Original Article

Effects of gender in automated urinalysis test results as predictors of true urinary tract infection in infants

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Objective: We sought to evaluate whether there is a gender difference for IRIS IQ200 test results as rapid diagnostic test in rouling out culture positivity in infants in a routine hospital laboratory.

Methodology: This retrospective cohort study utilized data from 2159 unselected consecutive patients aged 1 month to 1 year, who attended the Sevket Yilmaz Research and Education Hospital from January to December 2014 and received both urinalysis and culture for suspected UTI. A sample was considered culture-positive if it contained a pure culture of ? 50,000 10^4 CFU/mL. 272 patients, 39 culture positive and 233 culture negative females (median age 6 months), and 280 male patients, 28 culture positive and 252 culture negative, (median age 4 months) were evaluated.

Results: Nitrite alone had a PPV of 95% and an NPV of 90.9% in the infant group. PPV and SP were 100% in male infants. A positive leukocyte count (?4 cells/HPF) had a specificity of 90%, a PPV of 46.6% and an NPV of 94.5% in infants, which was 94.3 in boys. LE alone had higher specifity (95.1%), PPV (53.8%) and NPV (94.4%) in boys compared to girls, 89.2%, 50.0% and 94.4%

Conclusions: The results of urine tests should not be used alone for the diagnosis of UTI in infant without gender difference. (Rawal Med J 201;41:346-350)

Keywords: Urinary tract infections, sensitivity and specificity, leukocytes, microscopy.

INTRODUCTION

Urinary tract infections (UTI) affect up to 10% of children and are the most common types of bacterial infection in infants and young children worldwide. Newborn infants are at greatest risk of UTI, which is hard to diagnose in infants, since early stage symptoms may be nonspecific. In infants, UTI are more common in boys (3.7%) than girls (2%) and after age one, are more common in girls.[1-3] Positive bacterial growth from urine obtained by suprapubic aspiration is considered the standard for the diagnosis of UTI in infants. Culture results, however, require at least 48 hours, indicating the need for more rapid methods of diagnosing UTI. Infants may be unnecessarily exposed to antibiotics while waiting for the results of urine culture, which leads to antibiotic resistance. On the other hand, missed diagnosis of UTI will result in kidney damage, if left untreated.[4-5]

Urinalysis with automated instruments that allow determination of the absolute number of particles per HPF and dipstick analysis at the same time are quick and inexpensive screening methods that are frequently used in conjunction with urine culture for diagnosing UTI.[6-8] Significant difference of reference limits has been described in different populations and genders using various automatic analyzers.[8-11] Several studies have been conducted to assess the usefulness of results of urinalysis in predicting culture positivity in infants but no reports on the gender difference for diagnosis of UTI in infants have been reported. Thus, we sought to evaluate whether there was a gender difference for IRIS IQ200 test results as rapid diagnostic test in rouling out culture positivity in infants in a routine hospital laboratory.

METHODOLOGY

All procedures were performed in accordance with the Second Declaration of Helsinki. Bursa Şevket Yılmaz Training and Research Hospital is a 1050-bed tertiary care setting in which approximately 150,000 urinalyses are requested per year. This retrospective cohort study utilized data from 2159 unselected consecutive pediatric patients aged 1 month to 1 year who attended the Sevket Yilmaz

Research and Education Hospital from January to December 2014 and received both urinalysis and culture for suspected UTI and stored in hospital databases.

Computer-based clinical records were retrieved and analysed by one investigator in order to find eligible patients. Patients were excluded if they had been hospitalized, had been prescribed antimicrobial agents whose activity spectra included all organisms isolated from the index urine culture, had been seen by physicians within one month prior to entrance into the study, or had chronic diseases predisposing them to colonization of the urinary tract. All of these attempts were performed to reduce reduce the number of false-negative culture test results.

A calibrated 0.001 mL bacteriologic loop was used to inoculate urine onto 5% (v/v) Columbia blood agar and Eosin Methylene Blue (EMB) agar plates (bioMerieux, Marcy l'Etoile, France) within 30 min of collection. Our hospitals quality policy includes a 24 hour working emergency microbiology laboratory and time of specimen is recorded in all patients. Inoculated plates were incubated aerobically at 37°C for 18 to 24 h. Bacterial concentrations were expressed as the numbers of colony forming units (CFU) per milliliter. A sample was considered culture-positive if it contained a pure culture of ? 50,000 10^4 CFU/mL [12]. The following organisms were considered urine pathogens: *Escherichia coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas aeuroginosa, Citrobacter profundii, and Enterococcus faecalis.* Pathogenic microorganisms were identified using the Vitek 2 automated system (bioMerieux, St. Louis, Missouri, USA), according to the manufacturer's instructions. The system includes an Advanced Expert System (AES) that analyzes MIC patterns and detects phenotypes for most organisms tested.

The Iris iQ200 Automated Urinalysis System is composed of an automated urine microscopy module, connected to an Aution Max AX-4280 (Arkray Inc., Kyoto, Japan) automated urine chemistry analyzer. The Iris iQ200 has a microscope and digital imaging software and can classify urine particles into 12 categories, reporting quantitative concentrations of red blood cells (RBC), white blood cells (WBC, WBC clumps, squamous epithelial cells, non-squamous epithelial cells, hyaline casts, unclassified casts, crystals, yeast, bacteria, sperm and mucus.[13]

Statistical analyses were performed using SPSS v 21.0 with culture results defined as the 'gold standard'. Samples were divided into two groups: culture negative and culture positive. Using positive culture results as the gold standard, sensitivity , specificity, positive predictive value (PPV; post test probability of an outcome for positive tests), and negative predictive value (NPV; post-test probability of an outcome for negative tests), accuracy (the proportion of true results in the population) , positive likelihood ratio (LR+) (the probability of a person who has the disease testing positive divided by the probability of a person who does not have the disease testing positive) and negative likelihood ratio (LR-) (the probability of a person who has the disease testing negative divided by the probability of a person who does not have the disease testing negative) were calculated .

RESULTS

Out of 2159 infants sent to laboratory, 552 suggested diagnosis of UTI and fulfilled the criteria for the study. Infants taken to the study were divided into two groups. Group 1 consisted of 272 patients, 39 culture positive and 233 culture negative females, aged 0 to 12 months (mean age, 5.7 months; median age, 6 months), and Group 2 consisted of 280 male patients, 28 culture positive and 252 culture negative, aged 0 to 12 months (mean age, 4.7 months; median age, 4 months). Among the isolated bacteria, Escherichia coli was the most common (Table 1).

Table 1. Microorganisms isolated from positive urine cultures.

Microorganism(s)	Male (n=28)	Female(n=39)
Escherichia coli	19	25
Klebsiella pneumoniae	4	4
Enterococcus faecalis	2	6
Staphylococcus aureus	2	3
Pseudomonas aeruginosa	1	1

The discriminative and predicting power of the each individual urinalysis variable is shown in Table 2. All variables had high specificity however, sensitivity values were considerably lower. Nitrite alone had a sensitivity of 28.3%, a PPV of 95% and an NPV of 90.9% in the whole infant group. PPV and SP are 100% in male infants. SE is very low in both genders.

LE alone has higher specifity (95.1%), PPV (53.8%) and NPV (94.4%) in boys compared to girls, 89.2%, 50.0% and 94.4% .

Table 2. Test characteristics of urinalysis in all infants.

Parameter	SE (%)	SP (%)	PPV (%)	NPV (%)	LR+	LR-
LE ≥1+						
İnfants (Boys+girls)	58.2	92.3	51.3	94.0	7.56	0.45
Girl	64.1	89.2	50.0	93.6	5.9	0.42
Boy	50.0	95.16	53.8	94.4	10.3	0.5
NO_2						
İnfants (Boys+girls)	28.3	99.7	95.0	90.9	136	0.92
female	23.0	100	100	88	i	0.76
male	39.2	99.5	91.6	93.5	97.4	0.60
WBC (≥3)						
Infants (Boys+girls)	65.6	84.8	37.6	94.6	4.3	0.4
Girl	71.7	80.6.	38.3	94.4	3.7	0.3
Boy	57.1	88.3	35.5	94.8	4.8	0.48
WBC≥4						
Infants (Boys+girls)	62.6	90.0	46.6	94.5	6.2	0.4
girl	66.6	85.4	43.3	93.8	4.5	0.4
Boy	57.1	94.3	53.3	95.1	10.1	0.4

The median values of urine WBC levels in males and females were 6 cell/HPF and 6 cell/HPF for WBC with iQ200 in culture positive group and 1 cell/HPF and 2 cell/HPF in culture negative group, respectively. When we analysed cutoff points for microscopy results, we found that leukocyte count of ?4 cells/HPF had best accuracy.

A positive leukocyte count (?4 cells/HPF) had a specificity of 90%, a PPV of 46.6% and an NPV of 94.5% in infants, indicating that use of this parameter alone as a marker of culture positivity would result in a large number of false negatives and some false-positives (Table 2). When we used this cut of point specifity was slightly better in boys for this parameter in boys 94.3% compared to girls 85.4%.

DISCUSSION

Screening test for early detection of urine culture-positive infants must have a 95% sensitivity and a 95% NPV, and that have a sum of sensitivity and specificity ? 170%.[14] None of the parameters we tested reach these levels. The present study found that nitrite and LE analyses had very high specificities but low sensitivities in infants. Our findings are in accordance with the specificity outcomes (with the nitrite specificity of 99%), ranging from 75.6% to 100%.[15,16] The 28.3% sensitivity of nitrite in this study is low as the previous estimates of sensitivity ranging from 16.2% to 88.1%.[15,16] Nitrite alone had a relatively poor negative likelihood ratio of predicting culture results in infants (0.9), suggesting that nitrite is not useful for ruling out disease in infants and there was no difference between both genders in our study groups . There was considerable heterogeneity in terms of LRs ($P < 0.001$) in the literatüre.[16]

Low sensitivity of nitrites for infants are due to frequent bladder emptying because 4 hours of bladder incubation are required for bacteria to convert nitrate to nitrite at reliably detectable levels. Meanwhile, althoug E Coli, Klebsiella and Proteus produce nitrite from nitrate, some other urinary pathogens do not reduce nitrate to nitrite. Therefore, nitrite tests are helpful only when they yield positive results.[4]

The LE dipstick test is a method of determining LE in neutrophils, an enzyme released by WBCs after 2 hours. The leukocyte esterase test is an indirect measure WBCs, therefore, may be falsely negative when leukocytes are present in low concentration. LE alone appeared to be a relatively poor test, both for ruling in (LR+ = 7.5) and ruling out (LR- = 0.4) infection in infants in our patient group. LR+ values range from 2.6 to 32.2 and LR? values ranged from 0.02 to 0.66 in the literature which is in accordance with our results.[16]

LE sensitivity ranged from 37.5% to 94% and

specificity ranged from 64 % to 97.8% in infants in the literature which our results are in agreement.[15,16] Our result with 92% specificity correctly reports most of patients without the disease as test negative (true negatives) but 8% patients without the disease are incorrectly identified as test positive (false positives). Recently, Velasco et al found that the LE test showed a greater mean positive predictive value for males than females (79.4% versus 58%) in febrile infants. In the present study LE alone has higher specifity and PPV in boys compared to girls. Sensitivity of LE seemed to be slightly higher in female patients which is similar to Velasco's findings.[17]

In our study, we found that ?4 WBC/HPF cutoff criteria provided the best discriminative value. Our sensitivity level 62% is compatible with previous studies which showed that 2 to 5 or more leucocytes/ HPF in sediment had an overall sensitivity of 73% (range 32100) in children younger than 12 months.[16,18] We found that when we use this cut-off level SE is better in girls but specifity is better in boys.

There are conflicting reports in the literature which[19] failed to observe any significant gender-related difference for WBC reference and earlier studies, which showed clear gender related differences for values of WBC.[9-11] In our group, we also couldnt find a gender related differences. Most variables attributable to anatomical differences between genders play a relatively minor contribution, whereas a clean urine specimen from an infant is hard, especially a girl or an uncircumcised boy, contaminated specimens are common.[18]

In our hospital, we used sterile urine bags to obtain urine samples from infants because collection of urine by this method is noninvasive and requires limited personnel time and expertise. This collection technique has a low contamination rate if the patient's perineum is properly cleansed and rinsed before application of the collection bag; the urine bag is removed promptly after urine is voided into the bag; and the specimen is processed immediately. Parents, rather than hospital staff members, participated in urine collection from their children, which may have resulted in false positive test results . Our PPV of 46.6% indicates that a positive leucouria may be attributible to contamination or asymptomatic bacteruria rather than a UTI. Contamination is very common in obtaining a urine sample from the vagina in girls or the prepuce in uncircumcised boys. The false-positive rate with such specimens dictates that before diagnosing UTI, all positive although a negative culture of a bag-collected specimen effectively rules out UTI, a positive culture does not document UTI. Moreover, a negative (sterile) culture of a bag-collected urine specimen effectively eliminates the diagnosis of UTI, provided that the child is not receiving antimicrobials.

We acknowledge several limitations of this study. First, use of pediatric bags for collecting samples in children, although a general consensus has been reached,[20,21] suprapubic aspiration (SPA) is the appropriate method of urine collection in infants but it is invasive and time consuming. Use of adhesive perineal bags to collect urine is suboptimal, as bacteria from fecal contamination or urethral colonization may be misinterpreted as UTI.[22] Urine samples are collected by the parents, rather than healthcare operators so that urinalysis is overall more susceptible to poor standardization of extra-analytical phases and preanalytical issues.[23-24] Secondly, the retrospective nature of the study prevented us from classifing the subjects as symptomatic or asymptomatic. And some children with asymptomatic colonization may be included in the study. Also, we didn't catogerize male children based on their circumcision status. Additionally, use of highly trained single technician for interpratation of the automated urinalysis results would improve our results; however, our hospital is based on a 24 hour working schelude so more than one technician interprated the specimens. Therefore, our results may not be generalized to other automated analyzers and differences in risk factors such as, race and temperature should be considered in generalizing these results to other clinical practices.

CONCLUSION

Positive urinalyses help health care personnel to determine the necessity of further investigations. However, in terms of ruling out a UTI, no test performs particularly well, with only marginal differences between dipstick and microscopy. Our

findings indicate that the results of urine tests should not be used alone for the diagnosis of UTI in pediatric patients. Rather, the combination of diagnostic laboratory tests and urine culture is required for the early and accurate diagnosis of UTI and to select appropriate treatment. Urinalysis cannot sustitute for urine culture but needs to be used together.

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