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Correlation of urine culture with urine microscopy, leucocyte esterase and dipstick nitrite in detection of Urinary tract infection

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ABSTRACT

Background. Improving diagnostic testing for urinary tract infections (UTI) helps to minimize unneeded antibiotics, which contribute to the rising issue of antibiotic resistance. As a result, the current investigation correlates urine culture with urine microscopy, leucocyte esterase, & dipstick nitrite in detecting UTI.

Method. Background. A prospective observational study performed to correlate the urine culture with urine microscopy, leucocyte esterase & dipstick nitrite methods in detection of UTI. Based on the detailed history, clinical findings, and laboratory data, along with inclusion, exclusion criteria, and with consent of parents, 76 children were eligible for the study. Urine samples were collected, and all samples were subjected to microscopy, dipstick leucocyte esterase and nitrite methods.

Results: Urine examination revealed 38 samples were culture positive and 38 were culture negative. Culture examination results show that the Organisms isolated were E. coli (n=31), Pseudomonas (n=4) and Klebsiella (n=3). Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of the urine microscopy were 97.37%, 18.42%, 54.41%, and 97.5% respectively; those of the urine dipstick leucocyte esterase were 76.32%, 63.16%, 72.73% & 67.44% respectively; and those for urine dipstick nitrite detection 39.47%, 100%, 100%, & 62.3% respectively. When any of the three screening tests were considered positive, sensitivity and negative predictive value increased to 100%. A total of 100 % could be due to a small sample size. Even then by considering any one of the screening tests positive as significant, sensitivity increased than each screening test using alone.

Conclusion. This study trying to emphasize that considering dipstick, esterase & nitrite as screening tests along with microscopy will reduce the chance of missing a case of UTI. Delay in diagnosing a case of UTI may lead to complications, further renal scarring and renal failure according to severity.

Keywords: Urinary tract infection, microscopy, E.coli, dipstick nitrite, leucocyte esterase

INTRODUCTION

Urinary tract infection (UTI) is a common bacterial infection in children. UTIs have been identified as a risk factor for the development of renal insufficiency or end-stage renal disease in children, although the significance of UTI as a standalone risk factor has been questioned, as only 2% of children with renal insufficiency [1] disclose a history of UTI. This contradiction may be due to increased awareness of the hazards of UTI and early identification and treatment. Furthermore, many children are given antibiotics for fever without a particular diagnosis (eg: to treat a doubtful otitis media), which leads to partially cured UTI. UTI is diagnosed mostly by symptoms and signs, but a positive bacterial culture of the urine is required for a definite diagnosis. Urine culture is both expensive and time-consuming, as the results often take 48-72 hours to reach the clinician. The quality of the urine sample will influence the capacity to detect bacteria and confirm the diagnosis of UTI [2].

Other UTI screening tests include dipstick leucocyte esterase and nitrite. The leukocyte esterase test is a semi-quantitative method that measures neutrophil-specific esterase activity produced by destroyed white blood cells. Nitrite reduction tests identify nitrite generated by urine bacterial infections. Nitrites are not normally detected in urine and are produced when urinary bacteria convert nitrates to nitrites. Many gram-negative & gram-positive bacteria are capable of accomplishing this [3]. A positive dipstick nitrite test indicates that such organisms are present in large numbers (>100,000 per mL) [4].

Urine analysis results, particularly leukocyte esterase and nitrite tests, are frequently used to establish whether or not treatment is required. In the event of a suspected urinary tract infection, a culture will be conducted. Many clinicians interpret positive test results as markers of possible infection and use them to guide patient therapy.

However, there is some disagreement over the effectiveness of urinalysis as a screening test for urinary tract infections. The current study compared the effectiveness of dipstick (leukocyte esterase, nitrite) tests, urine microscopy, & urine culture in detecting UTI in children aged 1 month to 12 years.

Material & Methods

Study site: outpatient department (OPD) & inpatient department (IPD) of Ekta Institute of Child Health, Shanti Nagar, Raipur, India.

Study population: Pediatric age group between 1 month to 12 year with clinical suspicion of UTI in OPD & IPD departments at EKTA institute of child health, Raipur, India.

Study design: A Prospective observational study

Sample size: All the children between 1month & 12 year with clinical suspicion of UTI in OPD & IPD of Ekta institute of child health during the study period and whose parents give consent.

Period of study: October 2014 to September 2016.

Inclusion criteria:

1. Children of age 1 month to 12 year with clinical suspicion of UTI.

Exclusion criteria:

1. Prior administration of antibiotic therapy in past 72hrs.

Method of collection of data:

Primary diagnosis will be determined at the time of outpatient department visit in patients with fever, frequent micturition, dysuria/baby crying during micturition, vomiting, anorexia, etc.

Urine sample collected either midstream catch, catheterized, or supra pubic aspiration [5] as feasible as possible. Along with them other lab parameters for infection will also be done. Specimens with squamous epithelial cells were not eliminated from analysis because other research shows that the presence of squamous cells does not impair the diagnostic accuracy of the test [6].

Urine sample is subjected to urine microscopy, dipstick leucocyte esterase, Nitrite & urine culture. Dip stick reports and [6,7] urine microscopy collected and followed for culture report & collected and analyzed.

Procedure:

Urine routine microscopy: Urine sample collected as explained above is subjected to centrifugation. After centrifugation, sediment at the base is collected and made a

slide with cover slip on the sample and observed in high power field [8]. Urinary microscopy is considered as suggestive of UTI if;

1) Pus cells are >10 in uncentrifuged sample,

2) Pus cells are >5/hpf in centrifuged sample.

According to Hoberman & Wald [9], pyuria (10 white blood cells/mm^3) & bacteriuria have a positive predictive value of up to 84.6% each. Because of its low sensitivity, negative urine microscopy does not rule out a UTI.

Urine leucocyte esterase is a good indication of leukocytes in urine. A positive reaction (small or bigger) with a reading duration of fewer than two minutes may indicate the existence of leukocytes within the urine. Esterases are enzyme found in granulocytic leukocytes catalyze the hydrolysis of the derivatized pyrrole amino acid ester, releasing 3-hydroxy-5-pyrrole. This pyrrole then reacts with a diazonium salt, resulting in a purple product.

Ingredients: 0.4% w/w derivate pyrrole amino acid ester; 0.2%w/w diazonium salt; 40.9% w/w buffer; 58.5% w/w non-reactive ingredients.

Urine dipstick nitrite: Dipstick nitrite is unique to nitrite & will not react with any other substance found in urine. The concentration of nitrite rises as the urine specimen remains in the bladder before to collection. A minimum of 4 hours of bladder incubation considerably increases the chances of getting a good outcome. This test is based on the conversion of nitrate to nitrite by gram negative bacteria in the urine. At the acid pH of the reagent area, nitrite in urine combines with p-arsanilic acid to generate a Diazonium molecule. This Diazonium molecule partners with 1,2,3,4-tetrahydrobenzo(h)quinolin-3-ol, producing a pink color.

Ingredients: 1.4% w/w p-arsanilic acid; 1.3%w/w 1,2,3,4,- tetrahydrobenzo (h) quinolin-3-ol; 10.8% w/w buffer; 86.5% w/w nonreactive ingredients.

Urine culture: Hicrome UTI agar is a differential medium designed to identify bacteria that cause urinary tract infections. Suspend 56.8 gr in 1000 mL distilled water. Heat to boiling point to completely disintegrate the medium. Sterilize by autoclaving for 15 minutes at fifteen lbs pressure (121^0 degrees Celsius). Cool to 50^0 Celsius. Combine thoroughly and pour into sterilized Petri plates. Cultural traits were observed following an 18-24-hour incubation period at 35-370 degrees Celsius.

Ingredients: 15gms/l of peptic digestion of animal tissue, 26.8gms/l of chromogenic mixture, and 15gms/l of agar. The final pH is 6.8 at 25^0c. The cutoff for significant bacteriuria was 10^5 cfu/mL [10].

Statistical methods:

Calculated sensitivity, specificity, and predictive values for dipstick tests for leukocyte esterase, nitrite, or blood, & microscopic urinalysis for RBCs, WBCs, or microorganisms. The sensitivity, specificity, & predictive values were computed as follows [11]:

Sensitivity=True positive/(True positive+False negative).

Specificity=True negative/(True negative+False positive).

Positive Predictive Value= True positive/(True positive + False positive).

Negative Predictive Value= True negative/ (True negative + False negative).

RESULTS

Out of 76 samples studied 36 (47%) were males and 40 (53%) were females.

Out of total children, 14 (18%) were infants and 62 (82%) were 1 to 12 yr.

Out of 76 samples, 68 turned out to be positive for microscopy and 8 were negative (Table 1).

Out of 76 samples, 43 were positive for leucocyte esterase & 33 were negative. Among 76 samples, 15 samples were positive for nitrite & 61 were negative. Out of 76 samples cultured, 38 (50%) were positive & 38 (50%) were negative.

Table1. Basic Characterization of sample & their distribution

	Frequency,n	%
Urine Microscopy		
Positive	68	89
Negative	8	11
Leucocyte esterase		
Positive	43	56
Negative	33	44
Nitrite		
Positive	15	20
Negative	61	80
Urine culture		

Positive(yield growth)	38	50
Negative(no growth)	38	50
Isolated organism		
E.coli	31	
Pseudomonas	4	
Klebsiella	3	

Out of total study population (n=76), there are 38 cases of culture proven UTI and 38 cases of suspected UTI which were culture negative where culture is standard. 37 cases were positive for microscopy (true positive) & one case was negative (false negative) when culture was positive. 7 cases with negative culture were negative for microscopy (true negative), while 31 cases in spite of no growth showed positive microscopy (false positive). Sensitivity of microscopy showed that positive microscopic results can correctly identify 97.37% of cases with UTI when culture is positive. Specificity of microscopy showed that negative microscopic results can correctly identify 18.42% of cases without UTI when culture is negative.

(PPV of microscopy is 54.41% probability that subjects with a positive test truly have UTI. NPV of microscopy is 97.50% probability that subjects with a negative test do not have UTI. A significant association was observed between microscopy & culture (Table 2).

Table 2. Assessment of sensitivity, specificity & predictive values of Microscopy with culture as Standard

		Culture				Total	$\chi 2$
		+	N	-	n		
Microscopy	Positive	True Positive	37	False Positive	31	68	5.02 Yates Correction 3.52 p<0.051
	Negative	False Negative	01	True negative	07	08	
Total			38		38	76	

	Sensitivity	Specificity	PPV	NPV

Microscopy	97.37%	18.42%	54.41%	97.50%

Out of total study population (n=76), there are 38 cases of culture proven UTI and 38 cases of suspected UTI which were culture negative where culture is standard.

29 cases were positive for leucocyte esterase (true positive) and 9 cases were negative (false negative) when culture was positive. 24 cases with negative culture were negative for leucocyte esterase (true negative), while 14 cases in spite of no growth showed positive leucocyte esterase (false positive).

Sensitivity of leucocyte esterase showed that positive leucocyte esterase results can correctly identify 76.32% of cases with UTI when culture is positive.

Specificity of leucocyte esterase showed that negative leucocyte esterase results can correctly identify 63.16% of cases without UTI when culture is negative.

PPV of leucocyte esterase showed 67.44% probability that subjects with a positive test truly have UTI.

NPV of leucocyte esterase showed 72.73% probability that subjects with a negative test do not have UTI (Table 3).

A significant association was observed between leucocyte esterase & culture.

Table 3. Sensitivity, Specificity & Predictive values of Leucocyte esterase

		Culture				Total	$\chi 2$
		+	N	-	N		
LET	Positive	True Positive	29	False Positive	14	43	12.05 p<.00005
	Negative	False Negative	09	True negative	24	33	
Total			38		38	76	

	Sensitivity	**Specificity**	**PPV**	**NPV**
LET	76.32%	63.16%	67.44%	72.73%

Table 4. Sensitivity, Specificity & Predictive values of Nitrite

	Culture				Total	$\chi 2$
	+	N	-	n		

NT	Positive	True Positive	15	False Positive	00	15	18.68 Yates
	Negative	False	23	True	38	61	Correction
		Negative		negative			16.28 p<.00005
Total			38		38	76	

	Sensitivity	**Specificity**	**PPV**	**NPV**
NT	39.47%	100%	100%	62.30%

Out of total study population (n=76), there are 38 cases of culture proven UTI and 38 cases of suspected UTI which were culture negative where culture is standard. 15 cases were positive for nitrite (true positive) & 23 cases were negative (false negative) when culture was positive. 38 cases with negative culture were negative for nitrite (true negative), while no cases in spite of no growth showed positive nitrite (false positive). Sensitivity of nitrite showed that positive nitrite results can correctly identify 39.47% of cases with UTI when culture is positive. Specificity of nitrite showed that negative nitrite results can correctly identify 100% of cases without UTI when culture is negative. PPV of nitrite showed 100% probability that subjects with a positive test truly have UTI. NPV of nitrite showed 62.3% probability that subjects with a negative test do not have UTI (Table 4). A significant association was observed between nitrite & culture.

Table 5. Sensitivity, Specificity & Predictive values of Either Leucocyte esterase or nitrite positive

		Culture				Total	$\chi 2$ TEST
		+	N	-	n		
LET/NT	Positive	True Positive	33	False Positive	14	47	$\chi 2$=20.12 P=0.000007
	Negative	False Negative	05	True negative	24	29	
Total			38		38	76	

	Sensitivity	Specificity	PPV	NPV
LET/NT	86.84%	63.16%	70.21%	82.76%

Out of total study population (n=76), there are 38 cases of culture proven UTI and 38 cases of suspected UTI which were culture negative where culture is standard.

33 cases were positive if either of esterase or nitrite considered positive (true positive) and 5 cases were negative (false negative) when culture is positive. 24 cases with negative culture were negative when either of esterase or nitrite considered positive (true negative), while 14 cases in spite of no growth were positive when either of esterase or nitrite considered positive (false positive).

Sensitivity when either of esterase or nitrite considered positive showed that positive results when either of esterase or nitrite considered positive can correctly identify 86.84% of cases with UTI when culture is positive (Table 5).

Specificity when either of esterase or nitrite considered positive showed that negative results when either of esterase or nitrite considered positive can correctly identify 63.16% of cases without UTI when culture is negative.

Table 6. Sensitivity, Specificity & Predictive values of any of Microscopy, Leucocyte esterase, Nitrite Positive

		Culture				Total	$\chi 2$
		+	N	-	N		
Micro/LET/ NT	Positive	True Positive	38	False Positive	32	70	6.51 Yates Correction 4.53 p<.0331
	Negative	False Negative	00	True negative	06	06	
Total			38		38	76	

	Sensitivity	Specificity	PPV	NPV
Microscopy/LET/NT	100%	15.79%	54.29%	100%

Out of total study population (n=76), there are 38 cases of culture proven UTI and 38 cases of suspected UTI which were culture negative where culture is standard. 38 cases were positive when any of microscopy, esterase, nitrite considered positive (true positive) and no cases were negative (false negative) while culture is positive. 6 cases with negative culture were negative when any of microscopy, esterase, nitrite considered positive (true negative), while 32 cases in spite of no growth were positive when any of microscopy, esterase, nitrite considered positive (false positive). Sensitivity when any of microscopy, esterase, nitrite considered positive showed that positive results when any of microscopy, esterase, nitrite considered positive can correctly identify 100% of cases with UTI while culture is positive. Specificity when any of microscopy, esterase, nitrite considered positive showed that negative results when any of microscopy, esterase, nitrite considered positive can correctly identify 15.79% of cases without UTI when culture is negative. PPV when any of microscopy, esterase, nitrite considered positive showed 54.29% probability that subjects with a positive test truly have UTI. NPV when any of microscopy, esterase, nitrite considered positive showed 100% probability that patients with a negative test do not have UTI (Table 6).

Table 6. Sensitivity, Specificity, Predictive values & Accuracy of Microscopy, LET, NT test

Test	Sensitivity (95% CI)	Specificity (95% CI)	Predictive vales (95% CI)		Accuracy (%)
			Positive	Negative	
Microscopy	97.37 (86.19;99.93)	18.42 (7.74; 34.33)	54.41 (41.88;66.55)	87.50 (47.35;99.68)	57.89
LET	76.32 (59.76;88.56)	63.16 (45.99;78.19)	67.44 51.46;80.92	72.73 (54.48;86.70)	69.73
NT	39.47 (24.04-56.6)	100 (90.75-100)	100 (78.20-100)	62.30 (48.96;74.39)	56.57
LET/NT	86.84 (71.91;95.59)	63.16 (45.99;78.19)	70.21 (55.11;82.66)	82.76 (64.23;94.15)	75.00
Micro/ LET/NT	100 (90.75;100.00)	15.99 (6.02;31.25)	54.29 (41.94;66.26)	100 (54.07;100)	57.89

DISCUSSION

This study performed to correlate urine culture with urine microscopy, leucocyte esterase and dipstick nitrite in detection of UTI.

In present study, out of 76 children studied, 47% were male and 53% female. 18% of the children in study were infants & 82% were between 1 and 12 yr.

Organisms isolated through culture were in order of E.coli (31), Pseudomonas (4) and Klebsiella (3) according to their frequency.

In our study, positive urine culture with significant bacteriuria (105) was found in 38 (50%) out of 76.

Adeleke et al study had 19 (29%) positive out of 65 urine sample cultured [12].

This variability in percentage of positivity of culture could be due to mode of collection of sample which has been done according to feasibility in all studies like collection of urine through sterile bags in infants in some studies. More positivity in our study can be due to strict clinical criteria utilization.

Current study got depicts the results regarding urine microscopy as sensitivity- of 97.37%, Specificity of 18.42%, PPV of 54.41%, & NPV of 97.5%.

Adeleke et al study had Sensitivity- 43%, PPV- 37.1% for urine microscopy method.

Hatice Yuksel, et al study shows Sensitivity of 91%, Specificity of 68%, PPV of 61%, NPV- 93% for urine microscopy [13] method.

More sensitivity and low specificity in our study can be due to small sample size and also can be due to observational variation by pathologists.

Our study got statistics regarding urine dipstick leucocyte esterase as Sensitivity of 76.32%, Specificity of 63.16%, PPV of 67.44%, and NPV of 72.73%.

Adeleke et al study had Sensitivity of 74%, and PPV of 87.2% for urine dipstick leucocyte esterase.

Hatice yuksel *et al* study had Sensitivity of 80%, Specificity of 60%, PPV of 52%, and NPV of 84% for urine dipstick leucocyte esterase.

Statistics for leucocyte esterase in our study correlate with other studies.

Current study outcome regarding urine dipstick nitrite as sensitivity of 39.47%, Specificity of 100%, PPV of 100%, & NPV of 62.3%.

U.S.Nayak [14] et al study shows Sensitivity of 50% for urine dipstick nitrite.

N Taneja [15] study shows Sensitivity of 73.5%, Specificity of 58.5%, PPV of 33%, NPV of 88.8% for urine dipstick nitrite.

Variation in sensitivity could be due to difference in duration of stasis of urine before

sample collection i.e. children less than 3-4 years cannot hold urine for long time. In our study we had to collect some samples even in day time because of noncompliant patients. 100% specificity can be because of small sample size.

Our study got statistics when considered urine dipstick leucocyte esterase or nitrite as positive as Sensitivity of 86.84%, specificity of 63.16%, PPV of 70.21% and NPV of 82.76%.

U.S.Nayak et al study had Sensitivity of 68% when considered urine dipstick leucocyte esterase or nitrite as positive.

N Taneja et al study had Sensitivity of 79.6%, specificity of 56.5%, PPV of 33.8%, and NPV of 90.9% when considered urine dipstick leucocyte esterase or nitrite as positive.

Any one of Microscopy, Leucocyte Esterase, Nitrite positive:

Our study got statistics when considered any one of urine microscopy, dipstick leucocyte esterase, nitrite as positive as follows: Sensitivity of 100%, Specificity of 15.79%, PPV of 54.29%, & NPV of 100%.

U.S.Nayak et al, study had Sensitivity of 75% when considered any one of urine microscopy, dipstick leucocyte esterase, nitrite as positive.

N Taneja, SS et al study had Sensitivity of 95.9%, Specificity of 52.3%, PPV of 35.9%, & NPV and 97.9% when considered any one of urine microscopy, dipstick leucocyte esterase, & nitrite as positive.

High sensitivity and low specificity in this combination in our study again probably due to small sample size.

CONCLUSION

Our study trying to emphasize that considering dipstick esterase and nitrite as screening tests along with microscopy will reduce the chance of missing a case of UTI. Delay in diagnosing a case of UTI may lead to complications, further renal scarring and renal failure according to severity.

Ethical statement & Informed Consent: The study was performed as per the Declaration of Helsinki, and written informed consent was obtained from all participants. The study protocol received approval from the Institutional Ethics Committee.

Conflict of interest: The authors declare no conflict of interest.

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