

Detection of Asymptomatic Bacteriuria by Nitrite and Leukocyte Esterase Tests versus Urine Culture during Pregnancy

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ABSTRACT

Background: Asymptomatic bacteriuria is a major risk factor for the development of urinary tract infections during pregnancy and with further risk of preterm birth and pyelonephritis if untreated. There is good evidence that treatment of asymptomatic bacteriuria will decrease the incidence of pyelonephritis. So an early detection of urinary tract infection by nitrite and leukocyte esterase dipstick can decrease these complications.

Objective: To evaluate the efficacy of nitrite and leukocyte esterase versus the urine culture for detection of asymptomatic bacteriuria in pregnant women.

Methods: A prospective study conducted in Al-Yarmouk Teaching Hospital for a period of one year from September 2013 to September 2014. A clean catch midstream urine specimen obtained from 500 pregnant women present to antenatal clinic. One portion of specimen is test for nitrite and leukocyte esterase activity by dipstick, second portion of urine is plated on blood and MacConkey agars for culture. Comparison of the results of the above mentioned tests were done.

Results: Asymptomatic bacteriuria was prevalent in 12% of 500 pregnant women who were evaluated in the current study. The current study revealed asymptomatic bacteriuria by nitrite with 68.3% sensitivity, 100% specificity and leukocyte esterase with 53.3% sensitivity, 99.5% specificity which were consider accepted tests in comparison with urine culture.

Conclusion: The nitrite test and leukocyte esterase tests had a high specificity (99.8) and negative predictive value (99.6) in comparison with urine culture for detection of asymptomatic bacteriuria. But nitrite test was more sensitive and specific than leukocyte esterase. In current study all positive cases of nitrite test were positive for urine culture, so it can be considered as a good test for detection of asymptomatic bacteriuria.

Keywords: Nitrite and leukocyte esterase, Dipstick tests, Pregnant women, Asymptomatic bacteriuria.

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Urinary tract infections are the most common bacterial infections during pregnancy and it may manifest as acute cystitis, pyelonephritis or an asymptomatic bacteriuria⁽¹⁾. Asymptomatic bacteriuria is isolation of a specified quantitative count of bacteria in urine specimen obtained from a person without symptoms or signs. The incidence during pregnancy varies from 2-7%⁽²⁾. It typically occurs during early pregnancy, with only approximately a quarter of cases identified in the second and third trimester⁽³⁾.

The organisms that cause UTI during pregnancy are the same as those found in non pregnant patients. *Escherichia coli* accounts for 80-90% of infections. Other Gram negative rods such as *Proteus mirabilis* and *Klebsiella pneumoniae* can also be cultured⁽⁴⁾. Asymptomatic bacteriuria is associated with adverse maternal outcomes include symptomatic cystitis, pyelonephritis (in up to 30%) and preterm labour and delivery. In addition, there are increased maternal risks reported for pre-eclampsia, anaemia, chorioamnionitis and post-partum endometritis. The associated adverse fetal outcomes include prematurity, low birth weight^(1,4). Antimicrobial treatment of

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asymptomatic bacteriuria during pregnancy will decrease the risk of subsequent pyelonephritis from 20-35% to 1-4% and the risk of having a low birth weight baby from 15% to 5%^(1,5).

Pregnant women should be screened for bacteriuria by urine culture at least once in early pregnancy, and they should be treated if the results are positive⁽²⁾. The diagnosis of asymptomatic bacteriuria is a microbiologic diagnosis based on culture of a urine specimen. For asymptomatic women, bacteriuria is defined as two consecutive voided urine specimens with isolation of the same bacterial strain in quantitative counts of 10⁵ cfu/mL⁽²⁾. Although urine culture remains the gold standard for screening and diagnosis of ABU in pregnant women, it is costly time consuming. Selection of mothers for screening with risk factors may reduce the necessity of urine culture for all pregnant women and may be used as an alternative strategy of management. A pregnant woman with a positive dip slide test is very likely to have a definitive diagnosis of asymptomatic bacteriuria, whereas a negative result effectively rules out the presence of bacteriuria⁽⁶⁾. Dipstick test is the generally accepted screening test for UTI. Chemical test strips usually operate by detection of leukocyte esterase (LE) and a nitrate (N) reductase activity.

The aim of this study is to evaluate the efficacy of nitrite and leukocyte esterase dipstick with the urine culture in detection of in asymptomatic bacteriuria pregnant women.

Methods

A prospective study conducted in the department of obstetrics and gynecology at Al-Yarmouk Teaching Hospital, Baghdad, Iraq; in cooperation with laboratory department (bacteriology unit); for a period of one year from the September 2013 to the September 2014

The study was approved by the ethical committee of council of the Arab Board for Medical Specialization of Obstetrics and Gynecology.

The study included 500 pregnant women who were attending the out patients clinic of Al-Yarmouk Teaching Hospital.

Verbal consent was obtained from all pregnant women before inclusion in the study.

Inclusion criteria: Women at any gestational age and irrespective of their age, race or parity who were attending the out patients clinic were included.

Exclusion criteria: Pregnancy-induced diabetes mellitus or hypertension, antibiotic administration within the previous seven days, amniotic fluid leakage, and bloody vaginal discharge were excluded.

A pre-tested, self-administered questionnaire was used to collect the socio demographic data including maternal age, gravidity, parity, past obstetrical, gynecological, medical, and surgical history. The gestational age was assessed by last menstrual period (LMP) and confirmed by ultrasonography. Complete general and obstetrical examinations were done for pregnant women.

The specific instructions for collection of midstream urine specimens were provided to enable patients to collect appropriate specimens. Urine sample was collected from pregnant women by clean catch mid stream urine after washing the vagina by clean tap water and separate the labiae minora, a suitable sterile container for urine collection was used, and the samples were examined within 30 minutes of collection.

Urine sample were subjected for the following tests: Chemical test strips (dipstick): Sample of 3-5 ml of urine was taken in white ordinary tube from the original sample as soon as possible without centrifuged and put the strip of dipstick in urine for two minutes and read the results for N. and LE by change in colour of strip, if the light off-white colour of strip for N. change to light pinks or dark pink it means positive results, trace or strong positive respectively. In order to

dark off-white colour for LE in the strip change to light brown, pink or violet it mean (+25, ++75, +++500 WBC/microlitre (MI)) respectively, if there's no changes in colour of strip of N. and LE labels' it was negative tests.

Other 0.1 ml of urine sample was inoculates in MacConkey or blood agar for urine culture. Conventional microbiologic quantitation of bacteriuria is performed by streaking a known volume of urine from a calibrated loop 0.1 ml on to Agar incubating at 37 C° for 24 hrs.

After collection of results, a comparison was done to evaluate the efficacy of N. and LE with the urine culture.

The data were reviewed and analyzed using Statistical Package Social Sciences (SPSS) version 20 and Microsoft Office Excel 2007. Categorical data represented by frequency and percentages and analyzed using Pearson's chi-square test. The continuous data presented as mean and standard deviation.

Reliability tests (sensitivity (SN), specificity (SP) and accuracy) were used to validate the urine tests. A level of p-value less than 0.05 was significant.

Results

Five hundred pregnant women have been included in this study, assessed for UTI in symptomatic and asymptomatic women

Figure 1 shows the percentage of the included pregnant women according to appearance of UTI among the 500 pregnant women. It shows that 14% of patients have UTI regardless having symptoms or not.

Figure 2 shows the percentage of the included pregnant women diagnosed to have UTI which was confirmed by positive urine culture according to the appearance of symptoms. Ten (14.3%) of the included women were symptomatic and 60(85.7%) were asymptomatic. Thus ABU diagnosed in 60 patients equal to 12% of 500 included pregnant women.

Table 1 shows the association between the demographic characteristics of patients. It appears that there is significant relationship between positive urine culture with GA and previous history of miscarriage, by using Pearson's chi-square test, p-value was 0.002 with GA and 0.001 with history of miscarriages.

Table 2 shows comparison of different diagnostic tests which were performed on the 500 pregnant women included in this study. As shown 70 out of 500 pregnant women diagnosed to have UTI confirmed by urine culture, 10(14.3%) of them were symptomatic and 60(85.7%) were asymptomatic with p-value 0.35.

UTI diagnosed by N: 47 patients of them, 6(12.8%) were symptomatic and 41(87.2%) were asymptomatic with p-value 0.72.

UTI diagnosed by LE test: 38 patients of them, 4(10.5%) were symptomatic and 34(89.5%) were asymptomatic with p-value 0.49.

By using combined test N. and LE UTI diagnosed 36 patients of them, 5(13.9%) were symptomatic and 31(86.1%) were asymptomatic with p- value 0.99.

Finally by using ME: 40 patients of them, 8(20%) were symptomatic and 32(80%) were asymptomatic with p value 0.47, using Pearson's chi-square and fisher's exact test.

Table 3 shows comparison between the SN, SP, positive predictive value (PPV), negative predictive value (NPV) of N to that of urine culture among 500 pregnant women. It appears that there are 5(50%) out of 10 patients with symptomatic UTI had positive N and urine culture tests with SN 50%, SP 99.8%, PPV 83.3% and NPV 99.8%, 41 patients out of 60 patients with asymptomatic UTI had positive N and urine culture tests with SN 68.3%, SP 100%, PPV 100% and NPV 95.8%.

Table 4 shows comparison between the SN, SP, PPV and NPV of LE to that of urine culture in 500 pregnant women. It appears that 3(30%) out of 10 with

symptomatic UTI had positive LE and urine culture tests with SN 30%, SP 99.8%, PPV 75% and NPV 98.4% and 32(53.3%) out of 60 patients with asymptomatic UTI had positive LE and urine culture tests with SN 53.3%, SP 99.5%, PPV 94.1% and NPV 93.9%.

Table 5 shows comparison between the SN, SP, PPV and NPV of combination of N and LE with urine culture in 500 pregnant

women. It appears that 4(40%) patients out of 10(100%) were symptomatic UTI had positive combined tests and urine culture tests with SN 40%, SP 99.8%, PPV 80.8% and NPV 98.6%, 31(51.7%) patients out of 60(100%) with asymptomatic UTI had positive combined tests and urine culture tests with SN 51.7%, SP 99.5%, PPV 93.9% and NPV 93.7%.

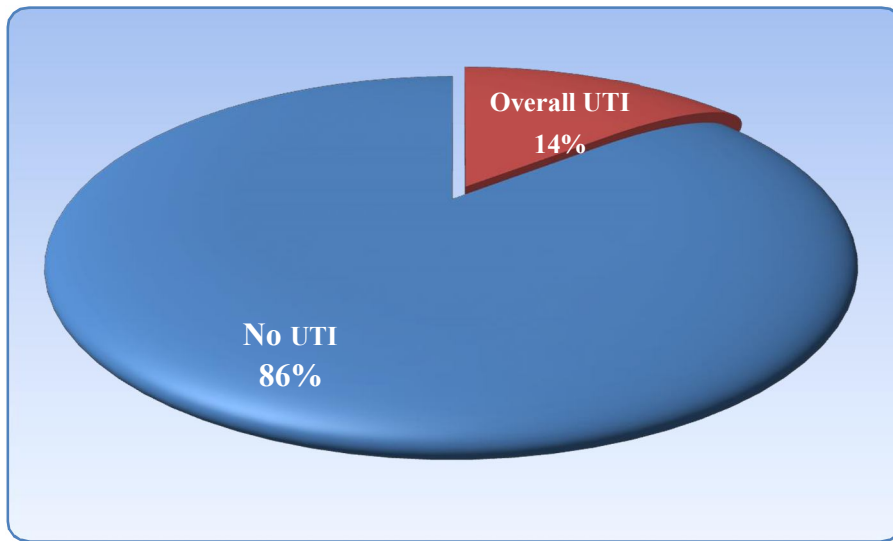


Figure 1: Percentage of the included pregnant women according to appearance of UTI, n=500.

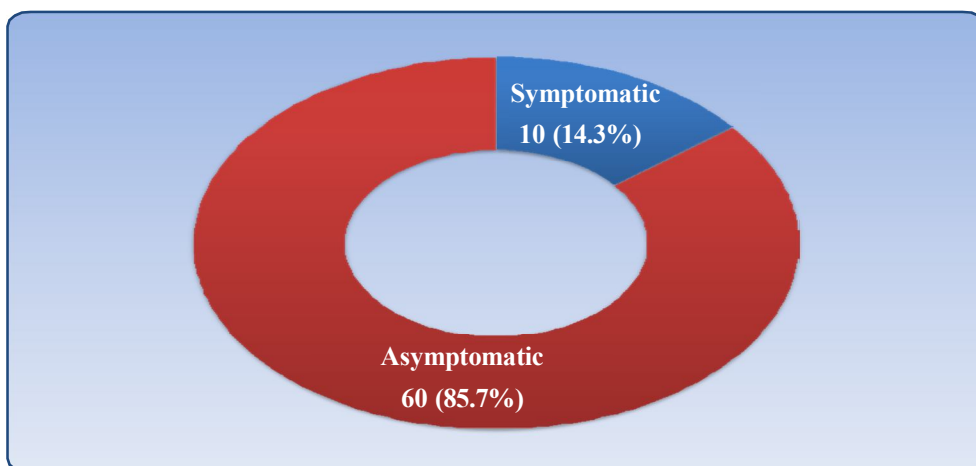


Figure 2: Percentage of the included pregnant women with UTI according to appearance of symptoms, n=70.

Table 1: Demographic characteristics of the study groups, n=500.

Parameters		Urinary tract infection		p-value ^a
		Overall UTI (n=70) Mean (±SD)	No UTI (n=430) Mean (±SD)	
Age (years)		28.7±(4.3)	29.1±(4.9)	0.520 (NS)
Gestational age (weeks)		9.3±(1.3)	9.7±(1.5)	0.012*
		No. (%)	No. (%)	p-value ^b
Parity	≤ 1	31 (44.3)	211 (49.1)	0.275 (NS)
	2 – 5	21 (30)	107 (24.9)	
	> 5	18 (25.7)	112 (26.0)	
Gravida	≤ 1	32 (45.7)	189 (44.0)	0.661 (NS)
	2 – 5	20 (28.6)	145 (33.7)	
	> 5	18 (25.7)	96 (22.3)	
Previous history of miscarriages	0	24 (34.3)	254 (59.1)	<0.001*
	1 – 2	34 (48.6)	101 (23.5)	
	≥ 3	12 (17.1)	75 (17.4)	

SD= Standard deviation, a: Pearson's chi-square, b: Independent t-test, NS: not significant, *Significant at $\alpha < 0.05$

Table 2: Comparison of positive diagnostic tests for UTI among included pregnant women according to the symptoms, n=500.

Positive tests	Symptomatic No. (%)	Asymptomatic No. (%)	Total	p- value
Urine Culture	10 (14.3)	60 (85.7)	70	0.35 ^P
Nitrite	6 (12.8)	41 (87.2)	47	0.72 ^F
Leukocyte esterase	4 (10.5)	34 (89.5)	38	0.49 ^F
Nitrite and leukocyte esterase	5 (13.9)	31 (86.1)	36	0.99 ^F
Microscopic examination	8 (20)	32 (80)	40	0.47 ^F

^P Pearson's chi-square, ^F Fisher's exact test

Table 3: Comparison between the results of N to that of the urine culture according to the symptoms, n=500.

Nitrite test	Urine Culture		Total No. (%)	SN	SP	PPV	NPV
	Positive No. (%)	Negative No. (%)					
Symptomatic							
Positive	5 (50)	1 (0.2)	6 (1.4)	50%	99.8%	83.3%	99.8%
Negative	5 (50)	429 (99.8)	434 (98.6)				
Total	10 (100)	430 (100)	440 (100)				
Asymptomatic							
Positive	41 (68.3)	0 (0)	41 (8.4)	68.3%	100%	100%	95.8%
Negative	19 (31.7)	430 (100)	449 (91.6)				
Total	60 (100)	430 (100)	490 (100)				

SN=Sensitivity, SP=Specificity, PPV=Positive predictive value, NPV=negative predictive value

Table 4: Comparison between the results of LE to that of the urine culture, n=500.

leukocyte esterase test	Urine Culture		Total No. (%)	SN	SP	PPV	NPV
	Positive No. (%)	Negative No. (%)					
Symptomatic							
Positive	3 (30)	1 (0.2)	4 (0.9)	30.0%	99.8%	75.0%	98.4%
Negative	7 (70)	429 (99.8)	436 (99.1)				
Total	10 (100)	430 (100)	440 (100)				
Asymptomatic							
Positive	32 (53.3)	2 (0.5)	34 (6.9)	53.3%	99.5%	94.1%	93.9%
Negative	28 (46.7)	428 (99.5)	456 (93.1)				
Total	60 (100)	430 (100)	490 (100)				

SN=Sensitivity, SP=Specificity, PPV=Positive predictive value, NPV=negative predictive value

Table 5: Comparison between the results of N and LE to that of the urine culture, n=500.

Nitrite and leukocyte test	Urine Culture		Total No. (%)	SN	SP	PPV	NPV
	Positive No. (%)	Negative No. (%)					
Symptomatic							
Positive	4 (40)	1 (0.2)	5 (1.1)	40.0%	99.8%	80.0%	98.6%
Negative	6 (60)	429 (99.8)	435 (98.9)				
Total	10 (100)	430 (100)	440 (100)				
Asymptomatic							
Positive	31 (51.7)	2 (0.5)	33 (6.7)	51.7%	99.5%	93.9%	93.7%
Negative	29 (48.3)	428 (99.5)	457 (93.3)				
Total	60 (100)	430 (100)	490 (100)				

SN=Sensitivity, SP=Specificity, PPV=Positive predictive value, NPV=negative predictive value

Discussion

The UTI is one of the most common health problems in the pregnancy because of the increased sex hormones beside anatomical and physiological changes during pregnancy^(7,8). Urine culture although remains the gold standard for screening and diagnosis of ABU in pregnant women, it is time consuming and patients may have difficulty in providing uncontaminated samples for testing. It would be useful to identify a rapid test with a high NPV value for ABU that could replace urine culture as a screening test. Urine cultures would then be performed only on patients with positive screening results⁽⁶⁾. The dipstick test with both the

parameters (N and LE) had the advantage of being simple, accurate and rapid take 2-5 minute for dipstick while 2-3 days for urine culture and less cost 3000-5000 Iraqi dinars (ID) for dipstick while 20000-30000 ID for urine culture, not requiring any expertise or equipment and must identify all positive cases, thus a sensitive test with a high NPV and SP is desirable (in Iraq)⁽⁶⁾.

The current study showed that the percentage of overall UTI was 14%, of them 12% had ABU. Mignini L et al 2009⁽⁹⁾ reported comparable level which was 15%, while Danilla et al 2002⁽¹⁰⁾ and paula et al 2005⁽¹¹⁾ reported lower incidence of over all UTI which was 10%. These differences in the reported incidence of over all UTI and ABU in pregnant women among the

above mentioned studies probably attributed to the difference in the climate, level of health care and educational levels between developed and developing countries⁽¹²⁾.

The current study showed that there was significant relationship between gestational age, previous history of miscarriages and UTI, while studies of Kovavisarach et al 2009⁽¹³⁾ and Demilie 2014⁽¹⁴⁾ showed no significant difference in the prevalence of ABU with respect to the trimester. The current study showed no significant relationship between maternal age, high parity and UTI, while Foxman study 2002⁽¹⁵⁾ showed that there was significant relationship between maternal age, high parity and UTI. Advanced maternal age ≥ 35 years was reported as risk factor for ABU in pregnancy⁽¹⁸⁾, which could be due to the fact that many women within this age are likely to have had many children before the present pregnancy and it has been reported that multiparity is a risk factor for acquiring ABU in pregnancy^(16,17).

For the purpose of diagnosis of symptomatic and ABU, the current study utilized different tests. So far the N test had 50% SN, 99.8% SP in symptomatic UTI and 68.3% SN, 100% SP in ABU, a result which is agree with Demilie et al study 2014⁽¹⁴⁾ were by the N test had 57.1% SN, 96.7% SP in symptomatic patients and 35.7% SN, 98% SP in ABU. Kacmaz et al study 2006⁽¹⁸⁾ had 60% SN, 99.2% SP were observed in N tests for ABU. In Kerbella study 2011⁽¹²⁾ found SN of N test in ABU was 66.6% and SP 97.7%. Sarah Coad et al study 2012⁽¹⁹⁾ had a higher result of N than current study with 81% SN for ABU. Also, Semeniuk H, 2004⁽²⁰⁾ had a higher result of N. SN 82.5% in ABU. Presence of N is highly specific for bacteriuria^(18,21). The N test is an indirect measure of nitrate reducing bacteria, which includes all Enterobacteriaceae, most non fermenters and gram negative cocci, provided the urine contains sufficient dietary nitrates and has been retained in the bladder for longer than 4 hrs. N test

showed high degree of agreement with culture results. The variability of the above mentioned studies for SN and SP of N test may be due to variable prevalence and variable causative organisms as not all uropathogens can reduce nitrates and some organisms convert nitrates to ammonia efficiently so that there is not enough N. during reduction to permit detection⁽⁶⁾.

For the LE test the current study had 30% SN, 99.8% SP in symptomatic and 53.3% SN, 99.5% SP in ABU, Demilie study 2014⁽¹⁴⁾ had higher SN 71%, SP 90% in symptomatic bacteriuria and 50% SN, 89.1% SP in ABU. Kerballa study 2011⁽¹²⁾ had 70.3% SN 88.5% SP in ABU. Kacmaz et al study 2006⁽¹⁸⁾ had 70% SN, 92.5% SP in ABU. Hosam M Hemedda et al study 2014⁽²²⁾ had 84% SN, 45.7% SP for ABU. The low sensitivity for pyuria suggests most probably but not always due to bladder colonization rather than acute infection, beside hypotonic urine or alkaline urine due to the presence of Proteus, Klebsiella and Pseudomonas can cause disintegration of pus cell. False positive findings may result from high urinary levels of ascorbic acid, albumin and phenazopyridine. Leukocyte has advantage to identify ABU even when the pus cells were disintegrated in the specimen. Also may be attributed to that the presence of leukocyturia does not correlate with bacteriuria, pyuria is not specific for UTI and may occur with other inflammatory disorders of genitourinary tract as vaginitis and infections with other uropathogens as Chlamydia and N. Gonorrhoea. Moreover, leukocyturia may continue even if bacteriuria had cleared^(18,23).

By using combined N and LE tests the current study had 51.7% SN and 99.5% SP in ABU, this agree with Mignini L et al 2009⁽⁹⁾ study had 53% SN and 99% SP for asymptomatic patients. While Masinde et al 2010⁽²⁴⁾ study were 38.9% SN and 86.7% SP in ABU had different opinion, their study showed combining test add no benefit and showed little agreement with

urine culture. Demilie et al 2014⁽¹⁴⁾ study had SN 22.9% and SP 99.1% for ABU.

In conclusion: the Nitrate test and LE tests had a high SP and NPV in comparable with urine culture for detection of ABU. But N test was more sensitive and specific than LE. In current study all positive cases of N test were positive for urine culture, so regard a good test for detection of ABU, it's accurate, rapid and less cost compared with the urine culture.

We recommend using N and LE tests instead of urine culture for early detection of ABU.

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