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ORIGINAL ARTICLE

Evaluation of diagnostic performance of routine automated urinalysis and association between urinary tract infection and leukocytosis

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Received 26 February 2015; accepted 24 April 2015; published online 22 May 2015

ABSTRACT

Objective: For diagnosis of urinary tract infection (UTI), urine culture has been accepted as the gold standard test. High numbers of unnecessary cultures have always been the obvious issue in clinical laboratory. Moreover, urine culture is labor- and time- intensive. In the present study we investigated the diagnostic performances of infection-related parameters of urine preliminary analysis (leukocyte esterase, nitrite, bacteria and leukocyte) in comparison to urine culture method and whether the presence of UTI causes leukocytosis.
Materials and Methods: 239.029 urinalyses were retrospectively examined. A total of 3427 patients that complete blood count (CBC), urinalysis and urine culture were requested on the same day were included in the study. Leukocyte count of CBC and leukocyte and bacteria in microscopy parameters of urine analysis were compared with urine culture. Diagnostic performance of the parameters for detection of UTI was estimated.
Results: 413 patients had positive urine culture results (12.0%). Among culture positive patients, leukocyte esterase and nitrite positivity were 85% (n=352) and 40% (n=166) respectively. Bacteria and leukocyte positivity on microscope were 31% (n=127) and 75% (n=310), respectively. Although negative predictive values were 80%, 62%, 75%, 58%, positive predictive values of leukocyte esterase (LE), nitrite, pyuria and bacteriuria tests were 69%, 97%, 74%, and 91% respectively. The highest specificity rate was estimated for nitrite (99%). Leukocytosis rate in patients with a positive urine culture were 23% (n=96). A strong association was detected between microscopic WBC and LE count ($r = 0.827$; $P < 0.001$).
Conclusion: Considering that most samples from the patients in our study have insignificant or no growth, urine microscopy and dipstick urine analysis can rule out UTI in these patients. We suggest that the investigation and application of a new algorithm in clinical practice could reduce unnecessary antibiotic prescriptions and in the clinical laboratory setting might reduce workload and cost.

Key words: Culture, urinalysis, urinary tract infection, leukocytosis.

Introduction

Urinary Tract Infection (UTI) has been recognized as the most common bacterial infection in the society. The frequency of UTI is high particularly in young women and the elderly [1]. Although UTI rarely cause kidney scarring, hypertension and renal failure, doctors often prescribe powerful antibiotics to minimize clinical complaints, based on positive rapid urinalysis without confirmation by culture [2,3]. Early diagnosis and on time treatment are considerable for patient's well-being [4]. Conventional semi quantitative urine culture, which has some difficulties, is the gold standard for diagnosis of urinary tract infection [5]. Bacterial growth requires at least 18 hours. This situation leads to a delay in treatment [6]. Culture is also labor-intensive, expensive and requires a microbiology technician [7]. Escherichia coli is the most commonly isolated bacteria in urine cultures [8]. Many studies are present in the literature concerning unnecessary culture requests [5, 9, 10]. Urine dipstick testing is superior at excluding a UTI when the results are negative when compared to approving a diagnosis of UTI when they are positive [11]. Dipstick urinalysis may rule out UTI. This technique is simple, fast and inexpensive [7]. Routine urinalysis may be a sufficient diagnostic instrument and can reduce

laboratory workload [1]. The lack of substantial pyuria is assumed as certain evidence of the absence of UTI [12]. As urine culture has manual work and is time-intensive, searching for a more practical way for investigating UTI has been required. At least the rate of unnecessary culture requests might be reduced. The aim of this study was to investigate the diagnostic performance of infection-related parameters of urinalysis (leukocyte esterase, nitrite, bacteria and leukocyte) in comparison to urine culture as the reference method and to investigate whether the presence of a UTI causes leukocytosis.

Materials and Methods

Data obtained from the electronic database of our hospital (Dıskapı Yıldırım Beyazit Training and Research Hospital in Ankara, Turkey) had been analyzed. During a year nearly 170.000 urinalyses had been requested at our hospital. This retrospective, observational study was conducted from July 2013 to December 2013. Assessment of patients; Electronic Database (SARUS LİS, Integrated Information Systems, Ankara, Turkey) included: age, sex, preliminary diagnosis, comorbid disorders, referred polyclinic and all test results. The causes for these requests were pre-hospitalization, screening or pre-surgery testing and emergency or symptomatic demands. The data of patients who had test results for both urine culture and urinalysis at the same time were evaluated. Patients with a urinary catheter and patients who took antibiotic medication in the previous three days were not included into the study. We collected samples from 3427 individuals [1447 men (42.2%) and 1980 women (57.8%)] with a mean age of 49,2 ±18,4 years. The Institutional Ethics Committee Review Board of the hospital approved the present study.

Mid-stream urine samples were obtained using the midstream clean-catch technique. For collection of the urine samples either evacuated sterile plastic containers for urine culture or non-sterile plastic containers (FıratMed Plastik, Ankara, Turkey) for rapid urinalysis were used. Contaminated specimens were excluded from the study. No patient had an abnormal urinary tract anatomy. After collection, every specimen was analyzed promptly and the outcomes had been registered in a database. 10 milliliters of sample was applied for rapid urinalysis. The parameters analyzed included; nitrite (by the test strips), qualitative measurement of leukocyte esterase (LE), and microscopic examination (bacteria and WBCs). For microscopic and dipstick testing, an automated urinalysis system (IQ 200 Elite, Iris Diagnostics, Chatsworth, CA, USA) was used. Urine specimens were tested with AUTION Sticks 10EA test strips (ARKRAY Factory, Inc., Japan) for the nitrite production as a determiner of bacteriuria and the existence of LE activity as an indicator of pyuria. The nitrite test (Greiss reaction) is an indirect measure of nitrate reducing bacteria. Dipstick test for white blood cell (WBC) determines LE of neutrophil granules ensured urine contained in vesica for longer than 4 h. WBC and bacteria were counted per high-power field (hpf, 400× magnification), however bacteria counts had been reported qualitatively as none, rare (0–1 /HPF, 0–6.8 /μl), few (1–5 /HPF, 6.8–27.7 /μl), medium (5–10 /HPF, 27.7–55.6 /μl) and plenty (>10 /HPF, >55.6 /μl). WBCs were reported quantitatively for urine microscopy (for example; 1/HPF or 67/HPF etc.). Urinalysis was finalized within 1 hour after taking samples. High and low quality control materials (iQ Control/Focus Set For in vitro diagnostic use with the iQ series, IRISpec CA/CB ichem urine chemistry control twin pack, Iris Diagnostics Chatsworth, California, USA) were utilized for internal quality control. Urine specimens were cultured by practicing of 10 μL on blood agar and eosin methylene blue agar broths (Oxoid, UK). Cultures were incubated at 37°C for 18–24 hrs and Phoenix (*Becton, Dickinson* and Company, USA) was utilized for definition. Bacterial counting was described as numbers of colony forming units (CFU) per mL. Specimens were accepted positive if either a pure of predominant culture of $>10^5$ CFU/mL, two organisms in similar proportions at $>10^5$ CFU/mL or 10^4–10^5 CFU/mL of a gram negative organism or two organisms where the gram negative obviously predominates [6]. The specimen was accepted contaminated (mixed growth), whenever three or more various colonies with no dominant type had grown (mixed flora), and were refused. As the reference for establishing the performance of the microscopy and dipstick data, culture was utilized. Negative and positive predictive values (NPV and PPV), diagnostic specificities, sensitivities, likelihood ratios (LR+ and LR–) and diagnostic odds ratios (DOR) of microscopy parameters and dipstick were estimated. Spearman correlation analysis was performed to test for a rank order relationship between two variables. As the data was not normally distributed, Spearman correlation analysis was performed (non-parametric test). ROC analysis for

Table 1. Defined pathogens in urine culture

Cultured urinary pathogens	Number	Percentage
Escherichia coli	291	70.4%
Klebsiella spp	43	10.4%
Enterococcus spp	21	5.0%
Staphylococcus spp	14	3.4%
Streptococcus spp	13	3.1%
Enterobacter spp	9	2.1%
Pseudomonas spp	8	1.9%
Candida spp	6	1.4%
Proteus spp	4	0.9%
Acinetobacter spp	1	0.3%
Stenotrophomonas	1	0.3%
Cedecea lapagei	1	0.3%
Serratia spp	1	0.3%

Spp: species

leucocyte esterase (LE) and Leukocyte count of CBC and Spearman correlation between parameters were applied by SPSS® for Windows ver. 17.0 (SPSS Inc., Chicago, IL, USA).

Results

3427 urine samples were evaluated in total. 3014 cultures (87.9%) were negative whereas considerable bacterial growth was determined in 413 (12.0%) specimens. E. coli was the most frequently isolated bacteria with a percentage of 70.2% (n = 291) (Table 1). 40% (n=166) of samples were nitrite positive, after defining cultured bacteria. Microscopic assessment of urine sediment from the 413 samples showed that 310 samples (75%) had ≥5 WBC per high-power field. The positive microscopy results for bacteria were 31% (n=127). LE and nitrite positivity in culture positive patients were 85% (n=352) and 40% (n=166) respectively. The results of the dipstick test for nitrite and LE and microscopic examination for bacteria and leukocyte were compared. The specificity, sensitivity, negative predictive value (NPV), positive predictive value (PPV) and Diagnostic Odds Ratio (DOR) of the test strips and microscopic examination results are shown in Table 2 and Table 3. Nitrite had the highest specificity (99%). LE had the highest sensitivity 85% (n=352). The specificity of all parameters were >61%. Leukocyte esterase had the highest negative predictive value (80%). The area under the curve (AUC) for the Leukocyte count was 0.818 (95% CI = 0.796–0.840) and 0.774 (0.753–0.796) for the LE and 0.798 (0.770–0.825) for the bacteriuria and for the nitrite count was 0.693 (95% CI = 0.661–0.726) and 0.542 (0.510–0.573) for the Leukocyte of CBC in the ROC analysis in which culture results were accepted as the reference for UTI (Figure 1). The specificity and sensitivity were also calculated with alternative cut-off values in order to ensure a low number of false negatives whereby the existence of leukocytes was very important in the estimation

Table 2. Diagnostic performance of strip and sediment microscopy parameters.

Test	Sensitivity	Specifity	PPV	NPV
Leukocyte Esterase*	85 %	61 %	69 %	80 %
Nitrite*	40 %	99 %	97 %	62 %
Leukocyte (/HPF)	84 %	62 %	74 %	75 %
Bacteria (/HPF)	70 %	83 %	91 %	58 %

PPV: Positive predictive value
NPV: Negative predictive value
HPF: High-Power Field
*Semi quantitative parameters (Negative,+,++)

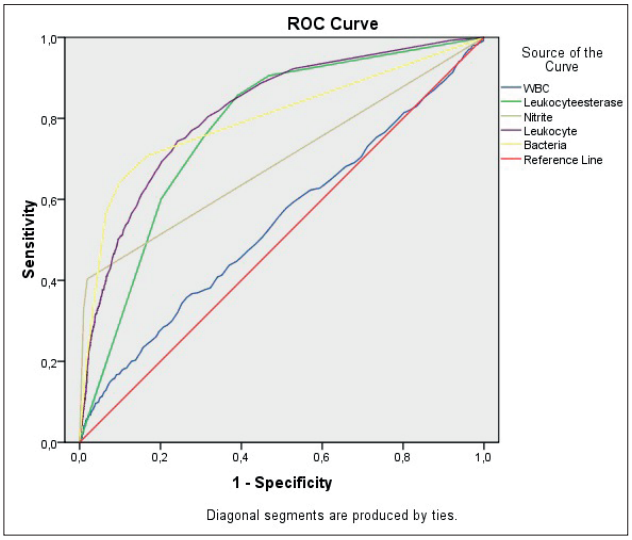

Figure 1. ROC curves for leukocyte, leukocyte esterase, nitrite, bacteria and Leukocyte of CBC. Area under curve (AUC) for leukocyte 0.818 (95% CI = 0.796–0.840), for Leukocyte Esterase 0.774 (0.753–0.796), for bacteriuria 0.798 (0.770–0.825), for nitrite 0.693 (0.661–0.726) and for Leukocyte of CBC 0.542 (0.510–0.573).

Table 3. Diagnostic performance of Leukocyte of CBC, strip parameters, sediment microscopy parameters and different bacteria rates.

Test	LR(+)(95%CI)	LR(–)(95%CI)	DOR(95%CI)
Leukocyte of CBC (10^3/mm^3)	1.09 (1.04–1.14)	0.54 (0.42–0.69)	0.49 (0.37–0.66)
Leukocyte Esterase	2.79 (2.36–3.30)	0.40 (0.37–0.43)	0.14 (0.11–0.18)
Nitrite	1.64 (1.51–1.78)	0.04 (0.03–0.06)	0.02 (0.02–0.04)
Leukocyte (/HPF)	3.94 (3.15–4.92)	0.44 (0.41–0.47)	0.11 (0.08–0.14)
Bacteria rate 1 (/HPF)	2.84 (2.44–3.31)	0.23 (0.21–0.26)	0.08 (0.06–0.10)
Bacteria rate 2 (/HPF)	2.52 (2.21–2.87)	0.15 (0.13–0.17)	0.06 (0.04–0.07)
Bacteria rate 3–4 (/HPF)	2.15 (1.92–2.40)	0.11 (0.09–0.13)	0.05 (0.04–0.06)
Bacteria rate 5–9 (/HPF)	1.40 (1.31–1.50)	0.09 (0.07–0.12)	0.07 (0.05–0.09)
Bacteria rate ≥10 (/HPF)	1.21 (1.16–1.27)	0.08 (0.06–0.12)	0.07 (0.04–0.10)

LR(+): Positive Likelihood Ratio, LR(–): Negative Likelihood Ratio, DOR:Diagnostic Odds Ratio, HPF: High power field

of UTI. The sensitivity increased to 88.6% but specificity decreased to 55.3% when a cut-off value of 3 WBC/hpf was chosen. Weak relationships between nitrite and LE ($r = 0.248$; $p < 0.001$), between leukocyte and nitrite ($r = 0.274$; $p < 0.001$), between bacteria and LE ($r = 0.314$; $p < 0.001$) were estimated in the correlation analysis. There was a moderate relationship between nitrite and bacteria ($r = 0.402$; $p < 0.001$). A relatively elevated correlation was determined between microscopic WBC count and LE ($r = 0.827$; $p < 0.001$).

Diagnosis of a UTI: Positive Likelihood Ratio (LR+) is accuracy rate of diagnosing the disease. The LR+ was higher for microscopy (leukocyte and bacteria) than dipstick (nitrite and leukocyte esterase) 3.94 [3.15–4.92], 2.84 [2.44–3.31] and 1.64 [1.51–1.78], 2.79 [2.36–3.30] (Table 3).

Ruling out UTI: Negatif Likelihood Ratio (LR-) is accuracy rate of diagnosis healthy. Nitrite is the most valuable parameter for excluding of UTI, because the lowest LR- was nitrite 0.04 [95% CI 11.44–64.21] (Table 3).

Discussion

The outcomes of urine culture and the outcomes of rapid urine analysis for UTI were crosschecked in this study. Findings of this retrospective study demonstrated that 12.0% of the culture requested patients had positive culture test results, which showed extremely unnecessary urine culture requests. Correct and prompt diagnosis is important for appropriate treatment especially in symptomatic patients. Giving a proper urine sample is a significant problem. Midstream clean-catch technique decreases the number of contaminated samples [13]. Spot mid-stream urine specimens were collected as required by our routine procedure and only 413 samples among 3427 were reported culture positive. This indicates the extremely elevated percentage of unnecessary urine culture requests (i.e. 87.9%). Kayalp et al. and Okada et al. and Christenson et al. reported excessive culture negative outcomes with a percentage of 97.7%, 80% and 82.1%, respectively [6,14,15]. Urine culture is an expensive diagnostic tool for UTI when compared to urinalysis, because cultures are time-consuming and increase length of stay in hospital, which leads to payment increase and treatment delay [3,16]. According to microbiological results, the most common identified pathogen for UTI is *E. coli* as 70.2%. Ducharme et al. and Kayalp et al. also revealed that E.coli was the most frequently isolated bacteria 60.4% and 54.5% respectively [3,6]. The most sensitive parameter is LE (85%) that was followed by leukocyte count with a powerful sensitivity of 84%. Each parameter has a specificity of 60% however nitrite has the highest specificity (99%). Furthermore microscopic outcomes of bacteria are more sensitive than dipstick outcomes of nitrite. Hughes et al. determined that bacteriuria has a sensitivity of 84.6% and a specificity of 65.0%

with using IRIS 939 UDx, although in our study we found a sensitivity and specificity of 70% and %83 respectively [17]. A study, investigating LabUMat with UriSed, showed that bacteriuria had a specificity of 97.8% and a sensitivity of 78.8% [6]. IRIS and UriSed systems assay (work) with different mechanisms. IRIS uses a flow cell arranged in the centrical plane of a mounted microscope with a video camera and caught microscopic pictures are categorized and quantified by a picture analysis program but yet UriSed discovers and enrolls excellent resolution pictures of microscopic areas (like manual microscopic sediment examination) [6]. Although our findings are in accordance with the specificity outcomes (with the nitrite specificity of 99%), we found a lower nitrite sensitivity than estimated. European Urinalysis Guideline dwells on that, low false negative rates for bacteria would determine true negative samples and reduce costs and the number of unnecessary culture requests [18]. For bacteria our false negative rate was similar with data estimated in the studies by Kayalp et al. and Christenson et al. in which false negative rates were 21.2% and 25% respectively [6,15]. However, for bacteria our false negative rate was higher [30%] than declared in the guideline (best<10%) [6,15]. We didn't distinguish between symptomatic patient or asymptomatic patient, therefore false negative rate was defined higher than supposed. Current studies show that nitrite had a higher specificity than LE, meantime sensitivity was seriously lower [6,7]. Outcomes of our study are parallel to these studies. The predictive value of rapid urinalysis parameters has major importance for occurrence of infection. Bolann et al. reported that the power of LE and nitrite to determine or externalize UTI was like with sediment microscopy parameters [19]. Similarly, in our study nitrite had the highest PPV (97%) and leukocyte esterase had the highest NPV (%80). Kayalp et al. and Okada et al. reported a low PPV and a high NPV of bacteriuria (45.4%, 99.5% and 63.0%, 90.7%, respectively) [6,14]. In contrary, a high PPV and a low NPV of bacteriuria were found (91.0% and 58.0%, respectively) in our investigation. Likewise in the study by Little et al. they showed that dipstick urinalysis poorly ruled out infection [20]. Another object of this study was to investigate whether the presence of a UTI caused leukocytosis. According to our knowledge this is the first study investigating relationship between UTI and leukocytosis (Increased Leukocyte count of CBC) of CBC. AUC for the Leukocyte count of the CBC in the ROC analysis was 0.542 [95% CI = 0.510–0.573]. The result was not as significant as we expect. There is need for further research on this topic.

One of the limitations of this study is that we couldn't classify patients as symptomatic or asymptomatic due to the fact that it was a retrospective study and had a high number of patients. The other limitation is that our automatic urine analyzer has no ascorbic acid test on strip. Therefore we were unable to evaluate the interferences.

In conclusion routine urine analysis is a very easy and quick test to apply. This test might be considered to have a high potential to be a candidate test, which could reduce unnecessary culture requests with predicting results. When the clinician would like to start empirical treatment without waiting for culture results, routine urine analysis might be reliable in preliminary diagnosis of UTI. In view of the fact that most samples have insignificant or no growth, urine microscopy and dipstick urine analysis can rule out UTI. Doctors should request only rapid urinalysis in the first examination. It is better if a new algorithm is developed in order to reduce unnecessary cultures, antibiotic prescriptions and to reduce workload and cost in the clinical laboratory.

Acknowledgment

We are grateful to Department of Medical Microbiology of Dıskapı Training and Research Hospital for their effective support during the research.

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