



SENSITIVITY AND SPECIFICITY OF NITRATE REDUCTASE AND LEUCOCYTES ESTERASE AS RAPID SCREENING TESTS FOR DIAGNOSIS URINARY TRACT INFECTIONS.

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ABSTRACT

BACKGROUND: Urine analysis particularly leukocyte esterase & nitrate reductase tests are often used to determine whether a culture will be performed or treatment is needed. This study was conducted to assess the usefulness of leukocyte esterase, nitrate reductase and quantitative microscopic urine wet mount examination in rapidly diagnosing urinary tract infections (UTI).

METHODS: Ninety nine urine samples were collected from adult patients presenting to Asia Center, from March to May 2010. The samples were tested by culture on Cysteine Lactose Electrolyte Deficient (CLED) medium. Microscopic examination of urine for significant pyuria, leukocyte esterase test (LET) and nitrite test (NT) were also performed. Culture was used as gold standard to evaluate the performance of direct microscopy and dipstick tests (1).

RESULTS: Nitrite test & leukocyte esterase test were found as specific for diagnosing UTI revealed 87.6% & 89.8% respectively. The sensitivity leukocytes esterase as simple diagnostic test for UTI was 50%, where as of nitrite test was 14.2%. The positive predictive value of a positive leukocyte esterase and Nitrite reductase tests were 66.6% and 8.03% respectively with high negative predictive value (91.02%, 97.7%) respectively.

CONCLUSION: Urinary dipstick testing for UTI in the near patient setting is a valuable resource to screen out negative urine specimens at the point of care. Negative result for one of test has a sufficient predictive value to exclude disease, and when both test results are positive there is sufficient evidence to rule in infection.

INTRODUCTION:

Urinary tract infections (UTIs) are the most common bacterial infection, accounting for 25% of all infections^(1,2). It is a significant health problem, both in community and hospital - based settings. It is estimated that 150 million UTIs occur yearly world-wide and are estimated to account for over 7 million office visits per year. In the health-care setting, approximately 40% of all nosocomial infections are UTIs^(3,4).⁽¹⁾⁽²⁾ and it is not surprising that urine specimens make up a large proportion of the samples submitted to a routine diagnostic laboratory⁽³⁾. A large laboratory may examine 200–300 urine samples each day, This heavy workload reflects the frequency of UTI both in general practice and in hospital settings. Many of these specimens will show no evidence of infection and several methods can be used to screen out negative samples given the limitations of the method used⁽³⁾.

Rapid diagnostic tests can screen out negative samples and can save valuable time and money, so this

study was conducted to assess the usefulness of leukocyte esterase, nitrate reductase and quantitative microscopic urine wet mount examination in rapidly diagnosing urinary tract infections (UTI).

Urine dipstick consists of chemically treated paper, which displays different colours indicating the presence of leukocyte esterase, nitrites, blood, and protein when dipped into urine sample, it is cheap, easy, readily accessible, gives instant results and can be competently carried out and interpreted by anyone^(6,7).

The leukocyte esterase relies on the reaction of leukocyte esterase produced by neutrophils and a positive result suggests pyuria associated with UTI⁽⁷⁾.

False negative results may be caused by contamination with vaginal discharge, elevated urine glucose or oxalic acid concentrations, in patients taking tetracycline, cephalexin or gentamicin, in urine with low specific gravity⁽⁶⁾. The detection of pyuria by this test may be more accurate than microscopy because enzyme activity is still retained when white cells have disintegrated⁽³⁾.

Many Gram-negative and some Gram-positive bacteria are capable reduces nitrate to nitrite and a positive test suggests their presence in significant numbers (i.e. more than 10^4 / ml). Negative result does not rule out a UTI ⁽⁶⁾.

False negative results can occur when bladder incubation time is shortened (less than 4 hours) gives the enzyme less time to act, in the absence of dietary nitrate, in the presence of nitrate-reductase negative organisms, when urine Specific gravity is elevated, pH is less than 6.0, and in the presence of urobilinogen and urinary vitamin C ^(7,8).

MATERIAL AND METHODS:

In this study 99 patients with symptoms and signs of UTI admitted to Asia hospitals, from March to May 2012 were enrolled.

The inclusion criteria were the patients with dysuria, urgency, and urinary frequency, loin pain, and suprapubic pain, and clinical suspicion of urinary tract infection.

Data were collected using structural interviewing questionnaire that covered general information & specific information (signs and symptoms).

Clean catch mid-stream urine specimen was collected from each patient in sterile, screw capped, wide neck, and leak proof disposable plastic container. A modified semi-quantitative technique was employed by spreading a standard (0.001ml) bacteriological loopfull of urine over the surface of Cysteine Lactose Electrolyte Deficient (CLED) agar plate. The plates then incubated aerobically at 37°C for 24 hours. The number of bacterial colonies were counted and multiplied by 100 to give an estimate of the number of bacteria present per milliliter of urine. A significant bacterial count was taken as any count equal to or in excess of 10^5 per milliliter ⁽⁹⁾.

The strip was immersed in fresh urine for few seconds then removed. The test area was compared to corresponding color chart on the bottle label within 60 seconds. A positive result of nitrite was indicated with pink color, while a positive result of leukocyte esterase was indicated by appearance of mauve color.

About 12 ml of urine was centrifuged at 2000 rpm for minute, and then 1 drop of urine were placed on a clean dry slide & covered with cover glass. The slide was examined by light microscope using high power field for presence of pus cells, red blood cells and bacteria.

A bacterial count of 10^5 organisms /ml or more CFU (100 or more colonies in medium) from fresh clean catch urine specimen indicates urinary infection (significant growth)

RESULTS:

A total number of ninety nine urine samples were collected from patients with symptoms of UTI, attending Asia Center. The study group had an age range between 25 and 55 years.

As shown in table (1) burning sensation was the most common symptoms among the study group that was found in 57.6% of patients.

The sensitivity & specificity of leukocyte esterase as diagnostic tools were 50%,89.8% respectively (Table 3). And for Nitrate reductase were 14.2%, 87.6%, Chi-square analysis showed statistical significant (<0.05).

The positive predictive value of a positive nitrite test result was 8.3%. In the instance of a negative nitrite test result, the probability of disease was 97.7%; addition of a negative LE test result decreased this probability to 91.02%, and a positive result increased it to 66.6%.

As indicated in table (2) leukocyte esterase was found positive in 10% of patient with significant pyuria compared with 7% negative results.

Table 1: Frequency of clinical findings among the study group (n=99)

Symptoms	Yes % n (%)`	No n (%)`
Dysuria	31.3	68.7
Frequency of urination	37.4	62.6
Burning sensation	57.6	42.4
Loin pain	55.6	44.4
Supra pubic pain	41.4	58.6
Fever	39.4	60.6
Chills	13.1	86.9

Table 2: The relation between leukocytes esterase & pus cells among the study group

Pus cells	Leucocytes esterase		Total
	Positive	Negative	
Less than 6 cells	11	71	82
More than 5 cells	10	7	17
Total	21	78	99

Table 3: Characteristics of Leukocyte esterase test for positive culture

Leukocyte esterase	culture		Total
	Positive	Negative	
Positive	7	14	21
Negative	7	71	78
Total	14	85	99

Table 4: Characteristics of Nitrite reductase test for positive culture

Nitrate reductase	culture		Total
	Positive	Negative	
Positive	2	0	2
Negative	12	85	97
Total	14	85	99

Table 5: Quality indicators for Leukocyte esterase & Nitrate reductase to detect infection

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Leukocyte esterase	50	89.8	66.6	91.02
Nitrate reductase	14.2	87.6	8.3	97.7

DISCUSSION:

The current study was conducted to assess the usefulness of urinalysis particularly leukocyte esterase & nitrate reductase as a simple screening test for diagnosing urinary tract infections.

Several rapid screening tests are used commonly to make a presumptive diagnosis of UTI, including dipstick: biochemical analysis of urine for nitrites or leukocyte esterase, as well as microscopic examination of urine for formed elements including white blood cells or bacteria. Use of dipsticks instead of urinalysis may decrease patient time and the cost of testing.

The diagnostic accuracy of microscopic urinalysis and urine dipstick for suspected urinary tract infections has been studied extensively, but results of these investigations have varied depending on patient population and laboratory techniques⁽¹⁰⁾. Our results show high specificity of Urinary dipstick test Nitrate reductase and leukocyte esterase (87.6% & 89.9%) respectively and sensitivity (14.2% & 50%). These findings were quite similar to that reported by Van Nostrand *et al.*⁽¹²⁾. In the present study the sensitivity of nitrite test was very low compared with that reported by this may be due in part to the insensitivity of the nitrite test to detect nitrate-reducing microorganisms or the urine was not remaining in the bladder long enough for organism to reduce nitrate to nitrite (12)

The negative predictive value of nitrate test was high (97.7%), with positive predictive value of 8.3%. The negative predictive value of the LE test was (91.02%) and positive predictive was (66.6%) which make them valuable resource to screen out negative urine specimens at the point of care.

CONCLUSION:

Urinary dipstick testing for UTI in the near patient setting is a valuable resource to screen out negative urine specimens at the point of care. If properly implemented this programme can result in improved use of laboratory resources and can aid clinicians in instant clinical decision making.

A negative test result for one of both tests has a sufficient predictive value to exclude disease, and when both test results are positive there is sufficient evidence to rule in infection.

REFERENCES:

1. Stamm, WE, Norrby, SR. Urinary Tract Infections: Disease Panorama and Challenges. *J Infect Dis.* 2001 Mar 1; 183 Suppl 1:S1-4.
2. Nicolle LE. Epidemiology of urinary tract infections. *Infect Med.* 2001; 18:153-162.
3. Smith's General Urology 16th edition 2004. Chapter 13. "Bacterial Infections of the Urinary Tract" Nguyen, Hiep. pp. 203 – 227.
4. Hooten, TM, Scholes, D, Hughes, JP, *et al.* A Prospective Study of Risk Factors for Symptomatic Urinary Tract Infection in Young Women. *NEJM* 1996; 335: 468.
5. Graham, J C., Galloway, A. The laboratory diagnosis of urinary tract infection. *J Clin Pathol* 2001; 54:911-919.
6. Flach, S., Longenecker, C., Tape, T., Bryan, T., Parenti, C., & Wigton, R. The relationship between treatment objectives and practice patterns. *Medical Decision Making*, . 2003; 23: 131-139.

7. Simerville JA, Maxted WC, Pahira JJ. Urinalysis: a comprehensive review. *Am Fam Physician* 2005; 71:1153-62.
8. David HL, Traore I, Feuillet A. Differential identification of *Mycobacterium fortuitum* and *Mycobacterium chelonae*. *J Clin Microbiol.* 1981 Jan; 13(1):6-9.
9. Cheesbrough M. 2000. District laboratory practice in tropical countries, 2^{ed} (2006), 7:39-76.
10. Ng Siew Hian, Angeline Yeoh Aing Chee, Jamilah Baharom *et al.* Health Technology Assessment Unit, Medical Development Division.
11. Oxman B, Barlow R, D'Arcy H, *et al.* Urinary tract infection: self-reported incidence and associated costs. *Ann Epidemiol.* 2000; 10:509-515.
12. Delanghe J.R., Kouri T.T., Huber A.R., *et al.* The role of automated urine particle flow cytometry in clinical practice. *Clin Chim Acta* 2000; 301:1-18.