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Original Article

Turbidity, pH, Direct Gram as Predictors of Symptomatic Urinary Tract Infections in Pregnant Women

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ABSTRACT

Introduction: *Urinary tract infections (UTIs) are common in the community, including among pregnant women. This study investigates turbidity, urine pH, and direct gram as predictors of symptomatic urinary tract infection in pregnant women.* ***Methods:*** *A total of 177 pregnant women with suspected UTIs based on clinical and laboratories at the community health center in Makassar were collected from September to December 2021. The Urine sample was assessed for turbidity, urine pH, direct gram, and urine culture.* ***Results:*** *Urine turbidity was found in 108 (75.5%) patients who had positive cultures and PPV of 94.7%. The mean pH of the urine from UTI patients was 6.42 and without UTIs was 6.45. Urine that grows Gram-positive bacteria showed the most alkaline pH (mean pH=6.71) and was significantly less acidic than urine with UTI negative. In contrast, urine with Gram-negative bacteria had the most acidic pH (mean PH=6.30). The gram direct test shows a significant association with UTIs.* ***Conclusions:*** *Turbid urine can predict urinary tract infections in pregnant women that provide clinical*

symptoms. In addition, leukocytes esterase test further selected the samples. Urine pH showed no significant correlation with UTI. Less acidic urine pH is particularly associated with Gram-positive bacterial infections. Furthermore, direct gram can predict the event of UTI.

1. INTRODUCTION

Urinary tract infections (UTI) are common infections in the community, especially in adult women. Lower urinary tract infection (cystitis) affects almost one in every two adult women (40%) during their lifetime, and there is a 25% chance of developing recurrent UTI within the following year.[1,2] The incidence of UTIs in women is four times higher than in men, and one of the risk factors is pregnancy.[2,3]

In pregnancy, there are anatomical and functional changes in the urinary tract that cause bacteria (bacteriuria). These changes increase the risk of complications in both mother and baby. Bacteriuria in pregnancy is associated with a 20 to 30% increased risk of complications to pyelonephritis and is related to a risk of preeclampsia, premature birth, and low birth weight.[4,5]

The gold standard for the diagnosis of a UTI is urine culture to determine the pathogen that causes urinary tract infections. The disadvantage of urine culture is taken up to more than 24 hours to get results, expensive, and not all laboratories in health centers can perform this method. Early examination is needed to establish the diagnosis of urinary tract infections.[6] The quick and simple tests are the macroscopic assessment of urine, dipstick, and microscopic.[7]

The previous study that has examined patients under the age of 21 concluded that clear urine could be used as one of the predictors of negative urine culture outcomes. The authors received a negative predictive value (NPV) for the absence of UTI of 97.3%.[8]

This study aimed to assess some predictors of UTIs through macroscopic features of urine (turbidity), examination of urine with dipstick (pH urine), and gram staining in the urine of pregnant women who experience symptoms of UTIs. This data is expected to support the previous study on diagnosing symptomatic urinary tract infections in pregnant women.

2. METHODS

Study design and population

This study is a cross-sectional study conducted on pregnant women with suspected urinary tract infections clinically (have symptoms of UTI) and laboratories (positive leukocyte esterase) at the community health center in Makassar from September to December 2021. We included 177 pregnant women with suspected UTIs based on symptoms that include urgency, lower abdominal pain, pelvic/waist pain, a sensation of heat/burning during/after urinating, fever, and hematuria. We exclude pregnant women who are currently on antibiotic treatment and experiencing pus and vaginal bleeding from this study. The subject fills out a written approval. Examination of leukocyte esterase and pH urine using urine dipstick (Verify 10 Parameters).

Study procedure

The study included all pregnant women in the community health center in Makassar with suspected UTIs during pregnancy clinically and laboratory. Each subject gave consent in writing and filling out a questionnaire. Researchers provided questionnaires to collect patient data, socio-demographic characteristics, medical history, including UTI symptoms, current drug use history, and UTI risk factors. The research team explained how to collect urine samples in a clean-catch midstream to the study subjects. Urine collected performed a macroscopic examination of color and turbidity and documentation of the urine collection. Turbidity is visually assessed by two observers on a white background and viewed under good lighting conditions. Leukocyte esterase and urine pH are tested by using a dipstick examination. On the clean glass slide, 0.05 ml of well-mixed urine was placed, allowed to air dry, heat-fixed, and then gram stained. Under oil immersion objectives, at least 20 fields were evaluated (100X). It was regarded significant if there were more than or equal to 1 bacterium per oil immersion field, which amounts to 100,000 organisms per ml of urine. Urine is then cultured on the Blood Agar medium using a 1 uL inoculation loop and MacConkey Agar and incubated at 37^{o}C for 24 hours. The number of colonies 10^4 CFU/mL is considered significant bacteriuria.

Statistical analysis

The analysis measured the accuracy of macroscopic urine examination, i.e., turbidity as a predictor of urinary tract infection. We use Microsoft excel and calculate sensitivity, specificity, positive predictive values, and negative predictive values of urinary turbidity. Sensitivity is a true positive value showing a positive confirmed case. Specificity is a true negative value that indicates a negative confirmed case. Positive predictive value refers to the proportion of patients who show positive results and suffer from the disease. Negative predictive value refers to patients whose tests show negative outcomes and do not suffer from the disease. Descriptive statistics were presented in the form of the mean (standard deviation) and median for continuous variables (e.g., urine pH) and as frequency and proportion (%) for categorical variables (e.g., age, pregnancy history, gestational age, educational background, turbidity, urine pH). We compared the characteristics and urine pH of the UTI positive and UTI negative groups by using the Chi-square test. We compared the mean urine pH bacteria gram with the mean pH of the UTI negative group by using a Mann-Whitney test. All analyses were performed using the SPSS version 25 program. The significance level was set at .05.

3. RESULTS

The sample was obtained from 177 pregnant women aged 18 to 42 years, 143 of which were positive cultures. The patient's characteristics are presented in **Tabel 1**.

Table 1. Characteristics of patients with urine culture examination results

Characteristics	All (n=177)	Non-UTI (n=34)	UTI[a] (n=143)	P value[b]
Age, years, mean (SD)	27.33 (5.99)	27.03 (6.11)	27.40 (5.99)	.641*
Pregnancy history, n(%)				
Primigravida	70 (39.5)	14 (41.2)	56 (39.2)	.829
Multigravida	107 (60.5)	20 (58.8)	87 (60.8)	
Trimester, n(%)				
1st	55 (31.1)	12 (35.3)	43 (30.1)	
2nd	71 (40.1)	15 (44.1)	56 (39.2)	.497
3rd	51 (28.8)	7 (20.6)	44 (30.8)	
Education level, n(%)				
Primary school	14 (7.9)	1 (2.9)	13 (9.1)	
Junior high school	23 (13.0)	4 (11.8)	19 (13.3)	.667
Senior high school	97 (54.8)	20 (58.8)	77 (53.8)	
College	43 (24.3)	9 (26.5)	34 (23.8)	

[a] UTI was defined as the growth of bacteria at a concentration of at least 10^4 CFUs per mL.
[b] P-value was calculated by Chi-square test and *Mann-Whitney test.
Abbreviations: CFU, colony-forming unit; UTI, urinary tract infection; SD, standard deviation.

Table 2 shows the urine testing results obtained by Turbidity, pH, gram stain, and urine culture.

Table 2. Distribution of urine turbidity, pH, and gram stain with urine culture examination

Urine test	All (n=177)	Non-UTI (n=34)	UTI (n=143)	P value[a]
Turbidity, n(%)				
Cloudy	114 (64.4)	6 (17.6)	108 (75.5)	**.000**
Clear	63 (35.6)	28 (82.4)	35 (24.5)	
pH, n(%)				
4.5-<5.5	8 (4.5)	1 (2.9)	7 (4.9)	
5.5-<6.5	73 (41.2)	16 (47.1)	57 (39.9)	.699
6.5-9.0	96 (54.2)	17 (50.0)	79 (55.2)	
pH				
Mean (SD)	6.43 (0.64)	6.45 (0.68)	6.42 (0.63)	.870
Median (Q1-Q3)	6.5 (6-7)	6.25 (6-7)	6.5 (6-7)	
Gram Stain, n(%)				
Positive	170 (96.0)	27 (79.4)	143 (100)	**.000**
Negative	7 (4.0)	7 (20.6)	0 (0)	

P-value that reach the significance level of alpha = 0.05 were labeled in bold.
[a] P-value was calculated by Chi-square test and mean urine pH by Mann-Whitney test.

The Comparison of gram examination to the urine pH can be viewed in **Table 3**.

Table 3. Distribution of UTIs urine pH in Gram Bacteria

Microscopically	N	%	Mean	**(**SD**)**	Median	**(**Q1**-**Q3**)**	P value[a]
UTI Positive	143		6.42	0.63	6.5	6.0-7.0	
UTI Negative	34		6.45	0.68	6.25	6.0-7.0	
Gram-positive	26	18.2	6.71	0.60	7.0	6.37-7.0	**.046**
Gram-negative	96	67.1	6.30	0.62	6.0	6.0-6.87	.402
Gram-positive, negative	21	14.7	6.61	0.54	6.5	6.0-7.0	.235

P-value that reach the significance level of alpha = 0.05 were labeled in bold.
[a] Mean urine pH gram bacteria was compared with the mean urine pH of UTI negative using Mann-Whitney test.

The sensitivity and specificity of urine turbidity in symptomatic pregnant women in predicting the presence of UTIs are 75% and 82.4%, respectively. Positive and negative predictive values are 94.7% and 44.4%.

4. DISCUSSIONS

Pregnancy is the risk factor for urinary tract infections. Some literature recommends screening and treatment for bacteriuria that occurs during pregnancy.[4] Several previous studies have examined the visual inspection of urine in predicting the growth of urinary pathogens. The study, conducted by Bulloch B et al., involved 159 urine samples of patients aged < 21 years by showed any or no symptoms of UTIs. Urine specimens collected from urinary catheters amounted to 44% and 56% of midstream urine. Of the 159 samples, 49 (31%) showed turbidity, and 110 (69%) were clear. Of the 110 clear samples, 107 produced negative cultures. The overall specificity value of urine was 82.3%. Clear urine on visual inspection has a negative predictive value (NPV) of 97.3%.[8] In another study, 100 samples were obtained from the urine of adult women aged 18 to 50 years. In fifteen samples with positive cultures, two samples showed turbidity, while of the 85 samples with insignificant bacterial growth, there were 3 cloudy and 82 clear urine. Eighty-one clear specimens had negative cultures with number of colonies >10,000 CFU/mL while 82 clear specimens grow >100,000 CFU/mL.[9] The positive predictive value (PPV) in both previous studies was low at 53% and 40% due to the less strict screening criteria.

This study used the number of colonies ≥ 10,000 CFU/mL to define significant bacteriuria among pregnant women with suspected urinary tract infections based on clinical or UTI symptoms and laboratory examination of leukocytes esterase positive. Strict criteria used in sample selection may alter the sensitivity of turbidity. Urine turbidity was found in 108 (75.5%) patients who had positive cultures (p=.000) (Table 2), and PPV of 94.7% was much higher than reported in previous studies. These data suggest that turbidity of urine can predict urinary tract infections in urine samples of pregnant women, provided that show clinical symptoms. In addition, leukocytes esterase test further selected the samples. The high PPV percentage was influenced by the high prevalence of UTIs included in this study.[8] UTIs were reported in 80.8 %.

The gram staining shows a significant association between a direct gram and urinary tract infections (p=.000) (Table 2). These findings support previous research by Kumar M *et al*., which found that direct gram staining in non-centrifugated urine provides a sensitive and specific screening test for identifying urinary tract infections.[10] Another study by Sartika *et al*., reported direct gram specific and accurate in diagnosing UTIs.[11] In one study, direct gram staining in non-centrifuged urine was sensitive to detecting bacterial concentration at 10^4 CFU/ml and 10^5 CFU/ml.[12] In our research, bacteria in direct gram may still be detected at 10^3 CFU/ml bacterial concentration. Our results are supported by Winquist *et al*. investigation, which reported gram staining at bacterial concentrations ≥ 10^3 can still be detected, although its sensitivity decreased by 69.6%.[13]

We further attempted to analyze the pH of urine based on gram bacteria. We discovered that urine that grew Gram-positive bacteria had higher pH (mean pH=6.71) than urine with Gram-negative bacteria (mean PH=6.30) (Table 3). Our findings are consistent with a study that associates urine pH with the most common uropathogen

causing UTIs. The study reported the pH of urine that grows *Escherichia coli* and *Enterobacter cloacae* showed a lower pH.

The combination of several factors determines the urine pH in UTIs.[14] Compared to the pH of urine that grew Gram-positive bacteria, we discovered that urine that grew Gram-negative bacteria had the most acidic pH. The possible mechanism is that the most Gram-negative bacteria identified in the urine of pregnant women, such as *Escherichia coli* and *Enterobacter cloacae*,[15] rarely produce urease and thus are unable to alkalinize the urine.[16] Urease activity catalyzes the conversion of urea to ammonia and carbon dioxide (CO_2). Ammonia reacts with water to form ammonium and hydroxide ions, thus raising the urine pH.[17] In this study, the proportion of Gram-negative bacteria that do not produce urease is 65%. Based on this data, it can be determined that almost two-thirds of these Gram-negative bacteria do not produce urease, indicating that their pH is more acidic. Further evidence of urease producer from the remaining Gram-negative must be provided. In contrast, Gram-positive bacteria such as *Staphylococcus saprophyticus, Enterococcus faecalis, Streptococcus aglactiae*, and *Corynebacterium urealyticum* produces urease and therefore can alkalinize the environment.[18]

This is the first study of the UTI predictors in pregnant women. One of the strengths of this study was screening by strict recruitment criteria, which had been set prior to the study based on interviews. This may reduce the potential of testing urine from an asymptomatic person. There are a few limitations of our study. First, dietary factors may impact urine pH (we did not standardize urine collection protocol, e.g., fasting urine sample). Dietary factors alter urine pH, such as milk consumption, because milk protein can lower urine pH.[19] However, this statement was not proven in two investigations that examined urine acid excretion following milk consumption. The study concluded that milk does not produce acid in urine.[20] Second, we did not microscopically examine the leukocytes on examination of the sample. The assumption was that they had been represented through the leukocyte esterase test.

5. CONCLUSION

Turbid urine can predict urinary tract infections in pregnant women that provide clinical symptoms. In addition, leukocytes esterase test further selected the samples. The gram staining indicates an association with UTIs. In contrast, Urine pH showed no significant correlation. Less acidic urine pH is particularly associated with Gram-positive bacterial infections. More large prospective studies are required to confirm our findings and determine whether urine pH is a risk factor for UTIs in pregnant women.

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Conflict of Interest Statement:

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.