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Screening Test for Detection of Urinary Tract Infections: Evaluation of the Urinary Leukocyte Esterase Dipstick Test

[İdrar Yolu Enfeksiyonlarının Saptanması için Tarama Testi: İdrar Lökosit Esteraz Strip Testinin Değerlendirilmesi]

SUMMARY
AIM/BACKGORUND: To compare the performance of leukocyte esterase dipstick test with microscopic examination in cases with clinically suspected urinary tract infections.
METHOD: Freshly urine specimens from 504 patients were analyzed for the presence of white blood cells (WBCs) by leukocyte esterase dipstick test and microscopically WBCs/high power field). A reaction of 1+ or more was taken as a definite indication of significant numbers of leukocytes.Urine dipstick tests were compared with pyuria for each sample. Results were statistically compared.
RESULTS: The sensitivity of dipstick leukocyte esterase was found to be 68.5%, whereas the specificity was 73.5%. Microscopic WBCs showed 34% sensitivity with 86.5% specificity. False negative results for leukocyte esterase could occur due to the heavy protienuria, and/or insufficient release of esterase from WBCs. In addition, false negative WBCs count might obtain in case of lyses.
CONCLUSION: Leukocyte esterase dipstick urine analysis was considered adequate screening tools in UTI cases. However, caution must be taken in interpreting factors that could affect the results of urine dipstick. Also, leukocyte esterase dipstick urine test was considered as a useful screening test that could be used in population based studies.

ÖZET
AMAÇ: Klinik olarak idrar yolu enfeksiyonundan şüphelenilen olgularda mikroskobik değerlendirme ile lökosit esteraz strip testinin performansını karşılaştırmak.
YÖNTEM: 504 hastadan alınan taze idrar örnekleri lökosit varlığı açısından lökosit esteraz idrar strip testi ile ve mikroskopla (lökosit/büyük saha) incelendi. Lökosit esteraz testinde 1+ veya daha büyük değerler önemli derecede çok lökosit sayısı için kesin gösterge olarak kabul edildi. Her örneğin idrar strip analiz sonuçları piüri sonuçları ile karşılaştırıldı. Sonuçlar istatistiksel olarak değerlendirildi..
BULGULAR: Lökosit esteraz strip testinin hassasiyeti %68,5 olarak hesaplanırken, seçiciliği %73,5 olarak hesaplanmıştır. Mikroskobik lökosit analizinin hassasiyeti %34, seçiciliği %86,5 olarak bulunmuştur. Ağır proteinüri ve/veya lökositlerden yetersiz esteraz salınımı lökosit esteraz sonuçlarının yanlış negatif çıkmasına neden olabilmektedir. Yine parçalanma durumlarında yanlış negatif lökosit sayımı meydana gelebilmektedir.
SONUÇ: Lökosit esteraz idrar analizi idrar yolu enfeksiyonlarının tanısı yeterli bir tarama testi olarak değerlendirildi. Bununla birlikte idrar lökosit esteraz analizinde karıştırıcı etkenlere dikkat edilmelidir. Ayrıca, lökosit esteraz idrar testinin toplumsal araştırmalarda kullanılabilecek faydalı bir tarama testi olabileceği değerlendirildi.

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Anahtar Kelimeler: tarama testi, idrar stripi, mikroskopik inceleme, piüri.

Key words: screening test, urine dipsticks, microscopic examination, pyuria.

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INTRODUCTION

Urinary tract infection (UTI) is defined as the presence of bacteria in urine along with symptoms of infection. Diagnosis of UTI usually involves the appearance of symptoms such as pyuria, and the culture of possible urinary pathogens. A microscopic analysis of urine that includes a leukocyte count and a gram stained smear provides high sensitivity and adequate positive predictive value for identification of positive urine samples (1). UTI is best defined by both a leukocyte count of at least 10/high power field (HPF) and growth of a single pathogen at a concentration of at least 50.000 colony forming units/ml (CFU/ml). These criteria almost always discriminate true UTI from bacteriuria resulting from either contamination of the urine specimen or colonization of the urinary tract (asymptomatic bacteriuria). The high predictive value of pyuria and bacteriuria for positive urine culture has permitted the initiation of antimicrobial therapy and the performance of diagnostic imaging procedures before availability of urine culture results (2). Dipsticks can be used to exclude a urinary tract infection when clinical symptoms are absent (3). The dipstick leukocyte esterase which detects esterase released from degraded WBCs is an indirect test for bacteriuria. UTI is the most common cause of bacteremia, which may be associated with a 10-30% fatality rate (4,5). It caused by a variety of gram-negative bacteria that ascend into the urinary tract and established bacteriuria often at levels greater than or

equal to 105 bacteria/ml of urine. Escherichia coli dominate as the causative agent of UTI (6). It seems very important to diagnose and treat urinary tract infection before renal damage has taken place. The validity of the microscopic urinalysis for diagnosing UTI and the criteria for pyuria and bacteriuria have been reported previously (7,8).

This study was conducted to compare the performance of leukocytes esterase dipstick with pyuria by high-power (HPF) and to evaluate the use of a new urine dipstick analyzer in detection of urinary leukocyte

MATERIALS AND METHODS

Subjects

The study group included 504 cases with signs and symptoms of UTI: 271(53.8%) female, 233(46.2%) male. Cases were seen at the King Hussein Medical Center during a two-year-period (from July 2004 to 2006). History of UTI and clinical findings were recorded. First-morning urine specimens for urinalysis were obtained by clean catch method, collected under aseptic conditions.

Urine dipstick for leukocyte esterase

Five hundred and four urine samples were tested with a urine dipstick for leukocyte esterase (MULTISTIX ® 10 SG, Bayer corporation, diagnostic Division, Tarrytown, USA). Fresh, uncentrifuged urine specimens were mixed, and the Bayer reagent strips were used. The chemical reaction was enzymatic-utilizing esterase present in granulocytic white blood cells. The dip-and-read test for leukocyte esterase was based on the splitting of the substrate 3-hydroxy-5-phenyl-pyrrole-N-tosyl-L-alanine ester by the enzymes to form pyrrole alcohol. Alcohol then reacts with a diazonium salt to produce a purple color. The reacted strips were matched to a color chart with four-color blocks of increasing color intensity from negative to 3+. The intensity of color was proportional to the amount of enzyme present and therefore directly related to the number of white blood cells. The sensitivity of the test has been adjusted to give a trace color with 5-15 WBC/HPF. A reaction of 1+ or more was a definite indication of significant numbers of leukocytes. Trace reaction was repeated on a fresh specimen. A strip was immersed in urine and placed on a feed load table and automatically drown into the instrument (Urine Chemistry Analyzer-Clinitck 200, Bayer corporation, Elkhart, USA).

Microscopic examination and urine culture

A sample of well-mixed urine (usually 10-15 ml) was centrifuged in a test tube at relatively low speed (about 1500xg) for 5 minutes until moderately cohesive bottom sediment was produced to be examined microscopically. The supernatant fluid was poured off and sediment resuspended in few drops of urine remaining, a drop of the urine was examined under 40x, a minimum of 10 to 15 high power fields were scanned for WBCs. Results were reported in terms of number of cells /HPF.

A calibrated 0.001ml bacteriologic loop was used to inoculate urine onto 5% Columbia blood agar and McConkey agar plates within 30 min of collection. The inoculated plates were incubated overnight aerobically at 37°C for up to 24 h (a minimum of 18 h). Uropathogens included genera of the Enterobacteriaceae family, group D enterococci, Staphylococcus saprophyticus, group B streptococci, and staphylococci other than S. saprophyticus when the patient was symptomatic. Urine colony counts were recorded as follows: (i) no growth, (ii) no significant growth ($<10^3$ CFU/ml), and (iii) significant bacteriuria ($\geq 10^3$ CFU/ml). Urines that grew contaminants (i.e., coagulase-negative staphylococci, lactobacilli, diphtheroids, and Streptococcus spp. other than group D spp.) were reported as demonstrating normal periurethral flora. Mixed growth was recorded for urines that grew multiple organisms (two or more). Significant urine bacterial isolates were identified by conventional biochemical procedures.

Urinalysis results were correlated to results of urine cultures. Urine cultures demonstrating significant bacteriuria (i.e., one or two uropathogens) were separated by the following colony count breakpoints for the performance analyses: (i) $\geq 10^3$ to 10^4 CFU/ml, (ii) $\geq 10^4$ to 10^5 CFU/ml, and (iii) $\geq 10^5$ CFU/ml. Performance of urinalysis tests was evaluated by calculating, using standard methods, sensitivity, specificity, and positive and negative predictive values.

Statistical method

Statistical analysis among the different laboratory methods was performed with the Mc Nemar test. Differences between methods were considered statistically significant if P-values were <0.05. Sensitivity, specificity and positive and negative predictive values (PPV, NPV) were calculated for the presence of pyuria and urine dipstick tests.

RESULTS

Leukocyte esterase

Urine samples from 504 patients with signs and symptoms of UTI were analyzed by the leukocyte urine dipstick. The sensitivity of the test has been adjusted to give a trace color with 5-15 WBC/HPF. A reaction of +1 or more is a definite indication of significant numbers of leukocytes. The results for WBC esterase are shown in Table 1.

Table 1. Number and percentage of urine samples with various leukocyte esterase results.

LeuLekocyte esterase	No. of cases n=504 (%)
Negative	321 (63.7)
positive+1	120 (23)
+2	31 (6.2)
+3	32 (6.3)

Microscopic examination and urine culture

Samples were analyzed for pyuria by microscopy, Leukocytes results in urine samples as seen by microscopic examination (Table 2). Statistical analysis revealed a significant correlation between dipstick results, Microscopic examinations (p=0.0001). The accuracy of the screening tests (dipstick and microscopic) in UTI cases was evaluated (Table 3).

Table 2. Leukocytes results in urine samples as seen by microscopic examination Pyuria WBC/HPF:

WBC/HPF	No. of cases N= 504 (%)
0-5	320 (63.5)
5-15	22 (4.4)
15-25	103 (20.4)
25-35	27 (5.4)
Abundant/Packed	32 (6.3)

Table 3. Sensitivity, specificity and positive predictive values(PPV), negative predictive values(NPV of pyuria and leukocyte esterase urine dipstick in UTI Cases.

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Leukocyte esterase	68.4	73.4	43.7	88.5
Pyuria (WBC/HPF)	34	86.5	43.5	81.3

All of the urine samples were mid-stream collections, but only 5% were first-void specimens. Only 95 (18.8%) urine cultures had a pure growth of one or two potential uropathogens, while 214 (42.4%) showed either no growth (63 cultures [12.5%]) or no significant growth (151 cultures [29.9%]). The rest of the urine cultures either grew contaminants or showed mixed growth.

DISCUSSION

The analysis of urine samples for the presence of significant pyuria can be used to made decisions regarding the need for urine culture.

Screening for pyuria is simple, inexpensive and an accurate method of diagnosis UTI (especially in situations where facilities for urine culture are unavailable. The presence of leukocytes (≥ 10 WBC/HPF) in the urine predicts a positive urine culture and hence indicates urinary tract infection (9) . A combination of leukocytes and bacteruria measured by HPF appears to be very useful marker in diagnosis of UTI (10). The absence or low level of WBCs in the urine is consistent with the absence of inflammatory response in colonized (rather than infected) individuals. In addition, false negative WBC count might obtain in case of lysis. The presence of pyuria has the highest specificity (86.5%) for identifying positive urine culture compared to dipstick test. Dipstick and microscopic urinalysis are generally considered adequate screening tools UTI cases (8) , while urine culture remains the standard screening test for bacteriuria. False negative results for leukocyte esterase are due to heavy proteinuria, insufficient release of esterase from WBCs. There is some controversy as to the best method of evaluating for UTI (7, 11). However, it has been reported that a combined leukocyte and nitrite test on a single dipstick has an improved sensitivity and specificity (9). Caution must be taken in interpreting the results of urine dipstick in certain population. For instance, positive results of leukocyte esterase or nitrite do not necessarily indicate infection. In addition, dipsticks and microscopic urinalysis are generally not considered to be adequate screening tools in pregnancy (12). A similar finding was observed in children with UTI (13). The absence of an internationally recognized reference measurement procedure is a serious drawback to their validation. In recent report, evaluation of certain urinalysis methods revealed that automated urinalysis system offers comparable results to the manual testing (98%), in addition to a reduction in methods variation (14). The most popular cite approach is to combine test strips with other methods (e.g. flow cytometers) for primary screening (15, 16). Physicians should send urine for

cultures from all patients and begin presumptive treatment only on those with significantly positive pyuria and dipstick results. The increased number of false-negative results and the relatively poor predictive value of a positive test make a single test less useful. Therefore, we conclude that a combination of the two criteria (≥ 10 WBC/HPF) is better for distinguishing an infected patient from a non-infected than any single test. The analysis of urine samples obtained by mid voided stream for the presence of significant dipstick leukocyte and nitrite results with pyuria could be used to guide decisions regarding the need for urine culture.

CONCLUSIONS

Urine analyses for leukocyte using urine dipstick test alone seems to be useful in all population to exclude the presence of infection in the results of leukocyte-esterase are negative.

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