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Original Research Article

COMPARATIVE ANALYSIS OF BACTERIAL PATHOGENS AND CRYSTALLURIA IN CASES ENROLLED IN A TERTIARY CARE TEACHING HOSPITAL, TIRUCHIRAPALLI, INDIA

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Abstract

Urinary tract infections correlated with crystalluria are highlighted recently to understand the real picture of infections by crystalluria or crystalluria by infections. This makes us to select this issue to perform the prevalence of crystals in urine samples and its association with bacterial urinary tract infections. The role of microscopic examination for identification is routine and identical for determining the different types of urinary crystals. The major aim and objectives of this investigation 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. Among 136 urine samples included, 74 (54.4%) were males and 62 (45.6%) 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 2 to 8. The correlation of the results of crystal formation with bacterial culture showed predominance of monomicrobial bacteruria among 27 cases (19.8%) and polymicrobial entities among 4 cases (2.9%). 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, pH, bacteruria, calcium oxalate, amorphous urates

Introduction

Crystalluria in individuals with anatomically and functionally normal urinary tracts is usually harmless because the crystals are eliminated before they grow large enough to interfere with normal urinary function. Even so, they represent a risk factor for kidney stones. Crystals that form in the urine following elimination or removal of urine from the patient often are of little clinical importance (i.e., crystals formed in the urine after leaving the body). Identification of crystals that have formed in vitro (in a lab environment) will not justify therapy[1]. 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[2,3].

Situations that will require further follow-up are instances where some types of crystals are detected in patients without symptoms (asymptomatic); large aggregates of crystals (e.g., calcium oxalate or magnesium ammonium phosphate) in apparently normal individuals are detected; or, when detection of any form of crystals in fresh urine collected from patients with confirmed kidney stones may have diagnostic, prognostic, or therapeutic importance.

The major causes of crystals are

- Concentration of crystallogenic substances in urine (which in turn is influenced by their rate of excretion and urine concentration of water).
- Urine pH is off balance – acidic or alkaline levels need to be balanced[4]
- Solubility of crystallogenic substances in urine.
- Excretion of diagnostic agents (e.g., radiopaque contrast agents) and medications (e.g., sulfonamides).
- Dietary influence—hospital diet may differ from home diet; timing of sample collection (fasting versus postprandial [after a meal]) may influence evidence of crystalluria.

The jagged edges of oxalate calculi injure the urinary tract epithelium and carry the growth of microorganisms 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 infectious renal stones[5].

Urinary pH is a major determinant for kidney stone formation. Changes in the balance of urinary pH can affect stone precipitation. Some studies suggested that urine pH approximately 6.0 reduces the risk of kidney stone formation. However, the risk of uric acid and calcium stone formation increases progressively at urinary pH<5.5 and >6.5, respectively. Generally, some crystals are found exclusively in acidic urine, while the others are found exclusively in alkaline urine. Uric acid, calcium oxalates and cystine stones thrive in acidic urine, whereas calcium phosphate, calcium carbonates and struvite stones thrive in alkaline urine[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 Tiruchirapalli.

Material and Methods

The formation of crystals may be 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 in a tertiary care teaching hospital at Tiruchirapalli were included for the investigation. The samples were analyzed for the presence of crystal formation and compared with the pH ranges and bacterial isolates. The patient history and demographic details were collected 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.

A battery of 136 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, the usage of separate dropper is advisable[7]. The indication of pH levels showed the amount of acid in urine; where abnormal pH levels may indicate a renal or urinary tract disorders[5].

For analyzing the pH, urine samples were numbered sequentially then the urine reagent strips were dipped in the urine 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.

Further, the samples were analyzed in the laboratory by centrifuging at 3000 rpm for 5 minutes, 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 (Adeltaplane AP40) for clear demonstration of crystals. The interpretation of the results included the types of crystals and its appearances.

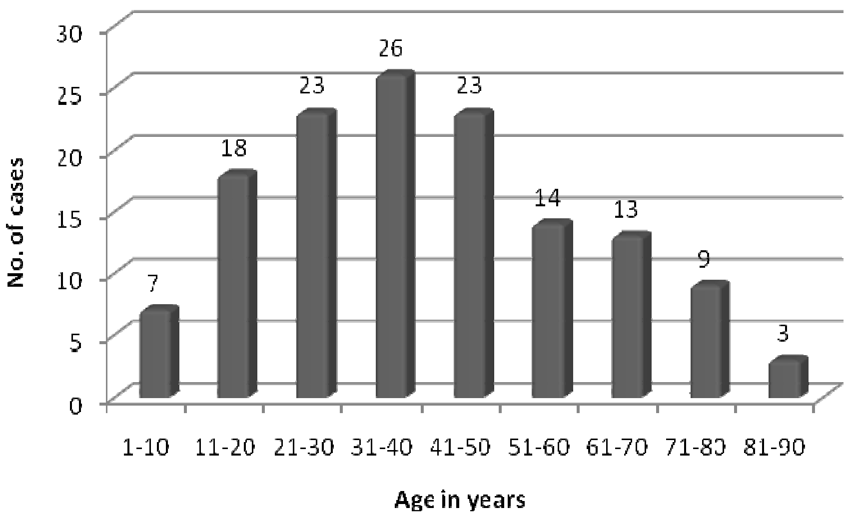
The bacteriological investigation concentrated mainly on identifying bacterial etiology for urinary tract infections; thus nutrient agar, *Pseudomonas* isolation agar, MacConkey agar and Eosin methylene blue agar medium were used. The bacteriological procedure and inoculation strategies were followed using standard loop technique and incubated. Further, the bacterial colonies were identified by colony morphology, Gram's staining and biochemical tests.

Results

Socio demographic details

Among 136 urine samples included in the study, the maximum cases was found in the age group of 31 to 40 (19.1%) followed by 21 to 30 and 41 to 50 (16.9% each).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=136)



A total of 74 male individuals (54.4%) and 62 females (45.6%) were included in the study where 46 males (62.2%) and 40 females (64.5%) supported crystal formation. In the subjects included, 21 (28.4%) and 10 (16.1%) 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=136)	Number of samples supported crystal formation	Number of samples positive for bacterial culture
Male	74 (55.7)	46 (62.2)	21 (28.4)
Female	62 (44.3)	40 (64.5)	10 (16.1)

Figure in parenthesis indicated percentage

pH analysis

The pH range was observed from 2 to 8. Further the pH range was determined in order to understand the crystal types and correlation with pH (acidic/alkaline). The descriptions of the pH in the samples were interpreted in table 2.

Table 2: Determination of pH among urine samples

S.No.	pH range	No. of samples (N=136)	Percentage
1	2.0-2.9	8	5.9
2	3.0-3.9	7	5.1
3	4.0-4.9	18	13.2
4	5.0-5.9	49	36.1
5	6.0-6.9	43	31.6
6	7.0-7.9	11	8.1

Urine crystal determination

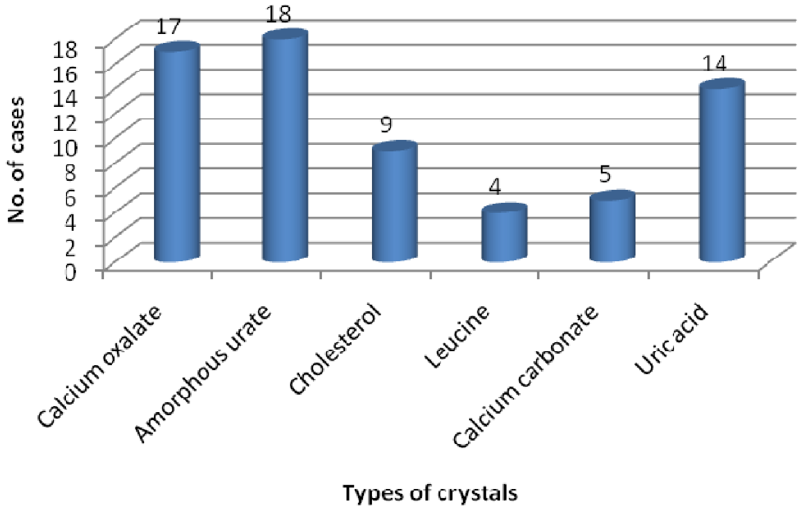
In general, many urine sediments harbor normal crystals. In some pathological conditions, the alteration and sudden change of normal crystals to abnormal showed the pathogenesis, stages and severity of the renal problems. This may leads to severe internal trauma to bleeding, further the chronicity depends upon the crystal types or bacterial infection correlated with crystal formation and/or vice versa. Further studies required to find out the types of crystals present depend on urine pH. 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 Calcium carbonate Amorphous urates	Cholesterol Leucine

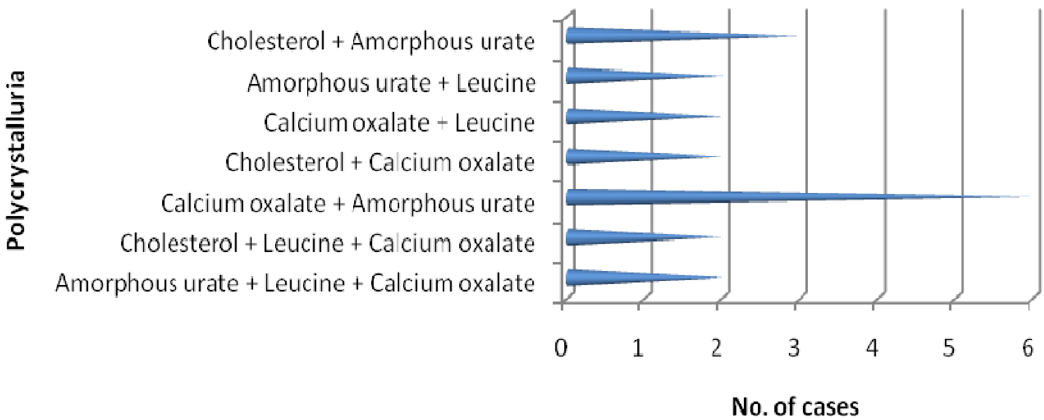
Among the crystals recorded, amorphous urate dominated followed by calcium oxalate and uric acid (Figure 2).

Figure 2: Type of crystals verses number of cases supported



The polycrystal formation either in two or three combinations were identified in five and two respectively. The bi-crystal formation with calcium oxalate and amorphous urate dominated among six samples followed by cholesterol and amorphous urate (Figure 3). The tri-crystal combinations were also identified among four samples where calcium oxalate and leucine found common and the details were impregnated in figure 3.

Figure 3: Number of cases supported crystal combinations



The correlation of pH with the crystal formation among populations was studied and it should be analyzed with theory (Figure 4). The microscopic crystal observation is depicted in figure 5. The crystals like calcium oxalate, amorphous urate and uric acid were observed.

Figure 4: Correlation of pH with positive crystalluria among population

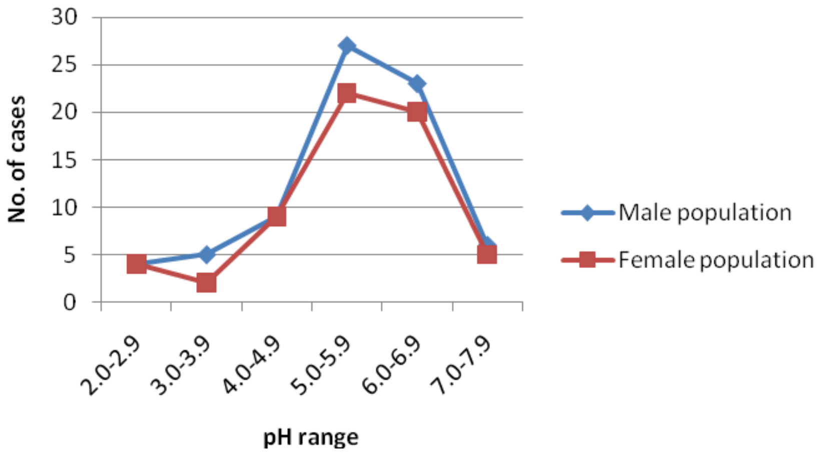
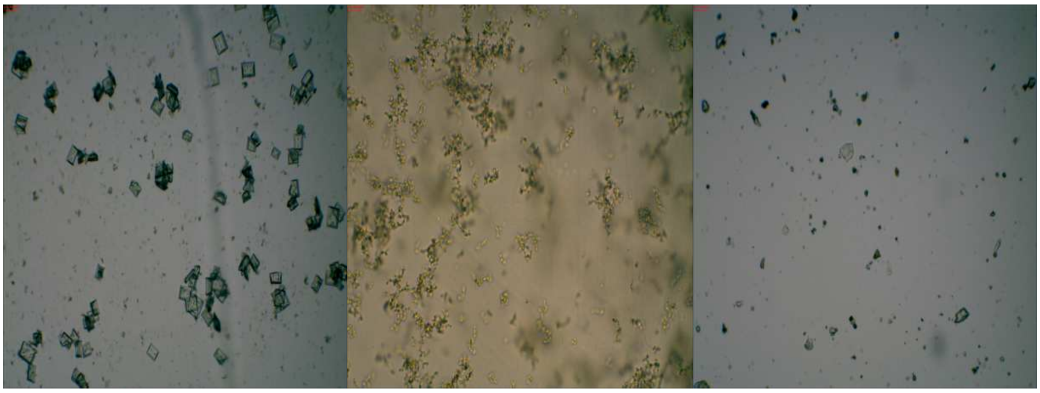


Figure 5: Micrographs of various urine crystals



A B C

[Crystals of A – Calcium oxalate; B – Amorphous urates; C – Uric acid]

The crystal formation was analyzed and demographic status of subjects was included and also correlated with pH. Among the subjects included (n=136), 86 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 46 samples and 31 samples supported bacterial culture. The prevalence of monomicrobial and polymicrobial bacteruria was 87% and 13% respectively. Of these, monomicrobial culture were found among *Escherichia coli* (51.6%), *Pseudomonas aeruginosa* (19.3%), *Klebsiella pneumoniae* (12.9%) *and Proteus mirabilis* (3.2%) (Figure 6). The polymicrobial culture was found among 4 samples in which the combination of *Escherichia coli* and *Klebsiella pneumoniae* were found among two samples (Figure 7). The integrated laboratory findings supported crystalluria correlated with infectious state was depicted in figure 8.

Figure 6: Prevalance of monomicrobial bacteruria

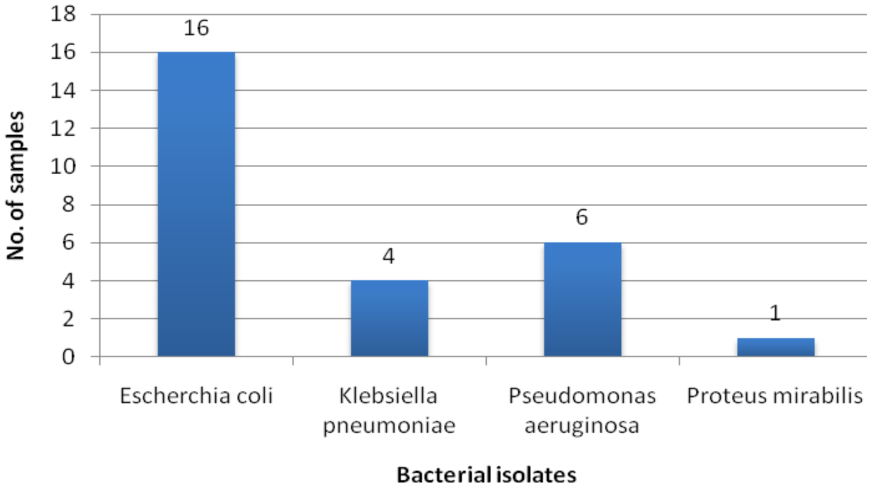


Figure 7: Prevalence of polymicrobial bacteruria

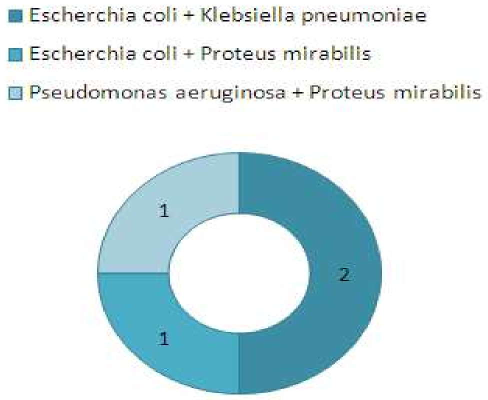
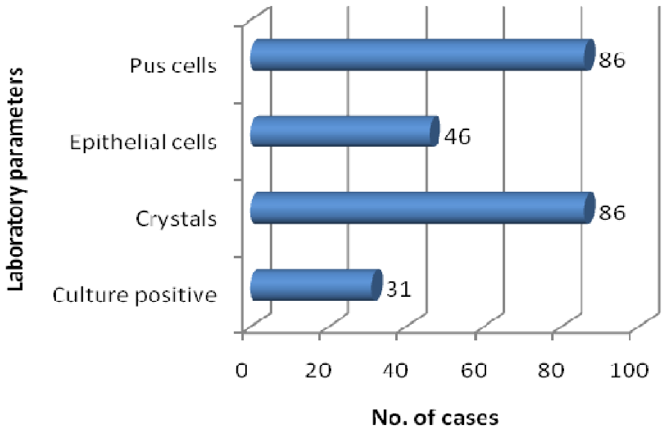


Figure 8: Integrated laboratory findings supported crystalluria correlated with infectious state (n = 136)



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 eighteen (18) and thirteen (13) cases observed in females. The relationship of age with crystalluria and bacterial culture was described in table 4.

Table 4: Relationship of age with crystalluria and bacterial culture

Age (in years)	Male population (n=74)			Female population (n=62)		
	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	3 (4.1)	-	-	4 (6.5)	-	-
11-20	10 (13.5)	5 (6.6)	-	8 (12.9)	7 (11.3)	3 (4.8)
21-30	12 (16.2)	10 (13.5)	2 (2.7)	11 (17.7)	7 (11.3)	4 (6.5)
31-40	12 (16.2)	6 (8.1)	2 (2.7)	14 (22.5)	7 (11.3)	3 (4.8)
41-50	10 (13.5)	9 (12.2)	8 (10.8)	13 (21)	10 (16.1)	3 (4.8)
51-60	9 (12.2)	6 (8.1)	4 (5.4)	5 (8.1)	5 (8.1)	-
61-70	10 (13.5)	6 (8.1)	-	3 (7.7)	4 (6.5)	-
71-80	8 (10.8)	4 (5.4)	2 (2.7)	-	-	-
81-90	-	-	-	3 (4.8)	-	-

Figure in parenthesis indicated percentage

Discussion

In this investigation, pH analysis was correlated with the crystal formation and bacterial growth, further it has been analyzed by changing the pH of urine samples from acidic to alkaline and vice versa. The mechanism behind the change of pH and crystal modification may found as serious pathological marker to understand the renal dysfunction and its pathology[7].

The highest prevalence of crystalluria was found in males and the maximum cases were found in the age group of 21 to 30 and 31 to 40, the same was studied in various investigations and highly correlated with the study comprised HIV patients aged between 40 and 50 years[8]. Other studies highlighted the maximum frequency of urine crystals observed in adults aged 40 years and above[9,10]. In India, the crystalluria cases found neglected due to lack of investigation in a regular consultation, the case may be critically investigated only during sub-chronic or chronic renal disorders[11].

The range of pH of this current study depicted the formation of crystals related to acidity and alkalinity of the urine, thereby maximum cases was observed in the pH range of 2 to 8 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 and bacterial growth[12].

The factor determining the crystal formation depends on the presence of crystals may or may not be pathologic and found inadequate clinical significance[13]. The other factors influencing the crystal formation are supersaturation, including solute concentration, ionic strength, urine pH and the presence of promoters or inhibitors; it may vary from day and night where during the day depending on fluid intake, dietary intake and body metabolism, the crystal formation raised. The common acidic urine crystals are calcium oxalate, uric acid and amorphous urate crystals[14,15]. Leucine crystals are always abnormal and are found in people who have leucinuria and who often have kidney stones in developed countries due to poor metabolism and very low water intake, further leucine crystals are also abnormal leads to liver diseases.

From the above review, the current investigation impregnated the maximum presence of amorphous urates, calcium oxalate followed by uric acid. Moderate cases of leucinuria were found where the chronic pathogenesis was not observed. In this study, the prevalance of normal crystal are higher than the abnormal crystals, where in some studies the depiction is controversy[7,16]. Managing the crystalluria by proper diet, medication use and nutrient intake prevent the formation of renal 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 appearance of urinary crystals in microscope 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]. Thus in this investigation, polycrystalluria of two and three crystals was found among fifteen and four samples respectively. The maximum combinations found along with calcium oxalate and amorphous urates[17]. 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 19.8% subjects showed positive to bacterial isolation with crystalluria whereas other study also highlighted more or less equal to our present investigation i.e., 18.7%[4,17]. The major principle behind this mechanism is mainly by urea-splitting bacteria in the development of the majority of crystals[18,19,20], but in this study the authors did not find any struvite crystals but amorphous urate (20.9%), calcium oxalate (19.8%), uric acid (16.2%), cholesterol (10.5%), calcium carbonate (5.8%), leucine (4.6%) 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. This study provided the satisfactory data indicated the identification of crystals correlated with pH and bacterial isolation to understand the pathophysiology of the renal function, in particular to specific crystals that affect 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 crystal formation leads to infection or vice versa. 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 leads to renal dysfunction to renal failure.

Conclusion

The authors found the importance of relationship between pH and 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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