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Value of Rapid Nitrite Screening Test with or without Leukocyte Esterase Test in Detection of Asymptomatic Bacteriuria in Thai Pregnant Women

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ABSTRACT

Objective: To determinethe sensitivity, specificity, positive predictive value, and negative predictive value of the rapid dipstick test for detection of asymptomatic bacteriuria (ASB) in pregnancy, and to investigate contributing factors that could relate to having ASB in obstetric patients.

Materials and Methods: Clean-catch midstream urine samples were collected from 414 pregnant women for bacterial culture and rapid screening tests : the nitrite and leukocyte esterase (LE) dipsticks.

ResultsS: Thisstudy showed the sensitivity of the nitrite test being 29%, the LE test being 47%. Wheneither the nitrite or LE test being positive, the sensitivity increased tobe64%.Both the nitrite and LE test had high specificity of 99.2% and 92.4%, respectively, and high negative predictive value of 97.0% and 97.6%, respectively. We did not find any significant differences in contributing factors between patients that had a positive urine culture and those that had a negative urine culture.

Conclusion: A combination of nitrite and LE testing improved sensitivity for the detection of asymptomatic bacteriuria (ASB), but may not be suitable for screening of ASB. However, the combination of nitrite and LE testing would be useful in monitoring patients after antibiotic treatment for ASB due to the high specificity and high negative predictive value of the combined tests.

Keywords: Asymptomatic bacteriuria, pregnancy, nitrite test, leukocyte esterase (LE) test

Introduction

Urinary tract infections (UTIs) are the most common bacterial infections during pregnancy. Maternal and neonatal consequences of UTI can be devastating. Asymptomatic bacteriuria (ASB) in pregnant women is an established risk factor for serious complications including pyelonephritis, preterm delivery and low birth weight. The incidence of ASB during pregnancy varies from 2-7 percent[1].

Routine screening and treatment for ASB during pregnancy are recommended. U.S. Preventive Services Task Force (USPSTF2008), as well as the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists (AAP&ACOG2012)

recommend screening for ASB at 12-16 weeks' gestation or at the first prenatal visit by urine culture as the gold standard[1,2].

Urine culture may not be as cost-effective when the incidence is low. Moreover, it is time-consuming and not available for the certain groups ofpatients, especially in rural areas. Many rapid screening methods have been studied, because full bacterial analysis could be reserved for those patients on screening test positive. There are dipsticks of nitrite test and leukocyte esterase (LE) that are commonly used for the screening of ASB[3, 4]. The nitrite test detects urine nitrites from nitrate reductase organisms. A positive nitirite test usually indicates the cause of UTI being gram negative organisms, most commonly Escherichia coli. The LE test detects esterase released from white blood cells (WBCs) or leukocytes, representing the presence of WBCs in urine. A positive LE test usually indicates bacteriuria[5]. Many groups have shown that a combination of the nitrite and LE tests may provide as an excellent screening for the presence of UTIs[3, 4, 6, 7]. The objective of this prospective study was to determine the diagnostic performances of the nitrite and the LE test-compared with that of urine culture in detecting ASB and to investigate contributing factors that could relate to having ASB in obstetric patients.

Materials and Methods

Urine sample collection

The study was undertaken after obtaining Hospital Ethical Committee's approval and conducted from February 17th through June 30th, 2014 at the antenatal care clinic of the Department of Obstetrics and Gynecology, Chonburi Hospital, Chonburi, Thailand. Pregnant women who participated in the study were screened for bacteriuria at 12-16 weeks' gestation or at the first prenatal visit according to USPSTF2008 and AAP&ACOG2012 recommendation. Patients with renal disease, symptomatic UTI, current antibiotics use, glucosuria, and proteinuria were excluded. A detailed explanation of the study was given to the subjects prior to obtaining informed consent. Demographic parameters of subjects were recorded. These included age (years), gestational age (weeks), BMI (kg/m^2), education level, income, history of preterm delivery, abortion, sexual transmitted disease, UTIs, multiple partners, and diabetes mellitus. Subjects were instructed to collect a clean-catch midstream urine specimen with a standardized technique. After collecting the urine sample, each specimen was divided into two portions. One portion was sent to the laboratory for urine culture and processed within 1 hour. The other was tested with the nitrite and LEdipstick tests. Urine culture was considered as the gold standard for diagnosing bacteriuria.

Urine culture

Blood agar and MacConkey agar plates were swabbed with urine and incubated aerobically for 24 hours at 35°C. Urine culture was defined as being positive if 105 CFU/mL of a single potential uropathogen was detected. Subjects who had either a single or multiple pathogens with colony counts less than 105 CFU/mL, were not considered as having a positive culture.

Urine nitrite and LE dipstick testing

The specimen was evaluated for nitrite and leukocyte esterase activity using the Uriscan dipstick and analysed by the Uriscan Optima urine analyzer (YD Diagnostics), according to the manufacturer's instructions. The colour changes of the dipsticks were measured after 2 minutes of contact with urine.

Comparative analysis and parameters

The comparative diagnostic value of the various components of the dipstick test and culture were evaluated in terms of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with urine culture as the gold standard. We used SPSS17.0 software for statistical analysis. Continuous variables are represented as mean±standard deviation (SD) and analyzed using the independent sample t-test. Discrete variables are represented as percentages (%) and analyzed using the Fisher exact test for intergroup comparison. $P<0.05$ was considered statistically significant.

Sample size calculation

We used Diagnostic test study formula to calculate the sample size. The sensitivity used in this formula taken from Robertson A.'s study (combined Nitrite and LE test had the sensitivity of 92%)[3]. The incidence used here was 2%[1].

$Z\alpha = Z0.05/2 = 1.96$ (two tail)
$n = Z\alpha^2 PQ/d^2$
P = sensitivity = 0.92
Q = 1 - sensitivity = 0.08
d = acceptable error = 20%P
$n = (1.96)^2 (0.92)(0.08) / (20\%0.92)^2 = 8$
sample size = n / incidence
n / 2 = 400

Results

A total of 414 pregnant women were screened in the study, of whom 17 (4.1%) were identified by urine culture as having asymptomatic bacteriuria. All were tested by nitrite and leukocyte esterase dipstick tests. 5 of 17 isolates (29%) were identified by nitrite test, and 8 of 17 (47%) were identified by leukocyte esterase test. Table 1 represents the calculated sensitivity, specificity, PPV, and NPV of the two screening urine tests as compared to urine culture results. Nitrite and LE testshad a sensitivity of 29.4 and 47.0%, respectively, in detecting ASB. When diagnosis was made based on a positive result of either the nitrite or LE test, the sensitivity of the assay increased to 64.0%. Both the nitrite and LE tests had high specificity values (99.2% and 92.4%, respectively) and high negative predictive values (97.0% and 97.6%, respectively).

The specific microorganisms isolated from the urine cultures are listed in Table 2. The predominant organism isolated from the urine samples was E.coli (47% of all uropathogens).

Table 1. Diagnostic performances of rapid screening tests

Test	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Nitrite	29.4	99.2	62.5	97.0
Leukocyte esterase	47.0	92.4	21.0	97.6
Leukocyte esterase and Nitrite positive	11.7	99.5	50.0	96.0
Either Leukocyte esterase or Nitrite positive	64.0	92.2	26.0	95.5

Table 2. Uropathogens isolated by urine culture

Organism	Positive culture (n=17)	Positive Nitrite (n=5)	Positive Leukocyte esterase (n=8)
Escherichia coli	8	4	5
Staphylococcus	3	0	0
Group B streptococcus	2	0	1
Enterococci	2	0	0
Enterobacter	1	1	1
Candida krusei	1	0	1

We compared the baseline characteristics of patients that had a positive urine culture with those that had a negative urine culture in the following parameters: age, gestational age, parity, BMI, history of preterm delivery/abortion, educational levels, income and history of STDs, UTIs, multiple partners and DM (Table 3). We did not find any significant differences in these parameters between the two populations.

Table 3. The contributing factors of patients that had a positive urine culture with those that had a negative urine culture

Associated factors	N = 17 Positive culture	N = 397 Negative culture	P
Age, years (Means±SD)	23.7±7.3	24.9±6.5	0.453 [a]
Gestational age, weeks (Means±SD)	20.2±6.9	18.4±6.4	0.363 [a]
Parity Nulliparity	7 (41%)	168 (42%)	0.559 [b]
Multiparity	10 (59%)	229 (58%)	0
BMI, kg/m^2	24.9±4.4	23.3±4.6	0.280 [a]
History of preterm	0	20 (5%)	0.303 [b]
History of abortion	1(5.8%)	83 (21%)	0.677 [b]
Education non educated	0	31 (8%)	0.166 [b]
Primary school	5 (29%)	82 (20%)	
Secondary school	9 (53%)	256 (64%)	
at least Bachelor degree	3 (18%)	28 (7%)	
Income per month, Baht			
< 5,000	2 (12%)	27 (7%)	0.717 [b]
5,001 - 20,000	12 (70%)	285 (72%)	
20,001 - 50,000	3 (18%)	85 (21%)	
History of sexual transmitted disease	1 (5.8%)	8 (2%)	0.326 [b]
History of urinary tract infection	2 (12%)	24 (6%)	0.298 [b]
History of multiple partners	5 (29%)	61 (15%)	0.165 [b]
Diabetes mellitus	0	4 (1%)	0.845 [b]

[a] Analysis by independent sample t-test, [b] Analysis by Fisher exact test

Discussion

An early detection and treatment of ASB are considered important not only to prevent acute cystitis and acute pyelonephritis in pregnant women, but also to reduce preterm birth and fetal low birth weight. Many urine dipstick tests have been studied as an alternative testing. In this study, four main indices (sensitivity, specificity, PPV and NPV) of urine dipstick test results using nitrite, LE or a combination of nitrite and LE were compared with the gold standard of diagnosing ASB, urine culture.

An ideal screening test should be simple, rapid and accurate to identify all positive cases, therefore, the high sensitivity with the high specificity and NPV of the test is desirable. We found the sensitivity of nitrite dipstick test and LE dipstick test were 29% and 47%, respectively. These are considered low and are not suitable as a single screening test for ASB. Others have also reported low sensitivities using the same LE reagent dipstick test (Uriscreen)[8]. However, there were also groups that reported higher sensitivity of the LE test[4]. This could be contributed from usage of different LE reagent dipstick tests.

The sensitivity of diagnosing ASB based on having a positive result for either nitrite or LE dipstick test was 64%, which gave higher sensitivity than diagnosis based on using only either nitrite or LE as individual tests. These were also observed in other studies with sensitivities between 68-92%[3, 4, 6-8].

A specificity of a diagnostic test is the probability that a patient without significant bacteriuria will have a negative result. A negative predictive value (NPV) of a test is the probability that an individual with a negative result does not have significant bacteriuria. Both nitrite and LE tests had high specificity (99.2% and 92.4%, respectively) and NPV (97.0% and 97.6%, respectively). These results are useful in monitoring patients after antibiotic treatment for ASB in place of repeated urine culture that will minimize the health care costs. A positive predictive value (PPV) is the probability that an individual with a positive test has significant bacteriuria. The PPV of the nitrite test, LE test and a combination of both tests were low (62.5%, 21.0% and 26.0%, respectively). Therefore, treatment should not be prescribed based on results from dipstick tests using nitrite and LE, but rather on a positive urine culture.

The low sensitivity of the nitrite dipstick test implies a high false negative value of the test. We believe that there may be bacteria that are present in urine that do not convert nitrate to nitrite, such as group B Streptococcus, Enterococci, Staphylococci and Pseudomonas species; of which bacteriuria caused from these bacteria species would not be detected using the urine nitrite dipstick test. Furthermore, urine prior to collection did not remain in the bladder for at least 4 hours and could contribute to inadequacy of time of bacteria to convert nitrate to nitrite[5]. Also, different diets may alter levels of nitrate in the urine, thus, resulting in lower amounts of urine nitrate to be converted to nitrite, despite the presence of bacteriuria[9, 10].

One of the reasons for a false negative result of the LE test is the presence of proteinuria and glucosuria (neutrophils are unable to release esterase, various drugs and chemicals include ascorbic acid, oxalic acid, cephalexin, cephalothin, gentamycin, and tetracyclin)[5]. However, patients with proteinuria and glucosuria were excluded from the study population.

False positive result of the LE test can be due to contamination of the urine with vaginal discharge or treatment with certain antibiotics (imipenem, meropenem, and clavulanic acid)[5]. In our study, each patient was carefully instructed regarding urine sample collection by a single nurse to clean the vulvar area prior to collecting the urine sample to avoid contamination. One of the limitations of the nitrite test, the first voided morning urine specimen is most suitable for sample testing, but sample collection was not practical in all patient populations. Also, a higher number of samples with a positive urine culture may be required to make a more comparable comparison of sensitivity and specificity between the urine culture positive population and urine culture negative population.

In conclusion, a combination of the nitrite and LE testing improved sensitivity of diagnosis of ASB when compared to using one or the other. However, the sensitivity of combined testing is insufficient to use as a screening tool for ASB and unable to replace urine culture. Due to the high specificity and high negative predictive values, the nitrite and LE tests were useful in monitoring patients after antibiotic treatment for ASB. Further studies with a larger sample population of urine positive culture are required in order to draw conclusions or perhaps a new candidate substrate is needed to replace nitrite and LE. Dipstick tests are easier to perform, very cheap, affordable by most patients as a screening tool and are independent of laboratory facilities. However, from our study, a positive mono-organism urine culture still remains the gold standard for diagnosis of ASB.

Conflict of interest

The authors declare that there was no conflict of interest.

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การประเมินค่าการตรวจคัดกรองไนไตรต์และลิวโคไซต์ เอสเตอเรส เพื่อตรวจหาภาวะติดเชื้อในปัสสาวะที่ไม่แสดงอาการในสตรีตั้งครรภ์ไทย

พชรพร ศรีเดช, พิมพิกา ตันสุภสวัสดิกุล

วัตถุประสงค์ *: การวิจัยเพื่อประเมินความไว ความจำเพาะค่าทำนายผลบวก ค่าทำนายผลลบของแถบทดสอบปัสสาวะไนไตรต์ (Nitrite) และลิวโคไซต์ เอสเตอเรส (Leukocyte esterase) ในการคัดกรองภาวะติดเชื้อในปัสสาวะที่ไม่แสดงอาการในสตรีตั้งครรภ์และหาปัจจัยที่เกี่ยวข้องกับภาวะติดเชื้อในปัสสาวะที่ไม่แสดงอาการในสตรีตั้งครรภ์*

รูปแบบการวิจัย *: Diagnostictestst udy*

วิธีการวิจัย *: สตรีตั้งครรภ์ 414 คน ที่แผนกฝากครรภ์ โรงพยาบาลชลบุรีผู้เข้าร่วมการวิจัย ทำการเก็บปัสสาวะส่งตรวจ 2 ส่วนคือตรวจหา Nitrite และ Leukocyte esterase ด้วยแผ่นทดสอบปัสสาวะ และส่งตรวจเพาะเชื้อจากปัสสาวะ*

ผลการวิจัย *: สตรีตั้งครรภ์ 17 คน จาก 414 คน (4.1%) ได้รับการวินิจฉัยว่ามีภาวะติดเชื้อในปัสสาวะที่ไม่แสดงอาการจาก ผลการเพาะเชื้อจากปัสสาวะเป็นบวก Nitrite และ Leukocyte esterase dipstick test มีค่าความไวเท่ากับ 29%, 47% ตามลำดับ เมื่อใช้ผลของ 2 การทดสอบร่วมกัน (Nitrite หรือ Leukocyte esterase test ผลอันใดอันหนึ่งเป็นบวก) ค่าความไวเพิ่มขึ้นเป็น 64% ทั้ง Nitrite และ Leukocyte esterase test มีค่าความจำเพาะสูง คือ 99.2%, 92.4% ตามลำดับ และมีค่าทำนายผลลบสูง คือ 97.0%, 97.6% ตามลำดับ การวิจัยนี้ไม่พบความแตกต่างอย่างมีนัยสำคัญของปัจจัยต่างๆ ระหว่างกลุ่มสตรีตั้งครรภ์ที่มีผลเพาะเชื้อจากปัสสาวะเป็นบวกและกลุ่มที่ผลเป็นลบ*

อภิปรายผลวิจัย *: การใช้ Nitrite และ Leukocyte esterase test ทดสอบร่วมกัน ช่วยเพิ่มค่าความไวให้สูงขึ้น แต่ยังไม่เหมาะสมที่จะนำมาใช้เป็นการคัดกรองหลักของการตรวจหาภาวะติดเชื้อในปัสสาวะที่ไม่แสดงอาการในสตรีตั้งครรภ์ไทยเนื่องจากทั้ง Nitrite และ Leukocyte esterase test มีค่าความจำเพาะและค่าทำนายผลลบสูง ดังนั้นการทดสอบนี้อาจมีประโยชน์ในการนำมาใช้ตรวจติดตามผลปัสสาวะหลังการรักษาภาวะติดเชื้อในทางเดินปัสสาวะได้*