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Full Length Research Paper

Evaluation of specimens in which the urine sediment analysis was conducted by full-automatic systems and a manual method together with urine culture results

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The aim of the present study is to assess leukocyte and bacterial counts, and urine strip leukocyte esterase and as well as nitrite results from IQ200 and UriSed full-automatic urine sediment analyzers, with urine culture results. Six hundred urine cultures were performed in accordance with the routine laboratory procedures of the hospital. A growth <10^4 CFU/mL in the culture was considered negative. After the inoculation of urine samples into the culture media, urine samples were analyzed by IQ200 and UriSed full-automatic urine analyzers, respectively. As 10^4 CFU/mL are considered the diagnostic criterion for UTIs, 39 (6.5%, n=600) urine specimens yielded positive cultures. The positive likelihood ratios (PLR) of the IQ200 were suitable for bacteria and nitrite measurements (PLR>10); however, negative likelihood ratios (NLR) were inadequate for both devices and the manual method (NLR >0.3 for all). However, when the utilization of two tests in combination was analyzed, the diagnostic odds ratio was 48 for the IQ200 (95% confidence interval CI, 12.1-190) and 168 for the UriSed (95% CI, 20.6-1369) in cases in which leukocyte, bacteria, and nitrite measurements were high together. The results of the IQ200, UriSed, and urine strip test do not accurately reflect the urine culture results. Nevertheless, it was concluded that negative nitrite and leukocyte results of both automated and manual tests could be utilized in ruling out a urinary tract infection (UTI) diagnosis.

Key words: IQ200, UriSed, KOVA® system, full-automatic urine sediment analysis, bacteriologic urine culture, urine microscopy

INTRODUCTION

Urinary tract infections (UTIs) are one of the most encountered clinical pathologies in the community and hospitals. The diagnosis of UTIs and treatment choices are important and complex pathologic processes requiring the collaboration of clinicians and laboratory specialists. The symptoms of the patient, urine analysis, and bacterial culture are used for the diagnosis of UTIs. One or more of these diagnostic materials are used together according to the situation (Ardic et al., 2004; Patel et al., 2005; Wilson and Gaudio, 2004).

Currently, bacteriologic urine cultures are the gold standard for the diagnosis of UTIs (Zaman et al., 2001). Nevertheless, analysis of bacteriologic cultures is a time-consuming and expensive method and requires skill and experience. In addition, the results of the bacteriologic culture are negative in 50 to 80% of the patients with UTIs (Zaman et al., 2001; Okada et al., 2000; Kellogg et

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Abbreviations: PLR, Positive likelihood ratios; **NLR,** negative likelihood ratios; **UTIs,** urinary tract infections; **LE,** leukocyte esterase; **WBC,** white blood cell; **DOR,** diagnostic odds ratio; **ROC,** receiver operating characteristic; **AUC,** area under the curve; **PPV,** positive predictive value; **NPV,** negative predictive value; **DPV,** diagnostic predictive values

al., 1987). Therefore, clinicians are in need of an appropriate screening test for patients with UTIs. Any screening test that can be applied prior to the analysis of the urine culture in patients with UTIs will save a considerable amount of time and cost, reducing the amount of unnecessary bacteriologic culture (Zaman et al., 2001; Okada et al., 2000).

Currently, with the increase in the number of patients admitted to hospitals, the need for fully automated systems that can perform both chemical and sediment analysis of the urine from a single specimen via a strip has increased and is widely used in clinical laboratories. In the present study, it was aimed to assess leukocyte esterase (LE), nitrites, and white blood cell (WBC) and bacterial counts by means of the IQ200 (IRIS International Inc., Chatsworth, CA, USA) and UriSed (77 Elektronika Kft, Budapest, Hungary) full-automatic urine analyzers that can automatically perform the chemical strip tests and microscopic examination of the urine and to compare them with the results of bacterial cultures, and to determine the predictive values of the two automated urine analyzers with respect to bacterial infections.

MATERIALS AND METHODS

Six hundred urine samples which had been collected in compliance with standard guidelines (Kauri et al., 2000) from the patients admitted to the Kecioren Research and Training Hospital with complaints of UTIs were examined in the present study. No limitations regarding age or gender were imposed for the samples. Patients who had been using antibiotics for any reason, who had structural urinary anomalies, who were being treated as inpatients, who had urinary catheter, and those who were pregnant, were excluded from the study. The approval of the Local Ethical Committee of the Kecioren Research and Training Hospital was obtained for the study (No: 20090324).

Samples were placed into sterile dishes and inoculated within 1 h for the culture. After inoculation, a 3 mL sample for the IQ200, a 5.5 mL sample for the Labumat, and a 10 mL sample for routine microscopic urine analysis were obtained. No protective substances were added to the samples. Samples were studied within 2 h after being obtained. Microscopic urine analysis was performed with a KOVA® system (Hycor Biomedical, Garden Grove, CA, USA) . In the KOVA® system, urine samples were centrifuged at 400 g for 5 min. In order to eliminate the differences that would result from the technician, all of the microscopic analyses were performed by the same technician using the same microscope. The results of the IQ200 and UriSed and KOVA® systems were reported with respect to low-power field (LPF, 100 X magnifications) and high-power field (HPF, 400x magnification).

For the urine culture analysis, 10 µL of an uncentrifuged urine specimen was inoculated onto blood and Mac Conkey agar plaques (Biomerieux, Marcy l'Etoile, France), and incubated under aerobic conditions at 36°C for 18-24 h. Growth results $<10^4$ CFU/mL were considered negative. In the case of growth of more than two microorganisms, it was reported as “mixed urethral flora,” ignoring the total bacterial count. The identification of pathogenic microorganisms was performed by means of a VITEK II automatic bacteria identification device (Biomerieux, Marcy l'Etoile, France).

Although the routine bacteriologic culture method used in the present study is able to detect the pathogens that are most frequently encountered in UTIs, bacteria that r arely cause UTIs, such as *Chlamydia* spp., *Mycoplasma* spp., *Ureaplasma* spp., *Mycobacterium* spp., and adenoviruses could not be detected.

The IQ200 is a fully automatic urine analyzer that can do chemical analysis via a strip (Aution sticks 10EA; Arkray, Inc., Kyoto, Japan) and microscopic analysis via the image flow cytometry method. The IQ200 can measure the urine content, classifying it into 12 categories via Auto-Particle Recognition (APR™) software.

The Labumat and UriSed combined system (77 Elektronika Kft, Budapest, Hungary) is a urine analyzer that is fully automatical and can perform chemical (LabStrip U11 plus; Analyticon Biotechnologies AG, Lichtenfels, Germany) and microscopic analyses of urine. The device centrifuges the urine sample at 2000 rpm for 10 s and provides the particles to be homogenously collected at the bottom of the basin. Thereafter, results are obtained with the examination of the samples in 15 fields via microscopy with 400x magnification.

The limit values of the IQ200 and Labumat and UriSed devices for WBC and bacteria are considered to be 5 WBC cell/HPF and >1 cell/HPF, respectively (Winn et al., 1997).

As statistical analysis for diagnostic evaluations, sensitivity (true positive [TP]/[TP+false negative {FN}]), specificity (true negative [TN]/[TN+false positive {FP}]), negative likelihood ratio (NLR=[100-sensitivity]/specificity), positive likelihood ratio (PLR=sensitivity/[100-Specificty]), and diagnostic odds ratio (DOR;TP and TN/FP and FN) were calculated. The accuracy of the tests were evaluated in terms of diagnosis according to the LR values, with NLR <0.1 and PLR >10. In addition, to compare the diagnostic test performances, the measurement of the receiver operating characteristic (ROC) curves and the area under the curve (AUC) are presented, and the confidence interval was calculated and presented as well (Bland and Altman, 2000). Statistical analyses were carried out by SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Considering 10^4 CFU/mL to be the threshold for the diagnosis of UTIs, growth in the urine culture was determined in 123 (20.5%, n=600) samples. Forty-eight urine samples in which mixed flora had grown were regarded as contaminated. Nevertheless, 36 samples with mixed flora, such as *Escherichia coli* and *Streptococcus viridans* (n=6), *E. coli* and coagulase-negative *Staphylococcus* spp. (CNS; n=6), *Lactobacillus* spp. and CNS (n=5), *Klebsiella pneumoniae* and CNS (n=4), *E. coli* + *Corynebacterium* spp. (n=2), *S. viridans, Lactobacillus* spp. (n=1), CNS (n=8), and *S. viridans* (n=2) and *Lactobacillus* spp. (n=2), were also regarded as contaminated. When the 84 cultures regarded as contamination were eliminated, growth was determined in the remaining 39 samples (6.5%, n=600) and regarded as the cause for the UTI. Organisms isolated from these cultures are presented in Table 1.

Bacteria and leukocyte counts obtained from the manual analysis performed by the KOVA® system, as well as those obtained from the IQ200 and UriSed devices, analytic performances of LE and nitrite results obtained from strip tests, and the results of the diagnostic evaluation obtained from the positivity of any or all of the methods are presented in Table 2.

When the obtained values were compared with the

Table 1. Microorganisms isolated from 600 urine cultures samples.

Microorganism	No	(%)
Escherichia coli	31	5.2
Proteus mirabilis	1	0.17
Klebsiella oxytoca	1	0.17
Pseudomonas aeruginosa	1	0.17
Klebsiella pneumoniae	1	0.17
Acinetobacter baumannii	1	0.17
Citrobacter freundii	1	0.17
Enterobacter agglomerans	1	0.17
Enterococcus spp.	1	0.17
Escherichia coli + *Streptococcus viridans*	6	1
Escherichia coli + CNS	6	1
Lactobacillus spp,+ CNS	5	0.83
Klebsiellae pneumoniae + CNS	4	0.67
Escherichia coli + *Corynebacterium* spp.	2	0.33
Streptococcus viridans + *Lactobacillus* spp.	1	0.17
CNS	8	1.33
Streptococcus viridans	2	0.33
Lactobacillus spp.	2	0.33
Mixed flora	48	8

Table 2. Diagnostic performance of IQ200, Urised and KOVA system results in comparison with urine cultures.

	TP	TN	FP	FN	Sensitivity	Specificity	PLR	NLR	DOR (95% confidence interval)	ROC, AUC (95% confidence interval)
IQ 200										
LE	26	371	190	13	66.7	66.1	1.97	0.50	3.91 (1.96-7.77)	0.69 (0.60-0.79)
leukocyte	28	456	105	11	71.8	81.3	3.84	0.35	11.1 (5.33-22.9)	0.85 (0.79-0.91)
Bacteria	18	542	19	21	46.2	96.6	13.63	0.56	24.4 (11.2-53.2)	0.71 (0.61-0.82)
Nitrite	17	549	12	22	43.6	97.9	20.38	0.58	36.0 (15.0-82.9)	0.71 (0.60-0.81)
leukocyte or nitrite or bacteria	34	433	128	5	87.2	77.2	3.82	0.17	23.0 (8.80-60.1)	
leukocyte, nitrite and bacteria	8	558	3	31	20.5	99.5	38.36	0.80	48.0 (12.1-190)	
Urised										
LE	24	407	154	15	61.5	72.5	2.24	0.53	4.21 (2.20-8.30)	0.69 (0.59-0.78)
leukocyte	25	483	14	78	24.3	97.2	8.62	0.78	11.1 (4.90-19.7)	0.83 (0.76-0.90)
Bacteria	91	33	470	6	93.8	6.6	1.00	0.94	1.06 (0.13-0.87)	0.65 (0.54-0.77)
Nitrite	17	550	11	22	43.6	98.0	22.23	0.58	37.9 (16.1-92.2)	0.71 (0.60-0.81)
leukocyte or nitrite or bacteria	35	89	472	4	89.7	15.9	1.07	0.65	1.65 (0.57-4.75)	
leukocyte, nitrite and bacteria	9	560	1	30	23.1	99.8	129.46	0.77	168 (20.6-1369)	
KOVA® system										
leukocyte	28	439	122	11	71.8	85.9	5.11	0.33	9.19 (4.4-18.9)	0.834 0.772-0.897
Bacteria	14	543	18	25	35.9	93.8	5.79	0.68	16.9 (7.6-37.8)	0.665 0.560-0.770

LE-Leucocyte esterase, TP-True positive, TP-True Negative FP-False positive, FN-False negative, PLR-Positive likelihood ratio, NLR-Negative likelihood ratio, DOR-Diagnostic odds ratio, ROC (*Receiver operating characteristic curves*) ile (AUC) area under curve.

results of the cultures, it was noted that the lowest FP percentages had been obtained from the bacteria and nitrite values. High FN rates (78.6%) were derived from the bacteria counts performed by the UriSed device.

When PLR were analyzed, it was noted that the evaluation of the infection according to the results of the bacterial count obtained from the IQ200 and manual methods was higher compared to the automatic analyzer. This is thought to have originated from the high FP rates in the bacterial count performed using the UriSed device (78.3%). Although the NLR of the results of the leukocyte count were higher than the other parameters, the NLRs were calculated to be inadequate for all measurements in general (for all parameters, >0.3). When the statistical values obtained by the common use of the measured parameters were examined, it was observed that the PLR values, obtained in cases of the positivity of leukocytes, bacteria, and nitrite measurements, were between the acceptable ranges for both devices. When the DORs obtained for all tests were evaluated, it was observed that adequate DORs could have been reached, particularly in the case of positive leukocyte, nitrite, and bacteria values in combination (Table 2).

ROC analysis revealed that the highest AUC values were obtained by leukocyte counts for all three methods.

DISCUSSION

The primary aim of the present study was to screen UTIs by means of an easy and reliable method in order to increase the probability of diagnosing UTIs prior to the culture tests, and to avoid unnecessary time and cost consumption as well.

Various rapid screening tests have been reported within the last 10 years, such as chemical strip tests (Clinitek 200) (Bowman and Riley, 1991), catalase activity (Uriscreen; 11), and microscopic analysis with gram staining (Cardoso et al., 1998).

In various studies, significant correlations have been found between LE, nitrite results, and positive culture results. In particular, the specificity of the nitrite test is 100% (Nostrand et al., 2000; Lohr et al., 1993) . Lenke and Dorsten (1981) determined the specificity of the nitrite test to be 100%, whereas the sensitivity was 22%, and concluded that the diagnostic value was limited. Similarly, Zaman et al. (1998) found the sensitivity of LE and nitrite was very low in the presence of bacteria. On microscopic examinations performed with gram staining, higher diagnostic efficacy was determined with a positive predictive value (PPV) of 97.6% and a negative predictive value (NPV) of 98.7% (Cardoso et al., 1998).

In the present study, the results of LE and nitrite analyses were similar for the IQ200 and Labumat analyzers, and the sensitivity was observed to be low, which was in agreement with other studies (Zaman et al., 2001) and inadequate since the NLR was quite high (Table 2). A low sensitivity and high NLR in nitrite measurement can be explained by two basic mechanisms: 1) bacteria can turn nitrate into nitrite within approximately 4 h, and if the waiting period of the urine sample is shorter, the nitrite level may be below the limits of detection; and 2) the infectious agent. Although bacteria, such as *Enterobacteriaceae,* produce nitrite, *Staphylococcus saprophyticus* and *Pseudomonas* spp. do not cause an increase in nitrite levels (Palmer et al., 1998).It is thought that FN nitrite results might be due to the anaerobic conditions or bacterial contamination.

However, when it was evaluated in terms of diagnostic predictive values (DPV) obtained via both of the automated methods, it was determined that nitrite can be one of the best diagnostic parameters because of its high specificity, despite its low sensitivity.

As the LE values obtained via the IQ200 and Labumat analyzers were examined, it was observed that the diagnostic performance values for both methods were inadequate. The FP and FN results that were observed for the LE values may be related to the leukocytes, eosinophils, and *Trichomonas* spp. passing to the urine from the vaginal fluid, as well as the amount of nitrites in the urine, hypotonic and alkali urine, abnormal urine pH, and high bilirubin, protein, and hemoglobin values (Palmer et al., 1998). Asymptomatic leukocyturia, which would occur in the presence of vesicoureteral reflux and urinary system-related invasive interventions, should also be taken into consideration (Cardoso et al., 1998; Nostrand et al., 2000).

When the leukocyte counts performed via the IQ200 and UriSed devices, and the manual method were examined, it was observed that the PLR, NLR, and DPV values were more appropriate compared to those obtained from the LE results; however, these values were not adequate in terms of diagnostic prediction. Nevertheless, on ROC analyses performed for clinical evaluations, the highest AUC values in all parameters were obtained from leukocyte counts. This indicates that leukocytes are one of the most important parameters for the diagnosis, even though leukocyteuria is not adequate in distinguishing culture negative samples alone.

When the results of the bacteria counts obtained from the two automated systems and from the manual method were examined, it was observed that only the PLR that was obtained from the IQ200 device can be considered adequate. It was observed that the UriSed analyzer had given the highest FP results for bacteria counts and it was inappropriate for the evaluation of the bacteria. It was observed that reference value studies must be performed even if the bacteria evaluation is carried out via a UriSed device. It was reported to have resulted from artifacts which were as big as bacteria are identified as if they are bacteria, by the device (Akin et al., 2009).

When the diagnostic performances that have been obtained using leukocyte and bacteria count values measured via two of the automation techniques and the

strip nitrite results, and/or using them in combination, were examined, it was determined that the probability of the culture being negative is quite high (99.5% for the IQ200 and 99.8% for UriSed) in the case of all being negative. In addition, the highest values of PLR and DPV were observed using the tests in combination.

According to the results of the present study, as well as the results of other studies, none of the urine analyses and automated methods are not adequate to predict infections. UTIs are a group of infections in which the diagnosis is quite complex and could not be accurately diagnosed by any test alone. It is understood that the high levels of bacteria and nitrites, which have been obtained via the IQ200 and manual measurements in particular, provide important information, and it would be favorable to evaluate these results in combination with the leukocyte count and clinical findings. An integrated approach according to the clinical appearance of the patient, laboratory facilities, and the results of the laboratory tests is required for the diagnosis of UTIs.

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