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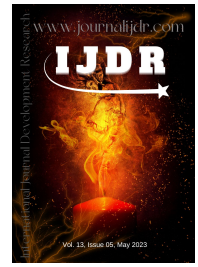
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RESEARCH ARTICLE

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EFFICACY OF NITRITE, LEUCOCYTE ESTERASE DIPSTICKS AND URINE MICROSCOPY IN THE DIAGNOSIS OF URINARY TRACT INFECTION IN FEBRILE CHILDREN AGED BETWEEN 1 YEAR TO 5 YEARS –A CROSS SECTIONAL DIAGNOSTIC STUDY

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ABSTRACT

Introduction: Recognition of urinary tract infection (UTI) in children may be difficult because of the non-specific presenting symptoms, particularly in younger children. Urine culture is still considered the gold standard in diagnosis of UTI, but culture takes 48 hours for the result, and culture facility may not be available in rural setup of our country. So dip sticks have the advantage of being quick and easy to perform, interpret and cost effective. Leucocyte esterase (LE) and Nitrite (NIT) Dip stick test have been commonly used in diagnosing UTI. Presence of one dip stick with high specificity may be a reliable and early method to diagnose UTI. So, this study was conducted to know the effectiveness the dipsticks over urine culture. **Objective:** To evaluate the effectiveness of Nitrite, Leucocyte esterase dipsticks and urine microscopy in diagnosis of UTI in febrile children between 1 to 5 years of age. **Material and Methods:** A cross sectional diagnostic study was done on 450 febrile children between age group of 1 to 5 years. Children who were on antibiotics 48 hours prior were excluded from the study. After obtaining valid informed consent from the parents, mid stream urine sample was collected in two sterile urine containers under aseptic precautions. One sample was processed for LE, NIT dipstick and urine microscopy and the other one for urine culture. The test could be read either visually by human eye or instrumentally using the SD Urometer urine chemistry analyzer. The test handling method and precautions as given in the Urocolor test manual were carefully followed. **Results:** Out of 450 urine samples, LE dip stick was positive in 186(41.3%) & there was statistically significant difference in positivity of LE dipstick in culture positive and negative samples. Out of 450 samples, urine culture was positive in 50 (11.1%). Out of 50 culture positive urine samples, NIT dipstick was positive in 32 cases. In 400 culture negative cases, NIT dipstick was positive in 43 (10.7%). E. coli was the predominant organism isolated in 35(70 %) out of 50 cases followed by klebsiella (10%). PPV, NPV, Sensitivity, Specificity of LE in predicting UTI was calculated. The combined sensitivity, specificity when Pus cells, LE, NIT dipstick were combined was 50%, 90% respectively. **Conclusion:** The present study suggests that a combination of leukocyte esterase and nitrite dipsticks is a reliable parameter in predicting UTI in febrile children. This study also suggests that, because of high specificity and NPV, LE and NIT urinary dipsticks could be used in daily OPD basis practice, along with urinary microscopy, to diagnose UTI.

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INTRODUCTION

Urinary tract infection is one of the most common bacterial sources of infection in children under five and it accounts for significant morbidity in pediatric patients (Hoberman, 1993). Roberts K.B. *et al.* reported overall prevalence of UTI as 4.1% in febrile children less than 2 years of age (Jeena *et al.*, 1996). Shaw K.N. *et al.* from U.S.A. reported 3.3% prevalence of UTI in febrile young (Downs, 1999).

Several studies have reported var (ying prevalence rates of UTI in children ranging from 3.3 in USA to 37.5% in Pakistan (Shaw *et al.*, 1998 and Anisur, 2008). The risk of developing UTI before the age of 14 years is approximately 1% in boys and 3-5% in girls (Hellerstein, 1995). The incidence of UTI varies with age, during the first year of life, male to female ratio 3-5:1 and beyond 1-2 years, there is female preponderance with male to female ratio of 1:10 (Shaikh *et al.*, 2008 and Wiswell, 1986). It may be difficult to recognize UTI in children because the presenting symptoms and/or signs are non-specific, particularly in younger children.

Although children with UTI tend to present with fever, it is often difficult on clinical grounds to distinguish UTI from other febrile illness in developing countries (Hoberman *et al.*, 2003 and Jeena *et al.*, 1996). That is why UTI is considered as one of the most often missed diagnosis in the Pediatrics in developing countries. Seeking laboratory confirmation of the diagnosis requires the initial step of collecting an uncontaminated urine sample and this is a challenge in infants and children who are not toilet-trained. Failure to consider a diagnosis of urine infection or delaying the antibiotic treatment of a urine infection can have the effect of producing an acute clinical deterioration and in addition it may result in long-term renal damage. Most UTI that lead to scarring or diminished kidney growth occur in children younger than age 4 years especially infants in the first year of life (Benador, 1994 and Berg, 1992). Risk factors for renal scarring is common in young infants especially when there is delay in initiating antibacterial treatment in recurrent UTI in Presence of moderate to severe vesicoureteric reflux (Hellerstein, 1995). Early diagnosis is important to preserve the function of growing kidney (Elder, 2004). Essential to the diagnosis of UTI is culture of urine (Seema Sood, 1999). Urine culture is still considered the gold standard in diagnosis of UTI, but culture has the disadvantage of taking at least 48 hours for the result, and culture facility is not available in rural setup of our country. More rapid methods of UTI diagnosis are therefore desirable. Several rapid screening techniques such as urinalysis plus gram stain, urine dipstick tests have been used in diagnosis of UTI (Antwi *et al.*, 2008 and Ayazi, 2007). The most widely used rapid tests are dipsticks. Analyte commonly tested by dipsticks include LE, NIT, Blood and protein. A Positive NIT test indicates that nitrite has been produced from reduction of nitrate by enteric bacteria, most commonly by Enterobacteriaceae family. LE detects WBCs via esterase, an enzyme released by WBCs. Positive test signals pyuria of the sample. Leucocyte esterase and Nitrite test have been commonly used in diagnosing UTI. Dip sticks with high specificity may be a reliable and early method to diagnose UTI. So this study was undertaken to evaluate the effectiveness of Nitrite, Leucocyte esterase dipsticks and urine microscopy in diagnosis of UTI in febrile children, which would help in rapid diagnosis and treatment of UTI.

MATERIAL AND METHODS

A cross sectional diagnostic study was done on febrile children between age group of 1 to 5 years. The study included 450 children by Purposive sampling and all the febrile children brought to Pediatric OPD or admitted with fever of greater than 38.3 degree Celsius for more than 24 hours, were included in the study, until the sample size was met. Children who were on antibiotics 48 hours prior were excluded from the study. UTI is a collective term for infections that involve any part of the urinary tract. The gold standard for the diagnosis of a urinary tract infection is the detection of the pathogen in the presence of clinical symptoms. The pathogen is detected and identified by urine culture (using midstream urine). According to KASS concept, in properly obtained midstream urine, 10^5 CFU/ml indicates significant urinary tract infection. After obtaining informed consent from the parents of study subjects, thorough clinical examination was done. Parents or Guardians of the study subject were explained about the technique for collecting midstream urine sample in sterile container. Before collecting the urine sample, perineal area was cleaned with warm tap water and cotton balls. Mid stream urine sample was then collected in two sterile urine containers. One of the samples was processed for dipstick and microscopy and the other for urine culture. Although nitrates are excreted by the kidney, nitrites are not normally found in urine. When bacteria reduce urinary nitrates to nitrites, the dipstick will identify this condition. One needs the presence of bacteria for the dipstick to register a positive nitrite. It generally requires more than 10,000 bacteria per ml to turn the dipstick positive, making it a specific but not a very sensitive test. A negative nitrite test does not rule out UTI, but a positive one strongly suggests infection. Dipsticks used were the SD Urocolor™ 10, manufactured by the Standard diagnostics, INC, Republic of Korea. SD Urocolor is a plastic strip which has several separate reagent areas

attached. Depending on the product being used, the strip provide tests for Blood, Bilirubin, Urobilinogen, Ketone, Protein, Nitrite, Glucose, pH, Specific gravity, Leucocytes in urine samples. The test could be read either visually by human eye or instrumentally using the SD Urometer urine chemistry analyzer. The test handling method and precautions as given in the Urocolor test manual were carefully followed. Reagent strips were kept in bottle with cap tightly closed. Strips were never removed from the bottle until it was to be used for testing. Cap was replaced immediately and tightly after removing the test strip. Dipsticks were stored at room temperature and never refrigerated. Human neutrophils produce proteins with esterolytic activity. Proteins with esterolytic activity hydrolyze ester substrates, which is the basis of LE tests. Leucocyte esterase reacts with agents on the dipstick to produce a blue color. After collecting the midstream urine sample in dry container, the tests areas of the reagent strip were completely immersed in the urine. The strip was laid on its side on tissue for 1-2 seconds to remove excess urine. The strip was held in horizontal position to prevent possible mixing of chemicals from adjacent areas. The color of the reagent areas on the test strip was compared to the closest corresponding color on the color chart on the bottle label. The test results were read at 60 seconds and after reading the test, strip was discarded.

The dipstick results were noted. The urine sample was labeled and sends for microscopic examination to the department of pathology. Urine microscopic examination was done on uncentrifuged sample. Urine was examined under microscope for Pus cells, casts, RBCs, crystals, and the report was noted. Pus cells > 10 in uncentrifuged sample were considered to be positive. The second sample of the urine was processed for urine culture, in department of microbiology. All the urine samples were processed for culture within 4 hours of collection. Urine culture was done using a standardized 1microlitre loop and urine from the loop was streaked on Urochrome agar plate. The plate was put for incubation for 48 hours and results were noted. Statistical methods applied as required, summary statistics was done using mean, median, Inferential statistics was done using sensitivity, specificity, Positive predictive value, Negative predictive value. P value < 0.05 was considered significant. SPSS version 22 was used for all measurements. Graphical presentation is done using Microsoft excel and SPSS.

RESULTS

The present study included 450 cases and majority (34.8%) of them were in the age group of 1 to 2 years. Out of 450 cases, 271 (60%) were males and 179 (40%) were females

Table 1. Age wise distribution of study subjects

		Number	%
AGE (Yrs)	1-2 yr	157	34.8%
	2-3yr	80	17.8%
	3-4 yr	62	13.8%
	4-5 yr	70	15.6%
	5-6 yr	81	18.0%

Out of 450 urine samples, 187 (41.5%) samples had no pus cells, 175 (38.9%) samples had < 10 pus cells and 88 (19.6%) samples had pus cells more than 10. Only 11 samples (2.4 %) had plenty of RBCs. Out of 450 cases, 317 (70.5%) urine samples had no protein, 92 (20.4 %) samples had 1+ proteinuria, 30 (6.7%) samples had 2 + proteinuria and only 2 (0.4%) samples had 4+ proteinuria. 47(10.4%) samples out of 450 urine samples, showed leucocyte esterase reading of 1 +, 27 (6%) samples showed reading of 2+, 63 (14%) samples showed reading of 3+. Out of 450 samples, Nitrite dipstick showed positive test result in 75 cases (16.7%) and negative in 375 (83.3%) samples. Out of 450 urine samples, 110 (24.4%) samples showed the growth of organisms and but of these 110 samples, significant growth of 10^5 was seen in 50 (11.7%) samples. E.Coli was the predominant organism isolated from the culture positive samples accounting for 70%, followed by klebsiella pneumonia, accounted for 10%. Nitrite dipstick was positive in total of 75(16.7%) samples out of 450. Out of

50 culture positive urine samples, Nitrite dipstick was positive in 32 cases and negative in 18 cases. In culture negative cases, Nitrite dipstick was positive in 43 (10.7%) samples and negative in 357 (89.2%). Out of 50 culture positive urine samples, RBCs were present in plenty in only 1 (2%) sample with P value of 0.4.

Table 2. Estimation of sensitivity, specificity, ppv and npv of nitrite positive dipsticks

Parameter	Estimate	Lower - Upper 95% CIs
Sensitivity	64%	(50.14, 75.86)
Specificity	89.25%	(85.83, 91.92)
Positive Predictive Value	42.67%	(32.1, 53.95)
Negative Predictive Value	95.2%	(92.54, 96.9)
Diagnostic Accuracy	86.44%	(82.97, 89.3)

Out of 450 samples, Leucocyte esterase dipstick was negative in 264 (58.7%) samples. There was statistically significant difference in positivity of leucocyte esterase dipstick in culture positive and negative samples. Out of 50 culture positive samples, Leucocyte esterase dipstick was negative in 8 (16%) samples, it was positive in 42 (84%) samples. $P < 0.0001$.

Table 3. Estimation of sensitivity, specificity, ppv, npv of leucocyte esterase dipstick

Parameter	Estimate	Lower - Upper 95% CIs
Sensitivity	84%	(71.49, 91.66)
Specificity	64%	(59.18, 68.55)
Positive Predictive Value	22.58%	(17.16, 29.11)
Negative Predictive Value	96.97%	(94.14, 98.46)
Diagnostic Accuracy	66.22%	(61.73, 70.44)

In the 50 urine culture positive samples, Only 4 (8%) samples had no pus cells, 11 (22%) samples had plenty of pus cells, and 16 (32%) samples had pus cells between 11-20 with P value of < 0.0001 indicating a statistically significant difference between presence of pus cells in culture positive and culture negative samples. Out of 50 culture positive samples, only 18 (36%) samples had no proteinuria, Urine protein was 1+ in 22 (44%) samples, 2+ in 7 (14%) samples and 3+ in 2 (4%) samples.

Table 4. Combination of various parameters and comparison with culture positive urine samples

PARAMETER		SIGNIFICANT GROWTH	
		Culture Positive	Culture Negative
		Count	Count
LES and Nitrite	Positive	32	43
	Negative	18	357
Pus cells, LES, And Nitrite	Positive	25	37
	Negative	25	363
Pus cells or LES, Or Nitrite	Positive	42	144
	Negative	08	256
Protein or Nitrite or LES	Positive	46	163
	Negative	04	237
	TOTAL	50	400

When both Leucocyte esterase and Nitrite dipsticks are positive, Sensitivity was 64%, Specificity was 89.25%, PPV was 42.6%, NPV was 95.2%, Diagnostic accuracy was 86.4%. When Pus cells, Leucocyte esterase, Nitrite Positive dipsticks are combined, Sensitivity was 50%, Specificity was 90.75%, Positive predictive value was 40.3%, Negative predictive value is 93.5%, Diagnostic accuracy is 86.2%. When Pus cells were combined with either leucocyte esterase or Nitrite Positive Dipsticks, Sensitivity was 84%, Specificity was 64%, PPV was 22.5%, NPV was 96.9%, Diagnostic accuracy was 66.2%. When Proteinuria were combined with either leucocyte esterase or Nitrite positive dipsticks, Sensitivity was 92%, Specificity was 59.2%, PPV is 22%, NPV was 98.3%, Diagnostic accuracy was 62.8%.

DISCUSSION

UTI is a common source of infection in children and infants in children < 2 years of age, both in the community and hospital setting (Hanna-Wakim *et al.*, 2015). The ideal screening test for significant bacteriuria should be rapid, inexpensive, simple to use, preferably independent of the organism grown, and should be accurate screening test because of its rapidity and low cost, easy to perform and interpret. Sensitivity, specificity and positive and negative predictive values of the dipsticks in relation to urine culture were determined in the present study showed positive correlation in the present study. Similar studies done by other authors were comparable with the present study, to quote a few, Laosuankoon studied 109 children and determined the sensitivity and specificity of urine LE, Nitrite test in an outpatient clinic (Laosu-ankoon, 2023). Sensitivity of combined LE and NIT as 66.7% Sensitivity of LE alone was 63.6%. Concluded that dipstick should be added in ER department for quick diagnosis of UTI especially in children to prevent potential sequel like hypertension and renal scarring the observations were comparable to the present study. R. Devaraja, P. T. Tamizharasu who studied 100 cases observed higher predictive values for LE and NIT.¹⁹ Sensitivity, specificity, PPV and NPV for NIT were 10.2%, 100%, 100% and 85.08% respectively, whereas that for LE were 61.22%, 98.8%, 90.91% and 92.88% respectively. The study has suggested that both dipstick urinalysis methods can be used for rapid diagnosis but the present study suggested that combined LE and NIT are more reliable in detecting UTI. Sundvall *et al.* in his study population of 100 determined the sensitivity, specificity, of LE, NIT for catheterized samples (Sundvall, 2009). Sensitivity of LE was 59% and NIT was 20%, while specificities were 84% and 97% respectively. Results showed that if both NIT and LE were negative, it was less likely that culture results were positive. Similar observation was noted in the present study. Manoj Sankar studied 100 children for reliability of urine LE and NIT dipstick analysis and microscopy as a predictor of UTI and noted that Sensitivity, specificity, PPV and NPV for NIT test were 10.2%, 100%, 100% and 85.08% respectively, whereas that for LE were 61.22%, 98.8%, 90.91% and 92.88% respectively. They concluded that Combination of LE and NIT and Pyuria are reliable parameters in predicting UTI in children (Reliability of Urine Dipstick Analysis and Microscopy as a Predictor of Urinary Tract Infection, 2015). LE and NIT Urine dipstick screening test done on 6394 children by Glissmeyer *et al.* showed higher predictive values for LE and NIT (Glissmeyer, 2014). They concluded that dipstick is a reliable screening tool, and could be used in emergency department for diagnosis of UTI and similar observation was noted in the present study (Benador, 1994).

Taneja *et al.*, who studied 450 febrile children noted the sensitivity, specificity, PPV, NPV of LE and Nitrite.²³ Sensitivity, Specificity, Positive PPV and NPV for LE were 73.5%, 58.5%, 33.0% and 88.8% respectively and for NIT were 57.1%, 78.7%, 42.7% and 86.8% respectively. Suggested that for faster diagnosis of UTI, dipstick tests for LE and NIT should be added in routine laboratory practices. In the present study, out of 450 samples, 50 urine cultures was positive amounting to 11.1%. Amongst the culture positive samples, E.coli was the most predominant organism grown in 70% samples, followed by klebsiella which constituted 10%. In the present study Sensitivity, Specificity, PPV, NPV of LE dipstick in predicting UTI were 84%, 64%, 22.58%, 96.97% respectively which was similar to the study done by Goldsmith *et al.* and Perry *et al.* (Goldsmith and Campos, 1990 & Coulthard *et al.*, 2010). In the present study sensitivity of NIT dipstick (64%) was lower compared to LE dipstick (84%) It could be because of low colony count forming unit or dilute urine. Muna *et al.* and Adeleke *et al.*, also have noted LE sensitivity ranged from 75-85%, and diagnostic accuracy of the test was 66.22%. (Muna, 2008 and Adeleke *et al.*). The present study revealed a higher diagnostic accuracy of 86.44%. compared to study by Muna *et al.* Gender wise distribution of our study was similar to the study conducted by Robert Anguyo *et al.* In the present study combined Sensitivity, Specificity, PPV and NPV and diagnostic accuracy of LE and NIT dipsticks were 64%, 89.25%, 42.67%, 95.2%, 86.44% respectively. It was observed

that these values were same as that of NIT dipstick alone. The reason was LE dipstick was positive in all the samples in which NIT dipstick was positive, therefore accounted for lower sensitivity, specificity rates when both the parameters were combined. In the present study, when 3 parameters like Pyuria, LE, NIT Positive dipsticks were combined Sensitivity was 50%, Specificity of 90.75%, PPV of 40.3 %, NPV of 93.5 % and diagnostic accuracy of the tests was 86.2%. Compared to other studies, it was found that sensitivity rate obtained was low, whereas Specificity and NPV was high in the present study. High rate of specificity, high NPV indicate that UTI can be ruled out if Pyuria, NIT and LE dipsticks were negative. NIT dipstick does not detect the organisms which are unable to reduce nitrate to nitrite such as enterococci, staphylococci species and acinetobacter which were isolated from few of our culture positive urine samples was the limitation of the study.

CONCLUSION

The present study concludes that a combination of leukocyte esterase and nitrite dipsticks is a reliable parameter in predicting UTI in febrile children. Dipstick test would ensure faster diagnosis and early treatment and also curb unnecessary use of empirical antibiotics. Because of high specificity and NPV of LE and NIT dipsticks, this study suggests that LE and NIT dipsticks along with urinary microscopy can be used to diagnose UTI in OPD practice.

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