Investigation of Crystalluria and Its Association with Urinary Tract Infections among Pregnant Women

Amaila Qaisar[*], Bibi Fatima[**], Tayyaba Khalid[**], Aliya Bibi[**], Dr Mohammad Raqeeb Khan [***], Tooba Khalid[**]

[*] Faculty of Rehabilitation and Allied Health Sciences, Riphah International University, Islamabad, Pakistan
[**] Faculty of Rehabilitation and Allied Health Sciences, Riphah International University, Islamabad, Pakistan
[***]Tawam Hospital affiliated with John Hopkins Medicine International, AlAin, Abu Dhabi

Abstract- Crystalluria is characterized by the presence of crystals in urine sediment. Urinary crystals are now recognized as an important biomarker in urinalysis for identifying various underlying health conditions. Urinary tract infection (UTI) is a collective term for infections of any part of the urinary tract, i.e. the kidneys, ureters, bladder and urethra. UTI are the most common bacterial infection affecting 150 million people each year worldwide. Urinary tract infection (UTI) is a common complication that arises during pregnancy. Around the 6th week of pregnancy, the ureters start to widen due to the physiological changes of pregnancy, reaching its peak at 22-26 weeks and lasting until delivery. *E. coli* is the most commonly grown pathogen seen in case of UTI. Approximately 85% to 90% of UTIs are caused by *Escherichia coli*. Other than *Escherichia coli, Klebsiella pneumonia*, *Proteus mirabilis*, *staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. were observed causing UTI. The study design used for this research was Experimental. This study was conducted at Railway General Hospital from January 2024 to June 2024. A total of one hundred and fourty four patient specimens were analyzed for the entire duration of research. Purposive sampling technique was applied to select the research participants. One hundred and fourty four early morning mid-stream urine specimens were taken for UTI analysis. The majority of the samples (69.44%) did not contain any detectable crystals. Among the samples with crystalluria, calcium oxalate was the most prevalent type, present in 27.08% of the cases. Amorphous urates and amorphous phosphate crystals were found in 1.39% and 2.08% of the samples, respectively. Amorphous urate crystals were least prevalent. *Escherichia coli* and *Staphylococcus aureus* are the main uropathogens causing UTI in pregnant woman that is 38% caused by *Escherichia coli* and 24% caused by *Staphylococcus aureus* respectively. Followed by *Klebsiella pneumoniae* and *Enterococcus faecalis* i.e. 16 % and 9% respectively. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the least common cause of UTI among pregnant women that is 4.2% and 2.8% respectively. The correlation coefficient of age is -0.015 showing negative correlation between age and crystal as shown in the table. The p value is 0.144 which shows that there is insignificant relationship between age and crystals. The correlation coefficient of specific gravity is -0.216** showing negative correlation whereas the p-value is 0.009 showing that is significant between crystals and specific gravity. The correlation coefficient of crystal and pH is -.091 which shows negative correlation between crystals and pH. The p-value is 0.47 showing insignificant association between crystals and pH. .Crystalluria occurs in UTI related pregnancies so should be properly diagnosed and treated.

Keywords: *Acinteobacter baumanni*, Amorphous Urates, Crystalluria, Calcium Oxalate, *Escherachia coli*, Pregnant Women, *Staphylococcus aureus*, Uropathogens.

I. Introduction

Crystalluria is characterized by the presence of crystals in urine sediment. It occurs due to super saturation of minerals in urine and may result in wide range of kidney problems and urinary tract infections (1). Urinary crystals are now recognized as an important biomarker in urinalysis for identifying various underlying health conditions (2). These crystals can vary in shape and composition, and their detection through microscopic analysis of urine can provide valuable information about the health of an individual. The first clinical manifestations of crystalluria is macroscopic hematuria, oliguria, intense burning during urination, and abdominal or lumbar pain. Acute renal insufficiency become manifest few days after the first clinical signs (3). According to research article published in 2015 in International Journal of Research in Medical Sciences, In the group of 88 urine samples examined there were 49 (55.6%) males and 39 (44.3%) females, across age categories, where 34 males (69.3%) and 29 females (74.3%) supported crystal formation. By urinalysis, among the normal crystals, calcium oxalate and

amorphous urates were found predominant followed by cholesterol crystals found among abnormal crystals (4).

When the urine volume is low or water content of urine is low and the mineral content of urine is high, urinary crystals are formed depending upon the type of mineral present (5). Growth of microscopic crystals is accomplished by movement of ions out of solution onto the growing crystal. The main urinary crystalline categories include: calcium oxalates, calcium phosphates, uric acids and urates, struvite, amino acids (cysteine) (6).

Cases of urolithiasis has significantly impacted women with occurrences now almost equal between genders whereas in the past it was more prevalent in men (10). Crystals or stones in urine, during pregnancy poses a health risk that may impact the overall health of both the mother and the fetus. It ranks as the most common cause of urological-related abdominal pain in pregnant women according to a research done in international journal of surgery (7). Throughout the pregnancy, mother experiences physiological and anatomical changes that add challenges to stone/ crystal management (8).

Urinary tract infection (UTI) is a collective term for infections of any part of the urinary tract, i.e. the kidneys, ureters, bladder and urethra (9).UTI are the most common bacterial infection affecting 150 million people each year worldwide (10). UTI occur when pathogen from the rectal area enter the urinary tract via urethra and multiply in the urinary tract damaging any of the urinary tract organ (11).

Pain in the suprapubic area, pain during urination, or hematuria are the typical symptoms of lower UTIs. Symptoms of an upper urinary tract infection (UUTI) can include fever (>100°F), chills, nausea, vomiting, and nausea, with or without cystitis symptoms. Fever is typically linked to more complex types of UTIs and is less frequent in lower UTIs (12).

According to the literature, the incidence of UTI in women is higher than in men, 40% to 50% of whom suffer at least one clinical episode during their lifetime (13). Anatomical factors and modifications contribute significantly to the pathogenesis of UTI in women. The close proximity of the urethra to the anus, along with its short length, creates an environment that allows bacteria to easily travel up the urinary tract (14).

Urinary tract infection (UTI) is a common complication that arises during pregnancy. Around the 6th week of pregnancy, the ureters start to widen due to the physiological changes of pregnancy, reaching its peak at 22-26 weeks and lasting until delivery. The levels of progesterone and estrogens rise during pregnancy, causing a decrease in ureteral and bladder tone. The increase in plasma volume during pregnancy results in reduced urine concentration and increased bladder volume. Glycosuria in pregnancy is another recognized factor that makes mothers more susceptible to UTIs (15). The growth and development of the fetus stimulate dramatic alterations in the maternal internal environment that might lead to UTI (16). If UTIs are not treated properly, they can lead to complications such as pyelonephritis, low birth weight infants, premature delivery, and in some cases, stillbirth. Therefore, it is crucial to promptly treat UTI in pregnant women (17).

According to the previous literature, gram negative bacteria are more prevalent in UTI unlike gram positive bacteria (18). *E. coli* is the most commonly grown pathogen seen in case of UTI (19). Approximately 85% to 90% of UTIs are caused by *Escherichia coli*(20). Other than *Escherichia coli, Klebsiella pneumonia*, *Proteus mirabilis*, *staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. were also seen causing UTI (21).

Persistent urinary tract infection may be the initial factors in the synthesis of crystals in urine. Maximum cases of UTI were seen in the pH range of 5 to 7 in both genders, indicating a correlation between the crystal formation and the acidity and alkalinity of the urine that occur during urinary tract infection (4). Urolithiasis can cause urine stasis, which allows bacteria to stick to the urothelium and grow, resulting in a urinary tract infection (UTI) (22).

The presence of struvite crystals, even in small amounts, in an alkaline urine (pH > 7) is indicative of urinary tract infection (UTI) with urease-producing bacteria, usually *Proteus mirabilis* or certain strands of *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa*, or less frequently, *Corynebacterium urealyticum*, responsible for encrusted cystitis and/or pyelonephritis (23).

According to statistical analysis, out of 75 patients, 26 patients had crystalluria and the other 27 had a urinary tract infection. This suggests that crystalluria is a significant risk factor for the development of stone and other urinary tract disorders (24)

II. METHODOLOGY

The study design used for this research was experimental. This study was conducted at Railway General Hospital from January 2024 to June 2024. A total of one hundred and fourty four patient specimens were analyzed for the entire duration of research. Purposive sampling technique was applied to select the research participants. One hundred and fourty four early morning mid-stream urine specimens were taken for UTI analysis.

To guarantee repeatable findings, sample preparation is crucial. A urine sample should be prepared for microscopic analysis by centrifuging 10–15 ml of recently voided mid- stream urine for five minutes at 1500–3000 rpm. The sediment is then resuspended in the liquid that remains after the supernatant has been separated. The urine is then inspected under low- and high-power magnification after one drop is deposited to a glass slide and a cover slip is placed on it. Cellular elements such as WBCs, RBCs, crystals, casts, and bacteria can be seen under high power field if the slides are made correctly (44). After that, the specimens were inoculated onto Cysteine Lactose Electrolyte Deficient medium (CLED) using a sterilized wire loop. For 24 to 48 hours, the specimens that were inoculated on CLED agar were incubated at 37°C. Following the incubation period, the CLED agar plate was checked for bacterial growth, and the colonies that developed were counted if there was noticeable bacterial growth. Following inoculation onto MacConkey agar and Blood agar plates (BAP), the colonies obtained from CLED agar were cultured for 24–48 hours at 37°C. Gram staining technique was employed to investigate the bacteria using the bacterial colony formed from the specimens of the participants used for research. To identify distinct species of bacteria based on their biochemical activity, a variety of biochemical assays were used. Triple sugar iron test, bile esculin agar, oxidases test, urease test, catalase test, coagulase test, and API 20 E etc. are the biochemical tests that were used in this investigation (47).

All the data was collected, and entered into an excel sheet and then was exported to SPSS version 25 for visual representation of data through tables, bar charts, pie charts etc. In order to see possible associations and correlation between different variables, different tests such as Chi square for association, Spearman's for correlation and Phi Cramer V for strength of association were computed on SPSS version 25.

III. RESULTS

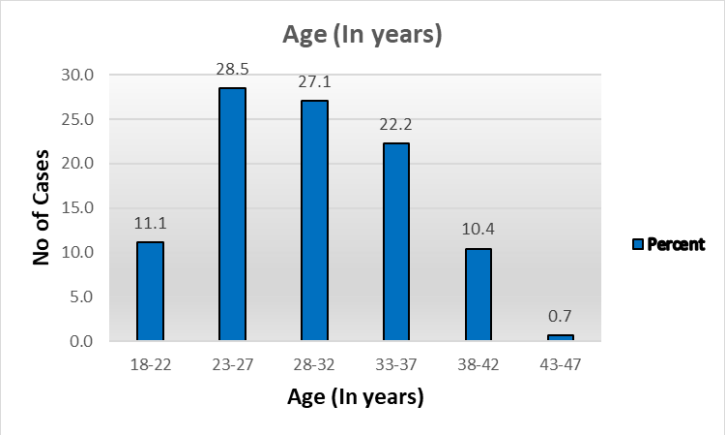

Figure 1: Age-wise distribution of Pregnant Women having UTI included for Crystalluria Analysis

Figure 1 illustrates the age distribution of the pregnant women having UTI who participated in the study. The highest representation was found in the age group of 23-27 years (28.47%), followed closely by the age group of 28-32 years (27.08%). Participants aged 33-37 years made up 22.22% of the study population, while those aged 18-22 years accounted for 11.11%. Participants aged 38-42 years made up 10.4%, followed by age group 43-47 years accounted for 0.7% having least cases represented. This distribution indicates that the study predominantly involved women in their mid-20s to early 30s, which is a common reproductive age range.

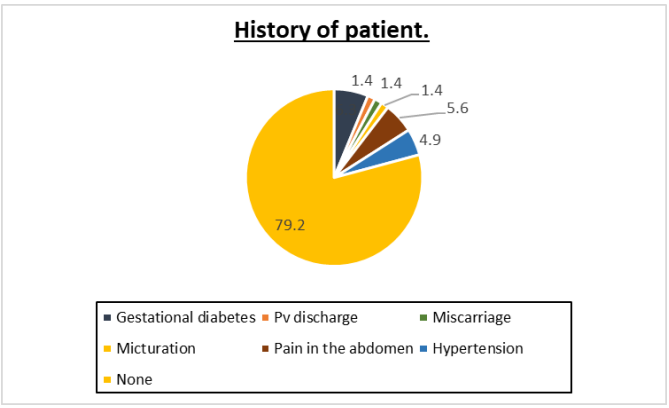

Figure 2: History of Pregnant Women

Figure 2 shows the history of 144 patients, 114(79.2%) patients were having no medical history of any illness while n=9(6.3%) patients were having gestational diabetes followed by N=8 (5.6%) patients having history of abdomen pain followed by n=7(4.9%) patients were having history of hypertension, n=2(1.4%) patients were having history of PV discharge,

n=2(1.4%) patients were having history of miscarriage and n=2(1.4%) patients were having history of micturition.

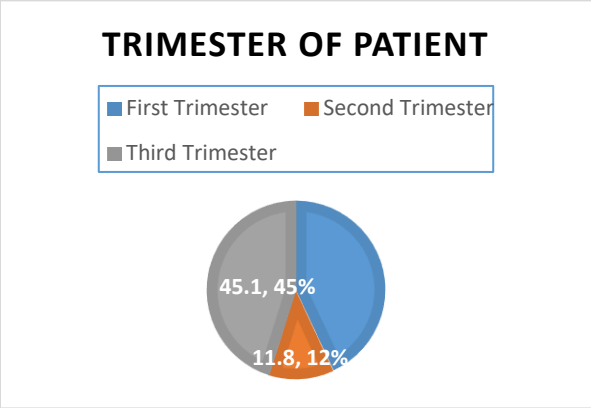

Figure 3: Trimester of Pregnant Women

Among the 144 pregnant women, n=65(45.1%) patients having UTI and crystalluria was highest in the third trimester of their pregnancy followed by n=62(43.1%) patients was in the first trimester and n=17(11.8%) patients which were in the second trimester of pregnancy.

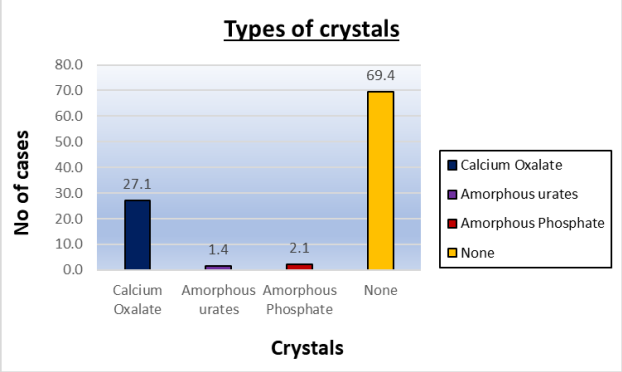

Figure 4: Predominant Types of Crystals observed among Pregnant Women having UTI

In Figure 4, the distribution of urinary crystals among pregnant women in the study is depicted. The majority of the samples (69.44%) did not contain any detectable crystals. Among the samples with crystalluria, calcium oxalate was the most prevalent type, present in 27.08% of the cases. Amorphous urates and amorphous phosphate crystals were found in 1.39% and 2.08% of the samples, respectively. Amorphous urate crystals were least prevalent.

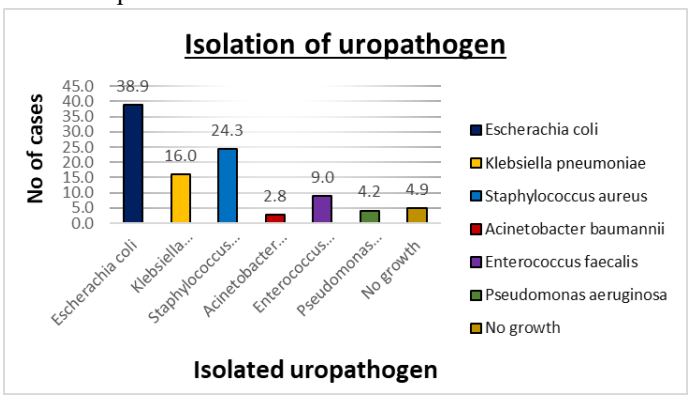

Figure 5: Prevalent Uropathogens Isolated from Pregnant Women having UTI

Figure 5 shows that among the six most common uropathogens causing UTI in pregnant women, *Escherichia coli* and *Staphylococcus aureus* are the main uropathogens causing UTI in pregnant woman that is 38% caused by *Escherichia coli* and 24% caused by *Staphylococcus aureus* respectively. Followed by *Klebsiella pneumoniae* and *Enterococcus faecalis* i.e. 16 % and 9% respectively. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the least common cause of UTI among pregnant women that is 4.2% and 2.8% respectively.

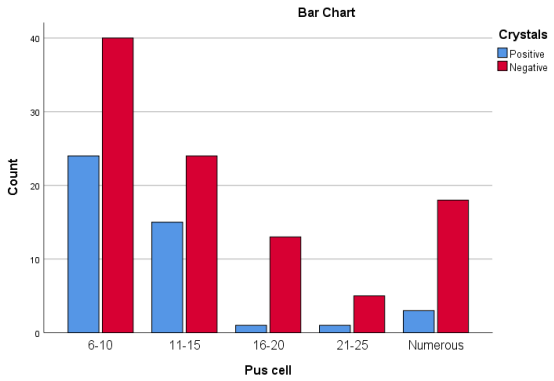

Figure 6: Association of Pus Cells with Crystalluria

Table 1: Correlation of crystals with Number of Pus Cells

		Number of Pus Cells							
		06-10	11-15	16-20	21-25	Numerous	p-value	Phi	Cramer's V
Crystals	Positive	24	15	1	1	3	0.05	0.05	0.05
	Negative	41	23	13	5	18			

Figure 6 and Table 1 represents the association between UTI and crystalluria. Highest positive number of crystals N=24 were seen between pus cells >6-10/HPF, followed by n=15 crystals in pus cells 11-15/HPF, least number of crystals, i.e. were n=1 seen in pus cells 16-20/ HPF, and 21-25/HPF. The p-value is 0.05 which shows that crystals and pus cells are having association. The Phi coefficient and the Cramer's V is 0.05 which indicates weak association between crystals and pus cells.

Table 2: Correlation of crystals with Age, pH, and Specific gravity of urine

	Crystals		
	Age	Specific gravity	pH
Correlation Coefficient	-0.015	-.216**	-.091
p-value	0.144	0.009	0.47

Table 2 shows correlation of crystals with age, specific gravity, and pH of urine. The correlation coefficient of age is -0.015 showing negative correlation between age and crystal as shown in the table. The p value is 0.144 which shows that there is insignificant relationship between age and crystals. The correlation coefficient of specific gravity is -.216** showing negative correlation whereas the p-value is 0.009 showing that is significant between crystals and specific gravity. The correlation coefficient of crystal and pH is -.091 which shows negative correlation between crystals and pH. The p-value is 0.47 showing insignificant association between crystals and pH.

IV. DISCUSSION

In our study of 144 samples, we found that the majority of patients (69.4%) of patients were having no crystals in urine which shows that crystalluria is not directly related to pregnancy however the in the remaining samples calcium oxalate (27.1%) were found to be the most predominant type of crystals in which some were having history of crystalluria in pregnancy. In the article (1) the researcher study crystalluria on 15 pregnant women, they perform urine RE on them in all trimesters and post-partum period. They found that the pregnant women had hypercalciuria in all three trimesters. Except for the first trimester, urine calcium levels in all trimesters were significantly higher when compared with the post-partum period and urine citrate (prevents urine calculi formation) were also found to be present in higher concentration in all trimesters aa compare to the post-partum period in addition urine oxalate (leads to calcium oxalate type crystals) were found to be observed in high concentration in the post-partum period than in pregnancy period. Hence, they observe that long time periods are needed for hypercalciuria to be able to lead to the formation of urinary calculi in pregnant women (except women having a positive family history) Our study findings about crystalluria in UTI also aligns with the article in which most of the pregnant woman (69.4%) were having no crystalluria and among the remaining calcium oxalate was found to be predominant type of crystals in urine of pregnant patients having UTI (2) Other crystals found in our study were amorphous urate (1.4%) and amorphous phosphate (2.1%), their presence may be due to transient supersaturation of urine, changes in urine pH and temperature or large amount od uric acid consumption. The article (9) also shows most crystals seen in UTI patients were calcium oxalate followed by amorphous urate and calcium phosphate.

In our study of 144 samples mostly cases having UTI in pregnancy fall in the age of 23 to 27 years (28.5%) followed by age 28 to 32 years (27.1%). The distribution indicates that majority participants were in their mid-20s to early 30s, a common reproductive age range. These studies showed the increased risk of UTI in the younger age. Our study aligns with the literature (3) shows that Women aged from 20 years to 30 years showed a higher incidence of urinary tract infection in pregnancy. In the article (9) most age group (21 to 34%) responsible for UTI was in 20 to 40 years.

In our study of 144 samples, majority (35%) of the pregnant females show their specific gravity in the normal range from 1.020 to 1.024. our study almost aligns with the study performed in tertiary care in Ratnagiri district of Maharashtra India which shows majority (94.69%) who's specific gravity was in the range

of 1.003 to 1.030 (4). In the article (9) the most samples with UTI were in the range of 1.020 to 1.025 which also aligns with our study.

We declare UTI on the basis of pus cells and culture of the urine samples In our study 84 patients were having leukocyte count positive and the remaining 60 was negative however in the article (5) the most important parameters to diagnose UTI on a dipstick are nitrite and leucocyte esterase they declare UTI in pregnant woman on the basis of leukocyte esterase nitrites and leukocyte count as many gram-negative bacteria produce the enzyme nitrate reductase, which converts urinary nitrate into nitrite indicating the presence of bacteria they aim to develop a diagnostic model based on all available evidence on leucocyte esterase, nitrite presence, bacteria presence, leucocyte count and symptoms. It differs from our study on the basis of nitrite and leukocyte esterase as we declare UTI on the basis of pus cells.

In our study to find the bacteriological profile of urinary tract infections we found that Escherichia coli (38%) was found to be the most predominant type of uropathogen causing UTI in pregnancy followed by staphylococcus aureus (24%). Our study aligns with the literature (6) which shows that *Escherichia coli* (*E. coli*) is the major etiological agent in causing UTI, which accounts for up to 90% of cases in their study. Our study also aligns with the literature (7) which also show cases with UTI, the organism grown was Gram negative in all with *Escherichia Coli* being the commonest, seen in 14 of 23 patients (60.8%) causing urinary tract infections. The literature (8) also shows that prevalence of UTI in pregnant women was 36.2%. It was also observed that *E. coli* (42.2%) was the most frequently isolated bacteria. Hence it shows that E coli is found to be the major uropathogen causing urinary tract infection among pregnant women.

In our study of 144 patients 121 patients were having no protein in urine, 126 we having no hemoglobin in urine, 134 were having no glucose in urine, 84 patients were having maximum leukocyte count in urine, 143 were having no casts in urine and 141 were having no ketones count in urine similarly in the article (11), 40.71% shows proteinuria, 8.62% shows glycosuria, 7.74% shows ketonuria and 15% shows the presence of casts in urine on microscopy it means protein, ketones, casts and glucose appears in very less quantity in the pregnant woman having UTI. The presence of leucocytes was seen only in 17.03% cases here this results differ from our article in such a way we found most of the patients (58%) having maximum leukocyte count. However In the article (9) pus cells epithelial cells and RBCs were most seen in the samples infected with UTI with the rate 80%, 83% and 45% respectively, this study aligns with our study on the basis of pus cells was found in UTI.

In our study the p-value for the relationship of pus cells and crystals is 0.05 which is significant and Phi Cramers V which shows the strength of association, we got 0.25 which shows there is a week correlation of pus cells and crystals. Our study aligns with article (10) which shows their study containing 10.17% cases crystals are crystals positive out of which, 86.95% showed calcium oxalate crystals, 59.6% cases were having ≥3 pus cells/hpf. It shows that there is a week correlation between pus cells and crystals in pregnancy of patients having UTI and crystalluria.

We also correlate crystals with urine pH, age and specific gravity, the age in groups shows insignificant relation with correlation coefficient -0.015 and p value 0.14. for correlation of correlation of crystals with pH, the correlation coefficient is -0.09 showing negative correlation and p-value 0.47 also showing insignificant correlation. While specific gravity with crystals also had negative correlation, correlation coefficient was -0,216** with P-value 0.009 which is significant.

V. CONCLUSION

Crystals formed in urine is referred as crystalluria. In our study most of the patients (69.4%) were having no crystals in urine and in the rest of patients calcium oxalate (27%) was found to be the most predominant type of crystal in urine of pregnant woman. some other crystals were also observed that includes amorphous urate (1.4%) and amorphous phosphate (2.1%). In urinary tract infections related pregnancies uropathogens provides a suitable environment for crystals formation. In our study the p-value for the association of pus cells and crystalluria is 0.05 which shows that crystals and pus cells having potential association with each other. The Phi and Cramer V which show the strength of association is 0.025 which indicates weak association between crystals and pus cells. Age and pH and specific gravity are negatively correlated with crystalluria. The age and pH have no significant correlation with crystalluria but the specific gravity is significantly correlated. UTI occurs due to the growth of the uropathogens in the urinary tract. In our study *E.coli* (38.9%) was found to be the most predominant type of uropathogen causing urinary tract infections in pregnancy followed by *Staphylococcus aureus*(24.3%), *Klebsiella pneumoniae*(16%) and the least common cause was *Acinetobacter baumannii* (2.8%) and 4.9% were having no growth on culture media. Crystalluria occurs in UTI related pregnancies so should be properly diagnosed and treated.

VI. ACKNOWLEDGMENT

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VII. CONFLICT OF INTEREST

I declare that there is no conflict of interest related to this research/study.

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IX. AUTHORS

First Author –Amaila Qaisar, M.Phil MLS, Senior Lecturer, Riphah International University, Islalmabad

Second Author – Bibi Fatima, BS MLT, Riphah International University, Islalmabad
Third Author – Tayyaba Khalid, BS MLT, Riphah International University, Islalmabad
Fourth Author – Aliya Bibi, BS MLT, Riphah International University, Islalmabad
Fifth Author – Dr Mohammad Raqeeb Khan, Graduated Internal Medicine resident, Tawam Hospital affiliated with John Hopkins Medicine International AlAin, Abu Dhabi,
Sixth Author – Tooba Khalid , Lecturer MS Biochemistry, Riphah International University, Islalmabad
Correspondence Author – Amaila Qaisar,