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Research article

Prevalence and evaluation of asymptomatic bacteriuria in pregnant women

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Abstract:
Introduction: Asymptomatic bacteriuria (ASB) is defined as the isolation of bacterial strain in quantitative counts ≥105cfu/mL in the urine without the presence of any symptoms. The aim of this study was to investigate prevalence and pathogen microorganisms of ASB, and the most appropriate diagnostic test in the first trimester of pregnancy.
Methods: In this study, 312 pregnant women were included. Midstream culture by conventional methods and urinalysis were done. By considering the urine culture as the gold standard, sensitivity, specificity, positive predictive value and negative predictive value of bacteriuria and pyuria were calculated.
Results: ASB prevalence was found at 6.7%. Specificity and sensitivity for bacteriuria were 77% and 100%. Specificity and sensitivity for pyuria was 65% and 61%. The common pathojens was *E.coli* (85.7%).
Conclusion: Pyuria and bacteriuria may not a major determinant of the ASB but may be useful as a screening test.
Key words: asymptomatic bacteriuria; pregnancy; pyuria; urinalysis; urine culture
Running title: Asymptomatic Bacteriuria

Introduction

Asymptomatic bacteriuria (ASB); without signs of infection in the urine is a count of colony more than 100,000 bacteria per ml [1]. The prevalence rates of ASB varies among pregnant women according to parity, race and socioeconomic status and geographical area, ranging from 2.5 to 12.5% [2, 3]. As a result of anatomical, hormonal and physiological changes in pregnancy, dilatation in the kidney collecting systems and ureters causes an increase in the volume of urine, ureteral reflux due to relaxation in the vesicourethral junction prepare the place for the proliferation of microorganisms [4, 5]. In addition, the decrease in the ability of the kidneys to concentrate urine reduces the antibacterial activity of urine. Physiological glycosuria and increased nutrients residues in the urine during pregnancy contributes to the proliferation of microorganism [6]. If ASB is not diagnosed and treated early, it causes 25-40% of symptomatic urinary tract infections and pyelonephritis, even urinary retention in the early postpartum period [7]. The most severe complication of ASB is acute pyelonephritis, which increases maternal and fetal morbidity [5, 8]. Wing et al. found that anemia, septicemia, acute pulmonary insufficiency, acute renal dysfunction, and spontaneous preterm deliveries were higher in patients with acute pyelonephritis [8]. ASB has been associated with preterm delivery, increased delivery of low birth weight infants, premature rupture of membranes, increased oligohydramnios/polyhydramnios, and increased incidence of recurrent pregnancy losses [9, 10, 11]. As a result of all this, both maternal and fetal morbidity is increased [11]. The diagnosis and treatment of ASB in the early stages of pregnancy can prevent at least two-thirds of these cases [12, 13]. For this reason, the American Academy of Pediatrics and American College of Obstetricians and Gynecologists recommend screening of bacteriuria in the first prenatal visit.3 Nevertheless, the benefits and cost of screening ASB by routine urine culture in gestations are discussed [12, 14]. The most common microorganism in the ASB is *Escherichia coli* [15, 10]. ASB is seen more often in women with previous episodes of urinary tract infections, patients with anemia, patients with diabetes mellitus, urinary calculi or those with a history of congenital anomalies, those with low socioeconomic level and advanced maternal age. Sexual activity also increases the frequency of ASB [16, 17]. In some studies, there is no relationship between ASB, parity and gestational age. [10]. A screening test is required to select for culture to reduce costs, to

win time, and to reduce the workload of laboratories and to alleviate serious complications.

The aim of this study was to investigate prevalence and pathogen microorganisms of ASB, and as well as the most appropriate diagnostic test in the first trimester of pregnancy and whether urinalysis was an effective alternative screening test to urine culture test.

Materials and methods:

Urinalysis and urine culture were performed simultaneously in the first trimester pregnant women. A total of 674 pregnant's files scanned retrospectively. In this study, 312 pregnant women with study criteria were included and evaluated. Gestational age, education status, occupation status, marital status, residence status (rural, urban), routine urine analysis and urine culture results were recorded from the files, including age, parity, urinary or urinary system complaints, use of antibiotics with any indications within the last 15 days (Table 1).

Variables	Number (n)	Percent (%)
Age		
15-24	87	27.9
25-34	180	57.7
35-44	45	14.4
Gestational stage		
First trimester	312	100.0
Marital status		
Married	307	98.4
Others	5	1.6
Occupational status		
Employed	172	55.13
Unemployed	140	48.87
Living location		
Urban	263	84.3
Rural	49	15.7
Educational status		
Read and write	10	3.21
Elementary	70	22.43
High school	171	54.81
Higher education	61	19.55
Total	312	

Table 1: *Socio-demographic characteristics of study participants*

Follow-up culture results of patients treated with ASB in their initial cultures were recorded. Routine urine examination and culture results were analyzed. Non-pregnant women, those who took antibiotics with any indications within the last two weeks, second and third-trimester pregnancies, complaints and clinical findings related to the urinary system, those had contaminated in urine culture were excluded from the study (Table 2).

Exclusion criteria	n	%
2.-3. Trimester pregnancy	299	82.60
Antibiotic use	33	9.12
Urogenital system complaint	18	4.97
Contamination	12	3.31
Total	362	100

Table 2: *Exclusion criteria for pregnant women*

Inclusion criteria were considered that pregnant women in the first trimester with first prenatal visits outside of the exclusion criteria. Pregnancies with study criteria and simultaneous routine urine analysis and urine culture at the first prenatal visit were included in the study. The

pregnancies were divided into groups according to their age and parity. Routine urinalysis and culture results were analyzed.

Midstream urine samples were taken after cleaning the urethral opening with an antiseptic towelette, with separation of labia and wiping from front to back. Urine specimens come to our laboratory within 15 minutes. Then routine urine examination, the culture, antibiotic susceptibility test was done. Patients treated with group B antibiotics according to the antibiotic susceptibility test. Following the treatment, the control culture was reconstituted after 1-2 weeks and a monthly culture was performed to monitor recurrence.

Nitrite was treated with the dipstick in urine and test was positive/negative according to color change. For the pyuria and bacteriuria, the 10 ml urine specimen was centrifuged at 1500 rpm for 5 min and then the sediment obtained was taken on a clean slide and examined under a microscope. If the dipstick urine test was positive for nitrite and bacteriuria in the direct urine microscope and those with ≥ 10 white blood cells (WBC) in every area scanned area were considered as positive. Urine samples were incubated on blood agar and EMB (Eosin-Methylene-Blue agar) for 18-24 hours at 37˚C. Positive urine culture was defined as ≥100 000 colony forming units of a single species. The isolated microbes were subjected to antibiotic susceptibility testing using the disk diffusion technique. Patients with bacteriuria and pyuria were compared with patients with positive urine culture results in the diagnosis of ASB [18, 19].

Statistical Analysis:

SPPS 16 package program was used for the analysis. We analyzed the sensitivity and specificity of bacteriuria and pyuria in urinalysis as an alternative to urine culture and descriptive statistics were expressed as mean±SD, nominal values as number and percentages. Pearson's Chi-Square exact test also was used for statistical correlations. $p<0.05$ was accepted as statistically significant. Prevalence of ASB, distribution according to parity and age range, and urine culture results were compared with pyuria and/or bacteriuria. By considering the urine culture as the gold standard, sensitivity, specificity, positive predictive value and negative predictive value of bacteriuria, pyuria were calculated. Nitrite positivity and proteinuria were not assessed statistically because nitrite positivity was seen in only one pregnant, proteinuria was seen in two pregnant.

Results

The socio-demographic findings of the studied pregnants were shown in Table 1. The distribution of cases not included in the study according to the exclusion criteria was shown in Table 2. According to urine culture; 21/312 (6.7%) pregnant women had ASB. When we evaluate the distribution of ASB frequency in age groups; ASB was detected in 10 out of 87 pregnancies (11.5%) in the age range of 15-24 (20 ± 0.45) years; 10 out of 180 (5.6%) pregnancies in the 25-34 (29 ± 2.65) age group; 1 out of 45 (2.2%) pregnancies in the 35-44 (38±3.15). When the results were evaluated by Pearson's Chi-Square exact test, the urine culture distribution was not different according to age groups ($p = 0.079$).

According to the results of culture, the distribution of pregnant women diagnosed with ASB according to parity; ASB was detected in 4 of 108 multiparous (3.7%) who delivered two or more deliveries, in 5 of the 99 nulliparous (5.1%), 12 of the 105 the primiparous (11.4%). According to the Pearson Chi-Square test, although the p-value of the primiparous seemed to be close to the significance level, ASB distribution was not different significantly according to parity ($p = 0.057$).

In the routine urinalysis, 1 case of nitrite test (4.76%), 2 cases (9.5%) of proteinuria, 114/312 (36.54%) of pyuria and 87/312 (27.88%) of bacteriuria were detected.

When we compared the results of the pyuria with the culture, culture positivity was found in 13 of the 114 (11.4%) pregnant women who had pyuria and 8 of 198(4%) pregnancies without pyuria (Table 3). The sensitivity, specificity, negative and positive predictive values of pyuria were calculated (0.62, 0.65, PPV = 0.11, NPV = 0.95), respectively, while culture results were considered as the gold standard. According to these results, 95% of the cases can be negative in culture.

PPV was so low that means culture was negative in many pyuria positive patients. Therefore culture positivity was not found in all pyuria positive patients. But NPV was found very high. This means that the probability of culture negativity was found as 95% in pyuria negative patients. When we compared the results of the bacteriuria with the culture that considered gold standard, we found that culture positivity in 21 (24.1%) of the 87 pregnant women who had bacteriuria and 0 (0%) of 225 pregnancies without bacteriuria (Table 3).

The specificity and sensitivity, PPV and NPV of bacteriuria were found at 77% and 100%, 0.24, 1.00 respectively. Bacteriuria has a very high NPV that means bacteriuria negative patients was found culture negative. None of the patients in bacteriuria negative group culture was positive. However, culture positivity was determined in 24 % of the bacteriuria (+).

Since our aim was not to investigate the predisposing factors for ASB, no comparison was made between ASB and socio-demographic characteristics of study participants except age and parity.

E. coli was found the most common microorganism in urine culture at a rate of 85.7 %, enterococci (9.5 %) in 2 cases, *Klebsiella pneumoniae* (4.76 %) in one case. It was determined that nitrite positivity in one case (4.76 %), proteinuria in 2 cases (9.5 %). ASB prevalence was found at 6.7 %. Patients with ASB treated with appropriate antibiotics for 5-7 days. After the end of treatment, no cases were positive in repeated urine cultures. Urinary tract infection has recurred only in one pregnant woman at later gestational weeks, she was followed by application of a long-term antibiotic treatment. Recurrences were not observed in follow-up with culture.

	Culture (-)	**Culture (+)**	**Total**
	n [%]	n [%]	n [%]
Pyuria (+)	101 [88.6]	13 [11.4]	114 [36.5]
Pyuria (-)	190 [63.5]	8 [4]	198 [63.5]
Bacteriuria (+)	66 [75.9]	21 [24.1]	87 [27.88]
Bacteriuria (-)	225 [100]	0 [0.0]	225 [100.0]

Table 3: *Rates of pyuria and bacteriuria in the culture-positive and negative cases*

Discussion:

ASB in pregnancy leads to high morbidity in the maternal, fetal and neonatal period if early diagnosis and treatment are not performed. So, screening of the bacteriuria was recommended at the first prenatal visit [3]. While urinary culture is the gold standard in diagnosis, urine culture in all asymptomatic pregnancies increases the laboratory burden, and cases such as contamination of the urine sample lead to delayed diagnosis and increased cost and a faster screening test are required before the urine culture. For this, midstream urine specimens collected in sterile

conditions to examine nitrite, leukocyte, and bacteria. We compared these results with urine culture results to investigate whether some findings in the routine urinalysis could be used as a screening test. If ASB was not treated, symptomatic urinary tract infections, acute pyelonephritis develop could develop in 30-40% of these cases. If ASB was treated, symptomatic urinary tract infections, acute pyelonephritis could develop in 3-4% of these cases.9 Detection and treatment of ASB in the early stages of the pregnancy can prevent at least two-thirds of these cases [13, 20]. The rate of ASB during pregnancy was reported as 2-12.5%.2, 3 in our study, it was found to be 6.7% and compatible with the literature findings. There were different notions about the predisposing factors for ASB in the literature and a full consensus was not provided [16, 17, 20, 21, 22]. ASB was not associated with age, parity, and prior urinary tract infections in some studies [10]. ASB was relatively high in the nulliparous group and 15-24 age group compared to other age and parity groups, but the result was not significant in this study. Thakur et al. found no association between age and parity and ASB [23]. Because of demographic data of the populations, statistical methods, number of cases, and differences in study designs, these differences in studies were seen. In our study, the specificity and sensitivity of pyuria were 0.65 and 0.62, PPV = 13/114 = 0.11 and NPV = 190/198 = 0.95, respectively. If the pyuria was positive, it was necessary to make a culture to confirm the presence of the ASB. Because leukocytes can be caused by inflammation in the genital system. However, someone with very high NPV and non-pyuria was likely to be negative for culture as 95% probability. The presence of the pyuria was not a prerequisite for the diagnosis of ASB, and the treatment according to the result of the pyuria will lead to unnecessary antibiotic use and side effects (allergy, diarrhea, candidiasis and antibiotic resistance development) [24]. In our study, the specificity and sensitivity of bacteriuria were 0.77 and 1.00, PPV = 0.24, NPV = 1.00, urine culture was negative in bacteriuria negative cases. According to these results, urine culture could not be done in the absence of bacteriuria. However, this study should be done in larger patient groups.

In our study, urine specimens were collected at any time of day rather than the first urine in the morning, and the evaluation was made based on whether bacteria were present, not by the number of bacteria.

We did not evaluate nitrite positivity in urine because it was detected only in one case. Although nitrites positivity may be used as a screening test, especially in the third world countries, the nitrite test was specific but not very sensitive [1, 25]. Many uropathogens *(E. coli, Klebsiella, Proteus* etc.) convert nitrate to nitrite and can be detected in the strip test. While nitrite positivity in dipstick test was significant, false positive results may develop. For example: consumption of certain foods that may affect the nitrite test, waiting for urine in the bladder more than 4 hours, contact of the strips with air can lead to false positives. After removing the strip, the cover should be closed immediately. On the other hand, diluted urine can be negative. Even if the result was negative, the urinary tract infection was not ruled out. In which case, other analysis should be made. The use of nitrite for screening urinary tract infections in particular ASB was associated with many false and negative results as compared to the gold standard culture method) [25, 26]

In some studies, recurrence was seen in one-third of cases had ASB in the first culture following treatment [27]. Our population was a more homogeneous group and there is no difference between ethnicity and religious belief. We thought that 232 (74.36%) of our cases had high school and high education, treated according to antibiotic susceptibility test result, perineum hygiene care, compliance with physician recommendations contribute to the absence of recurrence in our study.

The most frequent pathogens of ASB was *E. coli* (85.7 %) which is consistent with the literature [28, 29, 30].

Although urine culture remains the gold standard for diagnosis of ASB in pregnant women, it is time-and labor-intensive and patients may have difficulty providing uncontaminated samples. According to these results, pyuria and bacteriuria may not a major determinant of the ASB and culture positivity were not observed in any of the bacteriuria negative pregnant women. Because of the high NPV of pyuria and especially bacteriuria positivity for choosing the pregnant for urine culture, urinalysis may be useful as a screening test. *E. coli* is the most common cause of ASB.

Conflict Of Interest's Statement

The authors declare no conflict of interest and no funding.

Author contributions:

T.C., T.T.Ö., R.S., and M.Ö. participated in the design and conception of the study. T.T.Ö.,
R.S., and M.Ö. carried out the data collection. R.S. and T.C. performed the statistical analysis.
T.C., T.T.Ö., R.S., and M.Ö. contributed to the review and interpretation of the results. T.C.,
T.T.Ö., drafted the manuscript. All authors read, revised and approved the final manuscript.

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