ORIGINAL ARTICLE

DIAGNOSTIC PERFORMANCE OF REAGENT STRIPS AND MICROSCOPY FOR THE DIAGNOSIS OF URINARY TRACT INFECTION AMONG PREGNANT WOMEN ATTHE UNIVERSITY OF GONDAR COMPREHENSIVE SPECIALIZED HOSPITAL, NORTHWEST ETHIOPIA A CROSS SECTIONAL STUDY

*Getachew Belay,[3] Mulat Dagnew[1], Awgechaw ketema[3], Habtalem mequanint[3],Alebachew Fasil[2], Belete Biadgo[2], HabtamuWondifraw Baynes[2],Teklay Gebrecherkos[1]**

ABSTRACT

Background: *Pregnant women are susceptible to urinary tract infections due to changes in their urinary tract because the uterus lies directly on top of the bladder. The prediction of how to screen pregnant women for bacteriuriais a balance between the cost of screening sensitivity and specificity. The aim of this study was to evaluate the diagnostic accuracy of rapid dipstick tests and microscopy to predict UTI in pregnancy by comparing urine culture as a gold standard.*
Method: *A hospital based comparative study was conducted from March-April 2015 at Gondar University referral hospital. About 10-15ml of freshly voided midstream urine samples were collected from each participant. All samples were processed for urine dipstick tests, microscopic examination and cultured on CLED and identified by the conventional biochemical tests at the University of Gondar comprehensive specialized referral hospital Microbiology laboratory. Data was summarized and analyzed using SPSS version 20statistical software. The sensitivity, specificity, positive predictive and negative predictive and Kappa values were calculated to measure the diagnostic utility of urine diagnostic tests in the detection of UTI in pregnant women.*
Result: *A total of 282 pregnant women of whom11.7% (33/282) had ≥10^5colonies/ml on culture; sensitivity, specificity, positive and negative predictive values were 39.39% ,73.38%,16.45% and 90.10% for leukocyte esterase, respectively, and 18.18% ,96.77% ,42.86% and 89.90% for nitrite were included. Moreover, sensitivity, specificity, PPV and NPV of bacterial microscopy were 36.36%, 78.25%, 18.20% and 90.30%, respectively.*
Conclusion: *This study revealed many false positive and negative results of urine dipstick and microscopy when compared with the gold standard culture method. The low sensitivity and positive predictive values of urine dipstick and microscopy tests proved that culture should be used for the diagnosis of urinary tract infections.*

Key words: *Urinary Tract Infection; Bacterial culture; Leukocyte esterase; White Blood Cells.*

INTRODUCTION

Urinary tract infection (UTI) is caused by the presence and growth of microorganisms anywhere in the urinary tract. The infection occurs when bacteria inter from the digestive tract ascending the opening of the urethra and begins to multiply to cause infection either as symptomatic or asymptomatic (1).Asymptomatic bacteriuria (ASB) is characterized by bacteriuria without clinical symptom manifestations (2).

In pregnant women, untreated ASB leads to the development of pyelonephritis, intrauterine growth

[1]Department of Medical Microbiology, School of Biomedical and Laboratory sciences, College of Medicine and Health Sciences (CMHS), University of Gondar (UOG), P.O. box 196, Gondar, Ethiopia, [2]Department of Clinical chemistry, School of Biomedical and Laboratory sciences, College of Medicine and Health Sciences (CMHS), University of Gondar (UOG), P.O. box 196, Gondar, Ethiopia, [4]University of Gondar, College of Medicine and Health Sciences School of Biomedical and Laboratory Sciences
****Correspondence author:*** *Teklay Gebrecherkos, Department of Medical Microbiology, CMHS, UOG, Ethiopia, P.O. box 196, Tel. +251922795314; e-mail: estiftg17@gmail.com*

retardation, pre-term birth and low birth-weight infants (3, 4). Pregnant women are susceptible to UTI due to changes in the urinary tract because the uterus lies directly on top of the bladder. When the uterus grows, its increased weight can block the drainage urine from the bladder and causes infections (5).

The diagnosis of UTI is based on the isolation of a single colony of bacteria in a properly collected specimen of urine from without signs or symptoms of UTI (6).Clinical examination is less valuable in UTI diagnosis because in most cases the clinical signs are not supportive; so, it is important to do laboratory urinalysis tests to have a conclusive diagnosis. Until now urine culture has been the gold standard method for the diagnosis of UTI, and it has been used as a screening procedure for urinary tract infections in pregnancy (7). While Reagent Strip Testing (RST) is a simple and rapid test that can be done at bedsides of patients, it can minimize the number of samples which are sent for urine culture and make the patient care quick and better if it is effective (8,9).

Cysteine lactose electrolyte deficient (CLED) media in the form of Semi-quantitative culture was used as the gold standard for screening test UTI (10, 11).Urine Microscopy examination for (bacteria & white blood cells) is the other semi-quantitative diagnostic method to detect UTI in pregnant women that have the advantage of detecting bacteria which are non-nitrate producers, but it requires skilled manpower and electric supply (7).

Bacteriuria can be detected chemically using reagent strips when bacteria produce nitrite from nitrate. The biochemical reaction detected by nitrite test is related to the family Enterobacteriaceae (the pathogens responsible for UTIs), but the test has its own limitations because nitrite production is not associated with some pathogens, like *S.saprophyticus*, Pseudomonas species, or Enterococci (12).

Therefore, evaluating the diagnostic accuracy of rapid dipstick tests and microscopy to predict UTI in pregnancy by comparing urine culture as gold standard may contribute to the improvement of diagnosis, treatment and prevention of UTI infections in Gondar in particular and in Ethiopia in general. Hence, this study was designed to evaluate the diagnostic accuracy of rapid dipstick tests and microscopy to predict UTI in pregnancy by comparing to urine culture as the gold standard. As a result, the purpose of this study was to evaluate the detection capability of reagent strips and microscopy in the diagnosis of urinary tract infections among pregnant women at the University of Gondar comprehensive specialized referral hospital, northwest Ethiopia.

MATERIALS AND METHODS

Study Area and design: A cross-sectional study was conducted at the University of Gondar comprehensive specialized referral hospital from March to April 2015.Gondar is the capital of North Gondar zone in Amhara region. It is located 737km from Addis Ababa, the capital of Ethiopia, and 173km from the regional capital of Amhara, Bahir Dar. It has 21 kebeles with a projected population of 300,000.The town has historical sites, like the Fasil Castle and churches which contribute to income from tourism. In addition, the town has one teaching hospital and many government and private health institutions.

Study population, sample size and sampling technique: All pregnant women who visited the Antenatal Care clinic of the University of Gondar comprehensive specialized referral hospital at the time of the study were included. The sample size was calculated using the single population proportion formula: $N = z^2 p (1-p)/w^2$, where N = the number of pregnant women, Z = standard normal distribution value at 95

% CI which is 1.96, P = the prevalence of pulmonary tuberculosis among prison inmates (17.4%, previous report), W = the margin of error, taken as 5 %. Accordingly, the sample size calculated was 282.The systematic random sampling technique was used to select study participants.

Data collection technique and laboratory methods: Once eligible pregnant women were recruited for the study, socio-demographic characteristics were collected using structured questionee. To begin with, 10-15 ml of midstream urine was collected using sterile, wide mouthed glass bottles with screw cap tops; then, it was processed in the laboratory 30 minutes after collection.

Urine samples immediately after collection were poured into CLED (Cysteine Lysine-Electrolyte-Deficient) media and incubated at 37^0C for 24 hours. If significant bacteriuria were grown according to the standard, it was sub cultured into BAP, MAC and CAP (Oxoid Ltd Basingstoke, Hampshire, UK) and incubated at 37 ^{0}c for 24 hrs. The identification of microorganisms was based on their gram reactions, colony morphology and biochemical characteristics. Gram positive bacteria were tested by biochemicaly (Oxoid, LTD), like Catalase, Coagulase, and Novobiocin test. On the other hand, Gram negative bacteria were tested using a series of biochemical tests (Oxoid, LTD), like hydrogen sulphide production (H_2S), indole, urease, citrate, LDC (Lysine decarboxylase), production of gas and carbohydrate fermentation using triple sugar iron agar (TSI) (Oxoid Ltd Basingstoke, Hampshire, UK). The reagent strip test was performed by dipping the test strip in to a urine specimen which is immediately removed to drain off excess urine on a paper towel; next, the test strip was examined for color change and interpreted according to the manufacturer. Finally, microscopy was done on 10 ml of mixed urine specimen in a sterile conical test tube centrifuged at a speed of 1500-2000 for 3-5 minute. Then, after discarding the supernatant, a drop of urine sediment on a sterile slide was examined microscopically. WBC>5/HPF was reported as positive for bacterial urinary tract infection and as normal if the white blood cells were less than or equal to five of the high power fields.

Quality control: The questionnaire was originally prepared in English and translated to Amharic and back to English to maintain the consistency of the items. Pre-testing of the questionnaire was done for completeness and appropriateness before data collection and a half day training was given for data collectors. The reliability of the findings was guaranteed by implementing quality control measures throughout the laboratory work. The reference strains used as controls were *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *Staphylococcusaureus* (ATCC 25923).

Data analysis: Data was entered into Epi-Info version 3.5.4 and exported to SPSS version 20 for analysis. The sensitivity, specificity, positive and negative predictive values were calculated for microscopic urine analysis, leukocyte esterase and nitrites to measure their diagnostic utility in the diagnosis of bacteriuria compared to the gold standard urine culture. Descriptive statistics, chi-square, receptor operating curve (ROC) and logistic regression were used for the analysis of different variables. A value of $P \leq 0.05$ was considered significant.

Ethical consideration: Ethical clearance was obtained from the Ethical Review Committee of the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, the University of Gondar. Permission letter was obtained from the University of Gondar referral hospital Administration Office and written informed consent was also secured from each study participants. All information obtained from each participant was kept confidential.

The laboratory results of the participants were sent to their doctors for appropriate treatment.

RESULTS

Socio-demographic characteristics: A total of 282 pregnant women were includedand the predominant age group was 25-34(53.2%). Around 40.1% of the individuals were at second stage of pregnancy. Themajority, 96(34%),were unable to read and write (Table 1).

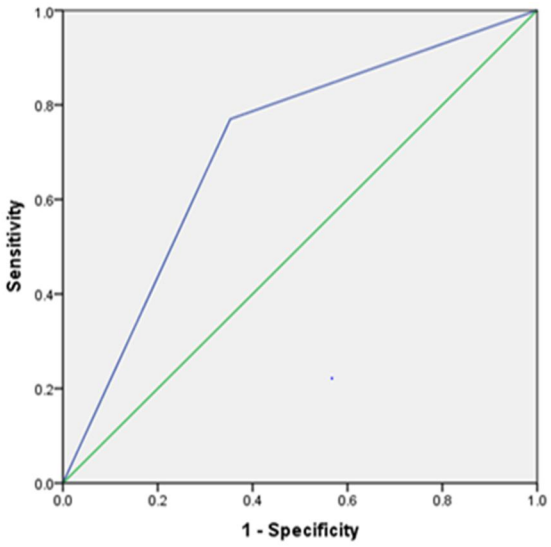
Performance characteristics of different methods for the diagnosis of UTI: Of the participants, 11.7% (33/282) had urine culture with $\geq 10^5$colonies/ml; of the282 samples examined using dipstick, 28% of the individuals were positive for leukocyte esterase,5% for nitrite and 23.4%for bacterial microscopy. The PPV and NPV values of leukocyteesterase were 16.45% and 90.10%,respectively, and the sensitivity, PPV and NPV of nitrite were18.18%, 42.86% and 89.90%,respectively (Table 2).

Leukocyteesterase was a variable that presented with the highest sensitivity (39.39%), while nitrite presented with the lowest (18.18%) and nitrite with the highest specificity (96.77%). Bacteria and microscopy also presented a high specificity and Leukocyte Esterase was with the lowest specificity (73.38%).Nitrite also had the highest positive predictive value, but almost all of the parameters used had the highest negative predictive values (around 90%). WBC microscopy and bacteriuriahad AUC of 0.393 (.310-.477)vs. 0.336 (.168- .504)], respectively. Leukocyte esterase had 0.709 (.610-.807) (Figure 1, Tables 2 &3).

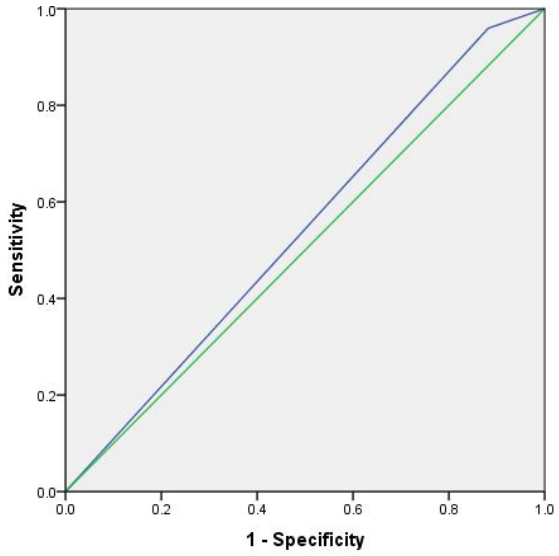
Table 1: Socio-demographic characteristics of pregnant women attending antenatal care follow up at the University of Gondar hospital, northeast Ethiopia 2015

Variable	Number	Percentage (%)
Age		
15-24	92	32.6%
25-34	150	53.2%
>=35	40	14.2%
Education		
Unable to read & write	96	34%
Read and write	36	12.8%
High school	53	18.8%
Diploma	71	25.2%
Degree	26	9.2%
Income/month		
<=500	75	26.6%
501-1000	114	40.4%
1001-2000	66	23.4%
>=2001	27	9.6%
Gestational stage		
<=3	66	23.4%
4-6	113	40.1%
7-9	103	36.5%

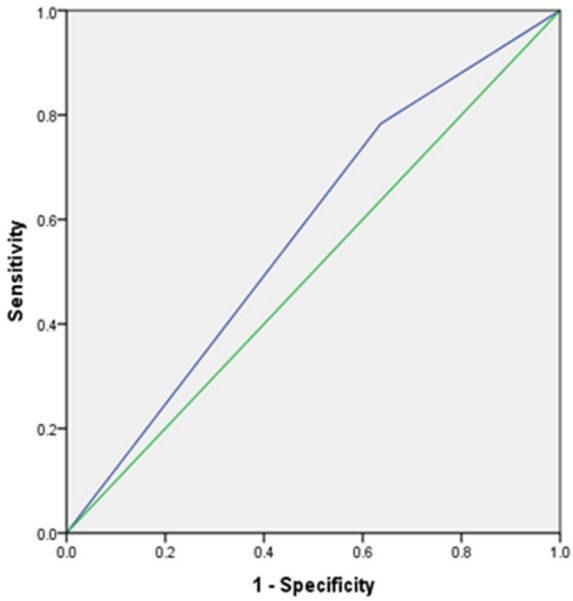
Key: Micro: Microscopy, LE: Leukocyte esterase Pos: Positive, PPV: Positive predictive value, Neg: Negative, NPV: Negative predictive value, Sens: Sensitivity, Spec: Specificity, WBCM: White blood cell microscopy



Bacteria Microscopy



WBC Microscopy



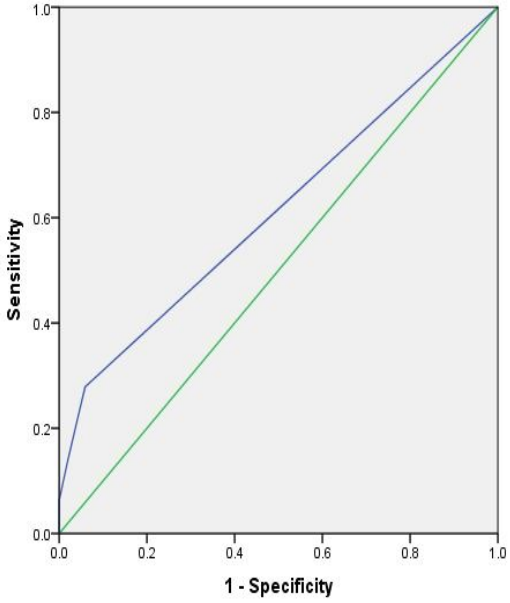


Figure 1: *Receiver operating characteristic (ROC) curve with 95% CIs of the leukocyte Esterase, nitrite, bacterial microscopy, WBC Microscopy, Bacteria UTI, in women attending ANC follow ups at the University of Gondar hospital 2015*

Table 2: Performance result of methods compared to the gold standard urine culture for the diagnosis of urinary tract infections among pregnant women attending ANC follow ups at the University of Gondar hospi-

Methods	**Culture**			**Sens**	**Spec**	**PPV**	**NPV**
	Pos	**Neg**	**Total**	**(%)**	**(%)**	**(%)**	**(%)**
Pos	13	66	79				
LENeg	20	183	203	79	39.39	73.38	90.10
Total	33	249	282				
NitritePos	6	8	14				
Neg	27	241	268	18.18	96.77	42.86	89.90
Total	33	249	282				
Pos	12	54	66				
MicroNeg	21	195	216	36.36	78.25	18.20	90.30
Total	33	249	282				
WBCM Pos	9	26	35				
Neg	24	223	247	27.27	89.50	31.57	90.20
Total	33	249	282				

Table 3: Sensitivity, specificity and AUCs for the positive results of the, leukocyte esterase, nitrite tests and parameters of WBC microscopy, bacteria microscopy, in diagnosing UTIs compared to gold standard urine culture among women attending ANC 2015

Tests and Parameters	**Sensitivity**	**Specificity**	**AUC**	**95% CI**	**P- Value**
Leukocyte Esterase	39.39	73.38	0.709	0.610-0.807	0.000
Nitrite	18.18	96.77	0.539	0.430-0.647	0.465
Bacteria Microscopy	36.36	78.25	0.573	0.465-0.682	0.171
Microscopy WBC	27.27	89.5	0.393	0.310-0.477	0.009
Bacteria UTI	31.24	75.65	0.336	0.168-0.504	0.039

Key: *CI: confidence interval AUC: area under the curve.*

Leucocyte Esterase Nitrite: In the logistic regression, there was a medium correlation between the gold standard culture and urine dipstick for nitrite with AOR7.2(0.056, 0.633) and p-value of 0.007 (Table 4).

Bacterial isolates: Out of the 282 samples examined, 33(11.7%) organisms were isolated in culture media and *E. coli* accounted for15 (45.4%)of the isolates, followed by *Staphylococcus saprophyticus*7 (21.2%) and *Staphylococcus aureus* (Table 5).

Table 4: The association between culture with leukocyte esterase, nitrite tests and parameters of WBC microscopy, bacteria microscopy among pregnant women attending ANC follow up at the University of Gondar hospital 2015.

Methods	**Culture**			
	COR (95% CI)	**P-value**	**AOR (95% CI)**	**P-value**
Leukocyte esterase	2.3(.845,3.82)	.125		
Nitrite	10.8(.048,.463)	.001	7.2(.056,.633)	.007
Bacteria microscopy	3.4(.95,4.5)	.065		
WBC microscopy	6.9(1.35,7.65)	.008	3.6(.97,6.78)	.057

Key: *COR: Crude Odds Ratio AOR:Adjusted Odds Ratio CI: Confidence Interval*

Table 5: Bacterial isolates among pregnant women attending ANC follow up at the University of Gondar hospital 2015

Organism	**Number of isolates**	**Percentage (%)**
E.coli	15	45.5%
S.saprophyticus	7	1.2%
S. aureus	5	15.2%
k. pneumonia	4	2.1%
P. mirabilis	2	6.1%
Total	33	100%

DISCUSSION

A total of 282 pregnant women were the target group for assessing the test performance of dipstick and microscope for biochemical; as well as morphological detection ability by comparing with culture as the gold standard.

In this study, the sensitivity and specificity of urine dipstick for leukocyte esterase was 39.39% vs. 73.38% which is low compared with a study in northwest India (14) which was 73.5% vs. 58.5% for sensitivity and specificity, respectively. This difference might be due to the fact that short stay of urine in the bladder and delay of samples may contribute to releasing correct results. A similar study conducted in Turkey on 250 patients showed that the sensitivity and specificity of bacteria in microscopy were 91% vs. 68%, whereas in leucocytes esterase they were 80% vs. 60%.Values were 84% for leucocytes esterase dipsticks and 93% for bacterial microscopy.

PPV was 52% vs. 61% for leucocytes esterase and bacteriuria(15). But in our study the sensitivity, specificity, NPV and PPV of leucocytes esterase were39.39%,73.38%, 90.10% and 16.45%,whereasa report from elsewhere showed that the sensitivity,

specificity, NPV and PPV of bacterial microscopy were36, 36%, 78.25%, 90.30%, and18.20%, respectively, which were lower than finding of the present study. The difference might be due to time of reading or aging of samples during the processes.

When we compare the sensitivity and specificity of leukocyte esterase and nitrite of the current study with that of a study conducted in Bahir Dar - Ethiopia, ours was by 10.61% and 17.52% and nitrite 15.82% and 1.23% lower, respectively (21).The difference from our result might be due to inappropriate material storage, technical error and personal bias.

In this study, the sensitivity and specificity of nitrite was 18.2% and 96.8%, respectively. The poor correlation of sensitivity between the culture and nitrite tests could be the result of non-nitrite reducing bacteria, such as *S.aureus, S. saprophyticus* and Enterococcus species or patients in vegetable free diet which is an important source of nitrite. On the other hand, there was no false positive or negative result. Ethiopian health care services use dipstick for routine diagnostic purposes when infections or chronic diseases are suspected even though this test has limitations in accuracy as recent studies indicate (16, 17).

This study also noted the poor detection capacity of microscopy for bacteria and white blood cells. In this study, the sensitivity of leukocyte esterase, bacteria and white blood cells in microscopy were 39.39%, 36.36%and 27.27%, respectively. The sensitivity of leukocyte esterase and microscope for bacteria was similar. But the sensitivity of leukocyte esterase was better than that of white blood cells in microscopy. This might be due to deformation, disintegration, lab personnel bias or lyses of white blood cells during the collection or examination of urine samples.

The area under the curve is one means of predicting the degree of diagnostic accuracy. In this study, the AUC of WBC microscopy and bacteriuria was 0.393 (0.310-0.477) vs. 0.336 (0.168-0 .504), respectively, which is different from that of a study done atMaxima Medical Centre, Clinical Laboratory(22), while leukocyte esterase had a similar finding which was 0.709(0.610-0.807).In the logistic regression analysis, there was a medium correlation between the gold standard culture and urine dipstick for nitrite with AOR7.2(0.056, 0.633) and p-value of 0.007 in the multivariate analysis which indicated that diagnosing using dipstick for nitrite had a comparable result as culture unlike leukocyte esterase, WBC microscopy and bacteria microscopy which have a poor correlation in line with the finding of a study done by Eigbefoh JO.et al (23).

Conclusion and recommendations: Using dipstick for nitrite and WBC microscopy for screening UTI was associated with low sensitivity and high specificity. There was a correlation between urine dipstick for nitrite and culture for diagnosing UTI. This is useful for poor resource settings, especially in the third world, where there is insufficient trained personnel and equipment for urine culture. But if there is enough resource, the study reveals that the low sensitivity and the positive predictive value of urine dipstick test and microscopy prove that culture should be used for the diagnosis of UTI.

Ethical Approval: It was approved by the Ethical Review Committee of the University of Gondar, School of Biomedical and Laboratory Sciences. And patients participating in the study provided written informed consent.

Competing interests: There is no competing interest among authors.

Authors' contributions: All the authors contribute their own role starting from drafting of the proposal to preparing of the manuscript and final write up.

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