed for the presence of crystal formation and compared with the pH ranges and bacterial isolates. Thus, patient history and demographic details were collected for this investigation to confirm the microbiological screening. The institutional ethical clearance was obtained to perform this analysis. The inclusion criteria are the urine samples received in the laboratory within four hours in a sealed urine container. The exclusion criteria are urine voided more than 4 hours, leaked container, less volume of urine, spilled urine and catheterized urine samples. The samples were stored at low temperature if the samples cannot be processed within 2-3 hours.

Battery of 88 urine samples was included in this study by which we can determine the presence of various crystals, its pH variations and bacterial isolates. To avoid the cross contamination and crystal overloading, usage of separate dropper is advisable and thus it should be followed in this investigation.[7] If accidentally any cross utilization of dropper may occur then samples are likely to be rejected (this is not happened in this study). The indication of pH levels showed the amount of acid in urine; where abnormal pH levels may indicate a kidney or urinary tract disorder.[5] After collecting demographic and history of the patient, the methodology was designed as follows,

pH analysis

The urine samples were numbered sequentially then the urine reagent strips were dipped in the urine. This set up was allowed for 20 seconds and observed the color change. Then the pH value of the urine sample noted separately for further analysis. The pH may get variations between the samples included in this study.

Microscopic urine examination

It involves the collection of 10ml of specimen of urine in a sterile wide mouthed container after taking necessary precautions. The sample was analyzed in the laboratory after centrifugation at 3000 rpm for 5 minutes, after draining the supernatant, a drop of the sediment was placed on a clean glass slide covered it with the cover slip. Then it was studied under low power and high power objectives of the microscopic lens (Adeltaplane AP40) for clear demonstration of crystals. The interpretation of the results for crystals including types of crystals and its appearances

Urine for culture

The main objective of this study is to concentrate mainly on identifying Escherichia coli bacterial urinary tract infections; thus only Eosin methylene blue agar medium is used to isolate the E. coli from the urine samples. The standard bacteriological procedure and inoculation strategies were followed using standard loop technique. After inoculation, plates are incubated at 37°C overnight. Further, organism is identified by biochemical tests.

RESULTS

Socio demographic details

Among 88 urine samples included in the study, the maximum cases was found in the age group of 41 to 50 (20.5%) followed by 21 to 30 (18.2%). The age-wise distribution of cases included for crystalluria analysis was depicted in Figure 1.

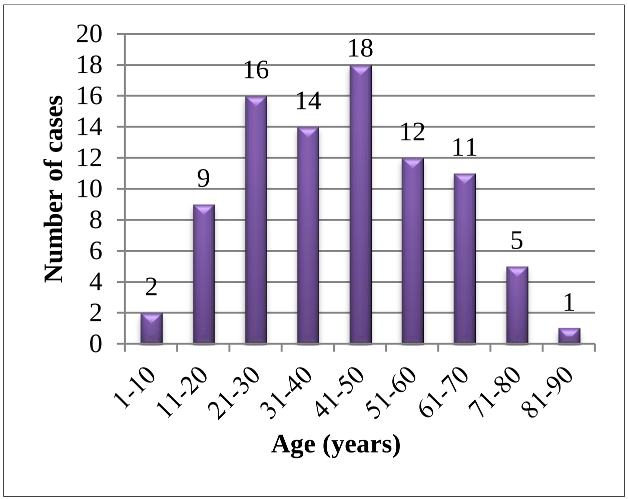

Figure 1: Age-wise distribution of cases included for crystalluria analysis (n=88).

A total of 49 male individuals (55.7%) and 39 females (44.3%) were included in the study where 34 males (69.3%) and 29 females (74.3%) supported crystal formation. In the subjects included, 12 (24.4%) and 5 (12.8%) urine samples supported positive to bacterial culture among male and female respectively (Table 1).

Table 1: Number of urine samples supported the crystal formation and bacterial culture.

Gender	Total number of cases (n=88)	Number of samples supported crystal formation	Number of samples positive for bacterial culture
Male	49 (55.7)	34 (69.3)	12 (24.4)
Female	39 (44.3)	29 (74.3)	5 (12.8)

Figure in parenthesis indicated percentage

pH analysis

The variations of pH among the urine samples supported the theory thereby the crystal formation is easy to correlate. The pH range was observed from 3 to 8. Further the pH range was very much applicable to determine the crystal types. Impregnations of the pH in the samples were interpreted in Table 2.

Table 2: Determination of pH among urine samples.

pH range	No. of samples (N=88)	Percentage
3.0-3.9	2	2.3
4.0-4.9	8	9.1
5.0-5.9	36	40.9
6.0-6.9	36	40.9
7.0-7.9	6	6.8

Urine crystal determination

Most of the studies highlighted many urine sediments harbor normal crystals. In some pathological situation, the normal crystals may become abnormal in its pathogenesis and cause various acute to chronic states. The types of crystals present depend on urine pH, further it was proved significantly. The various types of crystals including normal and abnormal identified in this study are depicted in Table 3.

Table 3: Types of crystals identified.

Normal crystals	Abnormal crystals
Uric acid	
Calcium oxalate	Cholesterol
Calcium carbonate	Leucine
Amorphous urates	

Among the crystals recorded, calcium oxalate and amorphous urate stands equal followed by uric acid (Figure 2).

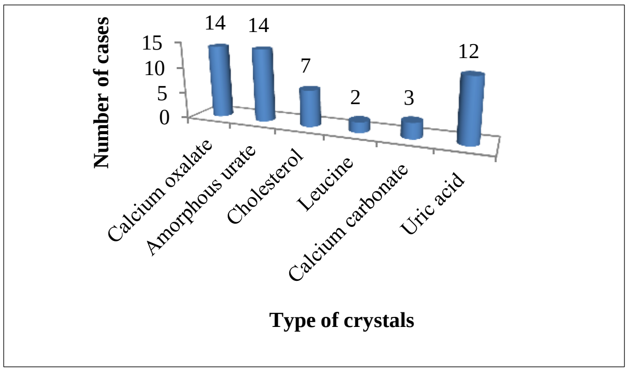

Figure 2: Type of crystals verses number of cases supported.

The crystal combinations with two crystals were identified in five combinations where calcium oxalate and amorphous urate dominated among four samples followed by cholesterol and amorphous urate (Figure 3). 3 crystal combinations were also identified among two samples and the details were impregnated in Figure 3.

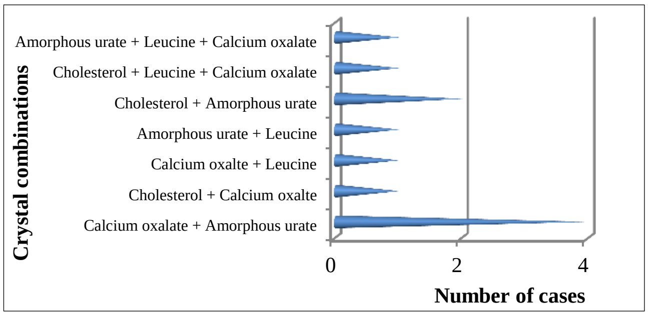

Figure 3: Number of cases supported crystal combinations.

The results of the correlation of pH with the crystal formation among male and female populations were well studied and further it gets proved with the existed experiments and its interpretation. The details of results were interpreted in the Figure 4. The observation of microscopic crystals is depicted in Figure 5. The crystals like calcium oxalate, amorphous urate, uric acid, cholesterol, calcium carbonate and leucine were observed.

The crystal formation was well analyzed with demographic status of patients included and also correlated with pH. Among the subjects included (n=88), 63 urine samples supported crystal formation and all the crystal positive samples were identified to have pus cells (100%). The epithelial cells above the normal values were found among 34 samples and 17 samples supported bacterial culture especially *Escherichia coli*. The integrated laboratory findings supported crystalluria correlated with infectious state was depicted in Figure 6.

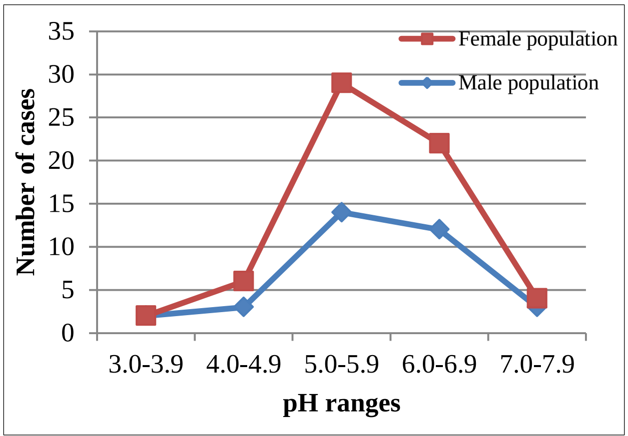

Figure 4: Correlation of pH with positive crystalluria among population.

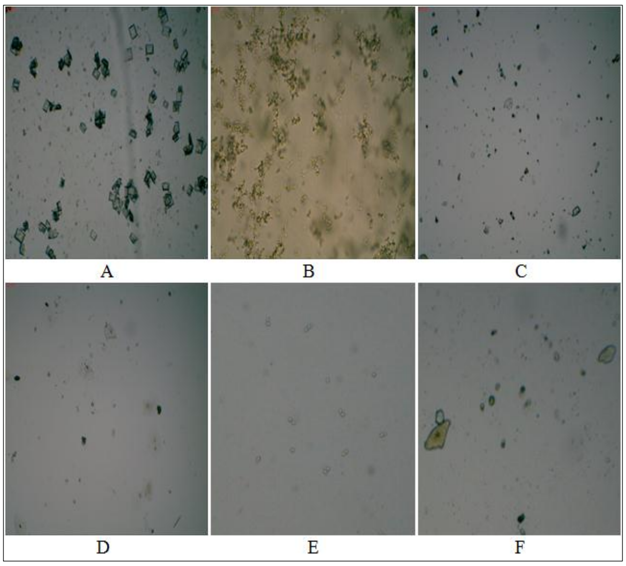

Figure 5: Micrographs of various urine crystals.

Crystals of A - Calcium oxalate; B - Amorphous urates; C - Uric acid; D - Cholesterol; E - Calcium carbonate; F - Leucine

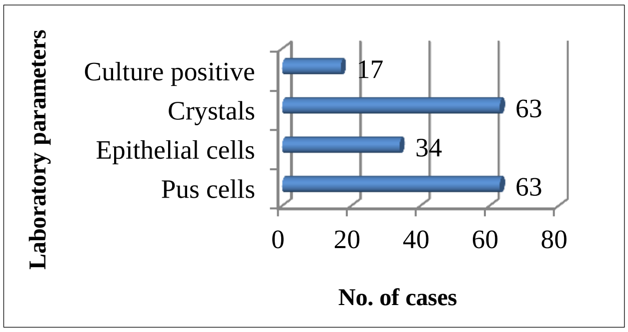

Figure 6: Integrated laboratory findings supported crystalluria correlated with infectious state (n=88).

Table 4: Relationship of age with crystalluria and bacterial culture.

Age (years)	Male population (n=49)			Female population (n=39)		
	Total cases	No. of cases supported crystalluria	No. of cases positive for bacterial culture	Total cases	No. of cases supported crystalluria	No. of cases positive for bacterial culture
1-10	1 (2)	-	-	1 (2.6)	-	-
11-20	5 (10.2)	3 (60)	-	4 (10.2)	4 (100)	1 (25)
21-30	9 (18.4)	7 (77.8)	2 (22.2)	7 (17.9)	5 (71.4)	2 (28.6)
31-40	7 (14.3)	4 (57.1)	1 (14.3)	7 (17.9)	5 (71.4)	1 (14.3)
41-50	7 (14.3)	7 (100)	5 (71.4)	11 (28.2)	8 (72.7)	1 (9.1)
51-60	7 (14.3)	5 (71.4)	2 (28.6)	5 (12.8)	4 (80)	-
61-70	8 (16.3)	5 (62.5)	-	3 (7.7)	3 (100)	-
71-80	5 (10.2)	3 (60)	2 (40)	-	-	-
81-90	-	-	-	1 (2.6)	-	-

Figure in parenthesis indicated percentage

Among the age groups, the maximum number of cases positive for bacterial culture was found in 41 to 50 years. The geriatric age group (71 to 80) was found positive for bacterial culture of two cases in males; whereas no cases observed among females. The total number of cases supported bacterial culture among male population was twelve (12) and five cases observed in females. The relationship of age with crystalluria and bacterial culture was described in Table 4.

DISCUSSION

The pH analysis is highly correlated with the crystal formation and growth, further it was measured in metastable solutions of oxalate and chlorides at different pH. Inhibition of calcium oxalate crystal growth in urine from patients with calcium oxalate stone disease increased with increasing pH.[7] The highest prevalence of crystalluria was found in males and the maximum cases were found in the age group of 21 to 30. This data is highly correlated with the study comprised HIV patients aged between 40 and 50 years.[8] Some study showed the higher frequency of crystalluria observed in normal adults aged 40 years and above in a cross-sectional study.[9] Incidence is equal between the sexes but men may be more severely affected.[10] Although the peak incidence of first cystine calculus is 22 years of age, almost 1/4 of cytinurics will develop stones in the first decade of life with 30-40% of patients having their first stone in the teenage years.[11]

The pH range of the current study stated the crystal formation correlated with acidity and alkalinity of the urine, thereby maximum cases was observed in the pH range of 5 to 7 in both genders. The other study by the same author found that the maximum cases were found in slightly acidic to alkaline condition.[7] The reasonable good correlation was obtained in the study where inhibition indices with correlation factor were directly proportional to crystal growth rate with different pH.[12]

Finding the crystals on microscopic examination of the urine is usual. Crystal formation depends on a number of factors and the presence of crystals may or may not be pathologic. Frequently, the presence of crystals in the urine is of inadequate clinical significance.[13] Supersaturation of the solute components of the crystals must occur for crystallization to initiate. A number of factors affect supersaturation, including solute concentration, ionic strength, urine pH and the presence of promoters or inhibitors. These factors vary from day and night where during the day depending on fluid intake, dietary intake and body metabolism, the crystal formation raised. Urine in normal individuals is often supersaturated with calcium oxalate, calcium carbonate and amorphous urate. Calcium oxalate, uric acid and amorphous urate crystals are typically found in acidic urine.[14,15] Leucine crystals are always abnormal and are found in people who have leucinuria and who often have kidney stones in developed countries. Leucine crystals are also abnormal and suggest liver disease. Prolonged standing of the urine may result in increased crystallization.

By comparing with the above review, the current study highlighted the maximum presence of calcium oxalate, amorphous urates followed by uric acid. Very low cases of leucinuria were found where the chronic pathogenesis was not observed. Here the normal crystal numbers are higher than the abnormal crystals where some study of the same author depicted the controversy.[7] Thus the patients may be affected with mild to severe renal disorders including renal calculi. The formation of the renal calculi and crystals leads to the kidney stone formation. Renal stones are closely associated with chronic kidney disease, thereby clinically can prevent the recurrence.[16] Managing diet, medication use and nutrient intake can help prevent the formation of kidney stones. To avoid the crystalluria, the urine should be alkalized or acidified according to the types of crystals by uptaking a diet high in fruits and vegetables, taking supplemental or prescription citrate, or drinking alkaline mineral waters; cranberry juice or betaine can lower urine pH.

The morphologic appearance of urinary crystals can often be the first indication of a specific pathologic process thereby pleomorphic, rhombic plates, or rosettes characterize common uric acid crystals, which may be observed in acidic urine favoring the conversion of soluble urate salt into insoluble uric acid.[7] The pathological peak was observed with the pathophysiological changes and clinical abnormalities in the renal assessment and functioning, further complications loaded with the combination of crystals in the single sample or cases. Thus in this study, mixture of two and three crystals was found among nine and two samples respectively. The maximum combinations found along with calcium oxalate and amorphous urates. In the triple crystal formation among two cases, calcium oxalate and leucine is common.[17] The abundance of cysteine crystals in urine constitutes a major risk of lithogenesis which should be reduced by appropriate therapeutic measures. Leucine and calcium oxalate crystals were found in children from 0-14 years old. The presence of leucine crystals are found in case of leucinosis, or Hartnup disease.[18] Among the bacterial culture included in this study, it was showed that 19.3% subjects showed positive to E. coli isolation with crystalluria whereas other study also highlighted more or less equal to our present investigation i.e., 18.7%.[4] These findings are consistent with what have been reported in the theory and literature.[17] The major principle behind this mechanism is mainly by urea-splitting bacteria in the development of the majority of crystals,[18-20] but in this study the authors did not find any struvite crystals but uric acid (29.4%), calcium carbonate (17.6%), cholesterol (11.8%), calcium oxalate (11.8%), amorphous urate (5.9%) and others showed mixed crystals. Among the crystals found in this study, leucine alone not correlated with the bacterial isolate. No studies so far highlighted the correlation of leucine with UTIs. Crystals of different chemical groups and combinations were found in urines of the patients regardless to their gender and age; sometimes in the presence of urinary tract infectious bacterial members.

This situation calls up to a meticulous research for crystals in laboratories and to their consideration in the interpretation of the laboratory results by the clinicians to correlate with infectious state. Indeed, this study allowed the identification of crystals correlated with pH and bacterial isolation to understand the pathophysiology of the renal function, in particular calcium, amorphous urates, uric acid and cholesterol crystals signs of variance in protein metabolism. Thus this study highlighted the importance of the analysis of the urine crystals compared with the pH and bacterial isolation. A controversial study must be undertaken in future regarding the inter relationship between the bacterial isolate with specific crystal formation. It is already proved nicely that the pH range of acidity, neutral and mild alkalinity playing a vital role in the specific crystal formation. The same authors already proved the same. Some samples showed the clear picture that the urine pH influences the crystal formation in kidney leads to renal stone to renal failure.

CONCLUSION

Through this investigation, the authors found the importance of cross testing the urine samples starting with pH to bacterial isolation in order to understand the clear pathology of renal functioning. It remains a mystery whether the crystal formation leads to the bacterial infection or the infection leads to crystal formation. It may provide the logical clearance to treat the patients appropriately. In the case of positive urine crystal analysis, the clinicians may consider the microbiological investigations to find out the real picture.

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Ethical approval: The study was approved by the institutional ethics committee

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