Open Science Journal of Clinical Medicine
2015; 3(3): 107-111
Published online May 30, 2015 (http://www.openscienceonline.com/journal/osjcm)

Predictive Validity and Reliability of Dipstick and Microscopy in Diagnosis of Urinary Tract Infections Among Febrile Under-Fives in Nsambya Hospital, Uganda

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To cite this article

Anguyo Robert DDM Onzima, Ocokoru Christine, Govule Philip. Predictive Validity and Reliability of Dipstick and Microscopy in Diagnosis of Urinary Tract Infections Among Febrile Under-Fives in Nsambya Hospital, Uganda. *Open Science Journal of Clinical Medicine.* Vol. 3, No. 3, 2015, pp. 107-111.

Abstract

The non-specific presentation of urinary tract infections amongst children poses a challenge in its diagnosis. Given the lower-relative frequency of urinary tract infections, more commonly occurring causes of fever have to be ruled out before considering it hence delayed diagnosis and treatment. Tests exist to aid clinicians timely-diagnose urinary tract infections. In this study we assessed if such tests could produce accurate and reliable results in diagnosing urinary tract infections among febrile children below 5 years (0-59 months) of age. *Objectives:* To determine predictive validity and reliability of urine dipstick, microscopy and their combination in diagnosing urinary tract infections among children 0-59 months of age. *Methods:* We conducted a descriptive cross-sectional study at the pediatric out patients' department of Nsambya Hospital from December 2013 to April 2014; and enrolled 302 febrile children aged 0-59 months with no antibiotic therapy 48 hours preceding hospital visit. We subjected urine samples from the eligible children to dipstick, microscopy and urine culture. We analyzed the data for predictive validity by computing the sensitivity, specificity and predictive values and employed the kappa statistic to assess for reliability using urine culture as our gold standard. *Results:* The combined urine dipstick and microscopy had sensitivity, specificity, positive predictive and negative predictive values of 98.8%, 95.0%, 87.9% and 99.5% respectively. Urine dipstick had 46.9% sensitivity, 95.5% specificity, 79.2% positive predictive value and negative predictive value of 83.1%. The sensitivity, specificity; positive and negative predictive values of microscopy were 95.1%, 98.2%, 95.1% and 98.2% respectively. Urine microscopy and culture; and combined dipstick-microscopy and culture showed close to perfect agreement (kappa=0.933 and 0.903 respectively). *Conclusions:* Urine microscopy is accurate and reliable in diagnosing urinary tract infections among children aged 0-59 months. Its use in screening for urinary tract infections among febrile under-fives in Ugandan hospitals could improve early diagnosis and treatment.

Keywords

Predictive Validity, Reliability, Urinary Tract infections, Febrile Under-Fives

1. Introduction

Urinary tract infections are common among children and present with non-specific signs and symptoms that include inter alia fever, irritability, vomiting and failure to feed [1]. This (non-specific) presentation of urinary tract infections (among under-fives) makes its diagnosis difficult, often requiring use of laboratory tests on urine samples of suspected patients. With this constraint, clinicians must have high index of suspicion in order to decide when to request for the designated laboratory tests. Accurate and timely diagnosis of urinary tract infections is critical to provision of timely and correct treatment; and to improvement of clinical outcome of the disease among children below five years of age. Fever is the most frequent sign and symptom of uncomplicated urinary

tract infections. In the majority of Sub-Saharan Africa (including Uganda), malaria and respiratory tract infections are reportedly the commonest causes of fever among children less than 5 years and other populations. Uganda Bureau of Statistics and ICF Macro [2] place urinary tract infections as the fourth leading cause of morbidity among children 0-59 months of age in Uganda: after malaria, respiratory tract infections and diarrhoea yet these (conditions) share fever as a common major symptom. This implies that clinicians presume urinary tract infection as a possible cause of particular episode of fever after mostly ruling out its more incident causes.

Use of urine dipstick, microscopy and culture are the commonly used diagnostic tests to confirm presence of urinary tract infections in Uganda. Urine culture is the most accurate and least accessible among all these methods because it is technically difficult to execute and requires a high-level laboratory set up. Such laboratories (with capacity to perform urine culture) are found in limited health institutions in Uganda. Urine dipsticks (to detect presence of nitrites and /or leukocyte esterase) are often used in the emergency department to screen for urinary tract infections due to ease of use, rapidity and low cost but may not be sensitive enough when used alone. Zorc, Kiddoo and Shaw in a clinical microbiology review of diagnosis and management of paediatric urinary tract infections recommend back-up urine culture to detect 12% of urinary tract infections missed by the urine dipstick [3]. Urine microscopy (to detect pyuria and bacteriuria) is another commonly used method for evaluation of patients with suspected urinary tract infection. The presence of pyuria (>5WBCs/hpf) on microscopic examination is less sensitive and less specific than bacteriuria while presence of both pyuria and bacteriuria make the likelihood of urinary tract infection greater [4].

We conducted this study to assess the predictive validity and reliability of urine microscopy and dipstick as independent tests, then in simultaneous combination using urine culture as gold standard. For ethical reasons, we employed bag collection and clean-catch-midstream urine since our study subjects were children and to prevent subjecting them to invasiveness of supra-pubic aspiration. Gopal, Rao, Hegde & Soans assert that, bag collection, clean catch midstream urine are the preferred methods of urine collection in children [5]. We are optimistic that this study has generated results that may lead to adoption of urine microscopy and/or dipstick in routine screening of urinary tract infections among febrile children 0-59 months in Uganda's ambulatory care clinics.

2. Methods

2.1. Study Setting

We conducted this study at the paediatric ambulatory care clinic of a Ugandan hospital. It is commonly called Nsambya Hospital and is a 361-bed private-not-for-profit, tertiary referral hospital.

2.2. Study Design

We employed a descriptive cross sectional study among children aged 0-59 months of age between December 2013 and April 2014. We approached parents or caretakers of the children presenting with fever, made inquires about prior antibiotic use as initial screening mechanism.

2.3. Inclusion and Exclusion Criteria

We included all children 0-59 months who presented with fever with no prior antibiotic use (within 48 hours); and whose parent or caretaker consented to participate in the study. We excluded all children whose parent or caretaker reported antibiotic use (within 48 hours) prior to hospital visit with the then episode of fever. In addition we excluded all children meeting the inclusion criteria and whose mother or care taker did not consent to participate in this study. All children aged 60 months and above were also excluded from participating in this study.

2.4. Sample Size and Sampling Strategy

We employed the Bouderer's formula and obtained a minimum sample size of 291 at 95% level of confidence, +/-5% desired precision, 20.3% prevalence of urinary tract infection [6] and 96.0% anticipated sensitivity. Prevalence of UTI in children 0-59 months based on a cross-sectional study conducted on febrile under-fives in Mwanza city, Tanzania [6]. We employed consecutive sampling to enroll children aged 0-59 months with temperature $\geq 37.5^{0}$C.

2.5. Collection of Urine

The principal investigator or trained research assistants (nurses) participated in collection of urine samples. We collected 2 urine specimens from each patient, one for dipstick and microscopy; and the other for culture. For children aged 2 years and below, the gold standard method of urine collection (supra-pubic aspiration) was not feasible due to its invasive nature since no parent/caretaker could consent to use of the procedure as such, we employed a standard adhesive sterile urine collection bag. In females, the bag was attached to the perineum after cleaning the area with normal saline; and gauze-drying it for the adhesion of the bag. In boys, we retracted the prepuce and similarly cleaned the surrounding and glans penis; and gauze-dried before attaching the bag to the perineum. We left the bags visible and one of the investigators kept observing for presence of urine in the bag and promptly removed it as soon as urine was seen. The urine was transferred into 2 sterile laboratory urine bottles. Each bottle contained 5mls of urine.

For older children (above 2 years of age) we collected midstream urine specimen (5mls each) into 2 sterile laboratory bottles. We swabbed the perineum of the girl child with normal saline and gauze-dried it before the child was left to squat over a clean pan and persuaded to pass urine. With the investigator's hands in sterile gloves, the female's labia were spread apart as she passed urine. When the urine was in full

flow, the investigator collected (it) in the 2 sterile bottles. For the male child, we cleaned the penis with normal saline and the foreskin of the uncircumcised male was retracted and the area underneath cleaned and dried with sterile gauze. The male was left to stand naked; as he passed urine and mid-way, it was collected in 2 sterile laboratory urine bottles.

The 2 urine samples were properly labeled and immediately taken to Nsambya Hospital microbiology laboratory and handed to the designated experienced laboratory technician in charge of urine examination. All the tests of urine dipstick, microscopy and culture were done by the same technician throughout the study period. A maximum of 15 urine samples were delivered per day to the laboratory between 8:30a.m and 1:30p.m.

2.6. Urine Testing

We performed tests using urine dipstick, microscopy and culture simultaneously. The urine dipstick and microscopy were performed on one of the urine samples and the second urine sample was cultured to identify organisms. We examined urine using dipstick and microscopy within 30 minutes of urine collection whereas commenced culture within 1-2 hours from the time of sample collection.

For dipstick, we examined urine for presence of nitrites and leukocyte esterase using a 10-parameter multi-reagent dipstick (multistix) strip. The examiner dipped a fresh test strip, from the multi-reagent strip container, into the urine sample – ensuring that all the test pads were immersed in the urine; and taken out immediately. The excess urine on the strip was removed by drying the edge of the strip on an absorbent paper. The examiner adhered to manufacturer's instructions by leaving the strip to stand for two minutes and then compared the colours appearing on it to the chromatin scale of the manufacturer. We classified urine as dipstick positive when either nitrite or leucocyte esterase or both, matched the colour on the chromatin scale.

The examiner performed microscopic urinalysis on a centrifuged-urine-sample using Olympus microscope by transferring 5mls of urine into a conical centrifuge tube before centrifuging at 3000 RPM for 5 minutes. The supernatant was decanted off into a jar containing a disinfectant, leaving the sediment in the tip of the tube. The sediment was shaken to make the mixture homogeneous.

We employed wet preparation and gram staining during microscopy. To examine the sample using the wet preparation, the examiner placed one drop of the homogenized urine sediment on a clean dry glass slide which was then covered by a cover slip. The wet preparation was examined under a high power (X 40) microscope for presence of leucocytes. Presence of ≥5 leucocytes per high-powered field indicated pyuria. For gram stain, the examiner further placed one drop of the homogenized urine sediment on a new clean dry glass slide, evenly spread it on the slide using the edge of a second glass slide in a sliding manner to make a thin smear. He then performed the staining according to the procedure described by Cheesbrough [7]. The dry smear was examined under a (X 100) microscope for bacteria. The gram positive bacteria appeared purple and the gram negative bacteria appeared pink. We considered positive microscopy with leukocyte count of ≥ 5WBCs/hpf or presence of bacteria on gram stain or both.

We cultured the bag or mid stream urine sample on Cystein-Lactose Electrolyte Deficient culture medium. The culture medium was dried at 37ºC for 15-30 minutes before we inoculated the urine specimen on the culture medium using a nicrome wire loop which held 1/200µl of urine. The culture plate was incubated at 35-37 ºC for 24 hours after which we subjected it to examination for colonies of isolates (after 24 and 48 hours).

We counted discrete colonies growing within clear zone of inhibition and picked single colonies out for sensitivity test; and identified them directly from the Cystein-Lactose Electrolyte Deficient plate. After 48 hours, we reported culture results and colony counts of more than 10^5 organisms/ml indicated infection.

2.7. Data Analysis

We entered and cleaned the data using Epidata 3.1 software using 2 data entrants before export to excel in which they were finally analysed. We employed urine culture (gold standard) as a basis for identifying children who had urinary tract infections. Cross tabulations using 2x2 contingency tables comparing each of the urinalysis markers towards the urine culture were constructed, as (table 2). We combined dipstick and microcopy simultaneously since our study aimed to identify a test that can identify as many sick children as possible. The sensitivity, specificity, positive predictive value and negative predictive value were calculated using the contingency table [8].

We assessed reliability of the tests using the Kappa test. Kappa statistics is a measure of agreement [9] which is often used to look at how accurately a test can be repeated [10]. Kappa is a way of assessing agreement between raters [11].In this study, it meant assessment of agreement between: urine dipstick and the gold standard (culture); microscopy and culture; or the combination of dipstick and microscopy and culture. It is only used with categorical data. Kappa statistic varies between 0 and 1 – with 1 showing perfect agreement and 0 showing agreement not different from chance. According to Di Eugenio, having a Kappa of greater than zero is not sufficient to assess the quality of agreement, as such, various scales have been proposed to assess quality of agreement [12]. In this study, we adopted a scale employed by Landis and Koch (1977) [13] for interpreting the kappa.

2.8. Ethical Consideration

We conducted this study after ethical approval of the institutional review board of Mother Kevin Postgraduate Medical School, Nsambya. We sought permission at all levels – including from mothers or caretakers of eligible children before collection of urine samples. Children whose mothers or caretakers did not consent to participate in this study were excluded.

3. Possible Limitation

We did not assess other (none urinary tract infection) causes of fever among our study subjects. This assumes that fever among all children who had positive urine culture was caused by urinary tract infections – having neglected occurrence of asymptomatic bacteriuria. This could have had a bearing on reliability of our findings: especially the sensitivity of dipstick – given identification of leucocytes and nitrites arises from pathology caused by the bacteria. In cases where we had asymptomatic bacteriuria, culture could have wrongly identified them yet this may have falsely caused reduction in sensitivity of dipstick.

4. Results

We enrolled 302 children and this represents response rate of 103.8%. Majority, 171 (56.6%) of the children were male. The mean age of the children was 22.6 months (SD=15.4 months). Children aged 1-12 months formed the highest proportion, 97 (32.1%) of the respondents. The mean fever duration was 2.6 days (SD=2.1 days).Table 1 shows the age and sex distribution of the study participants.

Table 1. *Age and Sex distribution of the study participants.*

Age category	Male	Female	Total
Less than 1 month	6(75%)	2(25%)	8 (2.6%)
1-12 months	47(48.5%)	50(51.5%)	97 (32.1%)
13-24 months	47(65.3%)	25(34.7%)	72 (23.8%)
25-36 months	36(54.5%)	30(45.5%)	66 (21.9%)
37-48 months	34(63.0%)	20(37.0%)	54 (17.9%)
49-59months	1(20.0%)	4(80.0%)	5 (1.7%)
Total	171(56.6%)	131(43.4%)	302 (100%)

Urine culture was positive among 81 children. We assessed the sensitivity, specificity, predictive values and global levels of agreement (accuracy) for the individual tests and their combination. We employed simultaneous combination of urine dipstick and microscopy since our target was to identify a test with as high net sensitivity and high positive predictive values. The sensitivities of dipstick, microscopy and the combined tests were 46.9%, 95.1% and 98.8% respectively. The test specificities (for dipstick, microscopy and the combined tests) were 95.5%, 98.2% and 95.0% respectively. At the current level of prevalence, the positive predictive values were 79.2% (dipstick), 95.1% (microscopy) and 87.9% (combined tests); and their corresponding negative predictive values stood at 83.1%, 98.2% and 99.5% respectively. The observed (global) levels of agreement between the screening tests and the gold standard were 82.5%, 97.4% and 96.0% for dipstick, microscopy and the combined tests respectively. The reliability assessments using the kappa test showed moderate level of agreement for dipstick (kappa = 0.487, $p< 0.001$); and close to perfect agreement for microscopy (kappa = 0.933, $p <0.001$) and the combined tests (kappa = 0.903, $p<0.001$).

5. Discussion

The sensitivity of our screening test was high for microscopy (95.0%) and very low for dipstick (46.9%). The net sensitivity of the tests after simultaneous combination rose to 98.8%. This proportion (for combined test) is just 1 % less an earlier projection [14]. In that study, Wu and Wong project that combining urine microscopy and dipstick could increase sensitivity to 99.8% [14]. On combination of dipstick and microscopy, our findings were similar to that of a study published by the American Academy of Paediatrics Journal [15]. In that study [15], the investigators assert that sensitivity of combined urine dipstick and microscopy was higher (94.7%) than the sensitivity of dipstick alone (90.8%). In such a condition (like urinary tract infection among children) with non-specific clinical presentation, employment of techniques with such high sensitivity in screening could aid timeliness of diagnosis; and consequently improve clinical outcome, though casts doubt on accuracy in clinical diagnosis of individual patients because of likelihood of false positives; – hence high treatment cost arising from treating false positive patients. Katz emphasizes that highly sensitive tests cannot help 'rule in' but rather 'rule out' diagnosis at clinical-level because it gives no clue that a positive test is false positive[8].The finding herein shows that urine dipstick may not even be of use in 'ruling out' diagnosis of urinary tract infection among children with fever given its low sensitivity.

The specificity of combined dipstick and microscopy is similar to that of dipstick alone, but lower than the specificity of microscopy alone. Our finding is not surprising because simultaneous combination of screening tests result into net increase in sensitivity and net reduction in specificity [16]. The high specificity of urine microscopy (when used in isolation) makes it suitable for 'ruling in' diagnosis [8] since a positive test in a highly specific test implies very low possibility of a false positive result. In this study, urine microscopy (when used alone) showed relatively high sensitivity and specificity; and this qualifies it for use in both 'ruling out' and 'ruling in' diagnoses. In a disease like urinary tract infection where wrongly diagnosing a case (false positive) may have minimum psycho-social or economic consequences on the patient and/or the family, a low specificity poses little public health significance though may confound and delay diagnosis; and treatment of the differential causes of fever.

The positive predictive value of combined dipstick and microscopy is higher than that of dipstick alone but lower than the positive predictive value of microscopy alone. The high positive predictive value of microscopy (when used in isolation) makes it of choice in clinical decision making since high positive predictive value depicts disease given a positive test result. This high positive predictive value (of urine microscopy) makes it most appropriate for use at clinical decision making at clinician-patient level since it shows evidence of minimal false positive errors. The negative predictive value of the combination of urine dipstick and microscopy is much higher than that of either dipstick or microscopy alone. Our estimates of predictive value are not generalisable, but may only be transferred to contexts with similar prevalence since prevalence of a condition affects predictive values [17].

From the kappa test, urine microcopy was most reliable despite higher sensitivity of the combined tests. The high false positive errors (associated with the combined test) accounts for reduction in its reliability. This finding confirms single use of microscopy in screening of urinary tract infections among children as the most efficient test since the health facilities are not only saved from the extra cost of the test kits (for combined tests) but also that arising from treatment of false positive children. One of the characteristics of a good screening test is that it must be affordable and in our study, urine microscopy has proved cheaper of the two effective methods.

6. Conclusion

The use of urine microscopy (in isolation) is highly accurate and reliable in diagnosing urinary tract infections among children aged 0-59 months in Uganda. Employing it in screening of all febrile under-fives in Ugandan hospitals could efficiently improve promptness of diagnosis, treatment; and treatment outcome among children infected with urinary tract infections.

Acknowledgements

The financing of this study was majorly from the co-author. We acknowledge the in-kind support of the study hospital through provision of human resources and laboratory reagents. Table 2 summarizes the data reported in this paper. The authors declare no conflict of interest in this study and the paper.

Table 2. *Summary of the screening test results.*

Screening test	Results	UTI	No UTI	Total	Kappa
Combined dipstick and microscopy	Positive	80	11	91	
	Negative	1	210	211	0.903
	Total	81	221	302	
Dipstick	Positive	38	10	48	
	Negative	43	211	254	0.487
	Total	81	221	302	
Microscopy	Positive	77	4	81	
	Negative	4	217	221	0.933
	Total	81	221	302	

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