Urinary Dipstick Nitrite And Leucocyte Estrase Test in Comparison To Urine Culture in Diagnosis of Urinary Tract Infection in Children

[1]Dr. Jaswir Singh, [2]Dr. Manpreet Sodhi, [3]Dr. Vasu Pandey
[1]M.D, FIMSA, [2]M.D, [3]M.D,
Department of Pediatrics, Government Medical College, Patiala, India

*Corresponding Author:
Dr. Manpreet Sodhi
M.D., Department of Pediatrics, Government Medical College, Patiala, India

Abstract

Objective: To compare accuracy of urinary dipstick nitrite and leucocyte estrase(LE) test for rapid screening of urinary samples in comparison to urinary culture in diagnosis of urinary tract infection in children.

Methods: It was a prospective observational study done in tertiary level hospital conducted over a period of 1 year. Children in age group of 2-18 yrs satisafying inclusion criteria were selected. Two clean catch urinary samples were collected. One sample was sent for culture and second was subjected to urinary dipstick test. The sensitivity, specificity, positive predictive value(PPV) and negative predictive value(NPV) of LE and Nitrite test was satistically calculated

Results: The sensitivity, specificity, PPV and NPV of nitrite test was34.6%,98.6%,97.2% and 60 % respectively whereas sensitivity, specificity, PPV and NPV of LE test was 80%,89.3%,88.2% and 81.7% respectively. When data was combined for both test sensitivity, specificity, PPV and NPV was 96%,98.6%,98.6% and 96% respectively.

Conclusion: The LE test was more sensitive in diagnosis of UTI whereas Nitrite test was more specific so helped in excluding UTI.Both test done together had both high sensitivity and specificity.PPV of Nitrite test was more than LE test close to PPV of both test together

Keywords: Leucocyte Estrase Test; Nitrite Test;Urinary Tract Infection; Urinary Dipstick

Introduction

Urinary Tract Infection(UTI) represents the most commonly encountered genitourinary disease in children and accounts for significant morbidity in pediatric patients. The risk of developing urinary tract infection before 14 years of age is 1% in boys and 3-5% in girls.[1] These infections may be present in children without any obvious presenting symptoms or may present atypically and because of paucity of clinical signs and symptoms, diagnosis may be difficult and delayed.[1] Although children with UTI tend to present with fever, it is often difficult on clinical grounds to distinguish UTI from other febrile illnesses in developing countries. This makes UTI one of the most often missed diagnosis in the pediatric ward in developing countries.[2,3] The gold standard method to diagnose UTI is urine culture. In it, if there is a significant number of bacteria, fungi, and virus present, then it is only diagnosed as UTI.[4,5] Accurate diagnosis is important in children to identify the disease , start proper treatment and prevent renal damage. Chemstrips (dipsticks) is a test strip that detects Leucocyte Estrase(LE) and Nitrite in the urine specimen. The leucocyte esterase test is specific to neutrophils that are present in urine due to inflammation of the urinary tract including kidney, bladder, urethra, and prostate, the esterase's, are not

present in normal serum, urine, or kidney tissue in absence of inflammation.[6].

Urine nitrite is produced by nitrate-reducing organisms present in the urine specimen and has been used for a long time as a screen for UTI. Rapid diagnostic tests can rule out urine infection and are inexpensive, less time-consuming, and are useful in areas where there is no culture facility available, they are also more rapid than culture in diagnosing complicated and uncomplicated UTI. A sterile urine sample is not required for dipstick test, therefore it is easy to collect samples especially in children by a non-invasive method.[7,8,9]

Keeping this in view, this study was planned to assess the diagnostic value of dipstick LE and Nitrite test in form of Sensitivity, Specificity,Positive Predictive Value(PPV) and Negative Predictive Value(NPV).

Subjects And Methods

This study was conducted at the Department of Pediatrics among outdoor and indoor patients in Rajindra Hospital/Government Medical College Patiala. It was a prospective and observational study over period from July 2019 to June 2020. The study was conducted on 150 children both from outdoor as well as indoor from 2-18 years age group.The study was approved by ethical committee of the institute vide letter no.8(109)2019/7856 dated 5/4/2019.Informed consent was taken from parents.

Inclusion Criteria:

Presence Of Urinary Symptoms In Children Of Age 2-18 Years Suggestive Of UTI.

1. Dysuria.
2. Urinary frequency.
3. Urgency.
4. Incontinence.
5. Dribbling of urine.
6. Vomiting.
7. Fever.
8. Abdominal or flank pain.[10]
9. In children having fever without focus.
10. Children with severe malnutrition.
11. Children with nephrotic syndrome.
12. With congenital renal malformation.

Exclusion Criteria:

1. Children already on antibiotic therapy

2. Having indwelling foley's catheter.
3. Symptomatic vaginal discharge.
4. Diabetes mellitus.
5. Immunodeficiency disorder.

Procedure

The case history was taken from reliable informant, mostly from the parents, clinical examination was done with parents consent, with special attention to the genitourinary system, Parents were given instructions regarding cleaning of the genitalia with soap and water. In male child, foreskin was retracted and glans was cleaned while voiding.In female child parents were advised to wipe from front to back and labia was spread while voiding.Clean catch, mid stream urine (MSU) samples were collected in sterile and wide mouthed bottle for dipsticks and in urine culture vial for urine culture and sensitivity.Culture samples were transported as quickly as possible and processed as soon as possible in microbiology laboratory.Dipsticks from SIEMENS , Multistix GP were used.

Chemstrip leucocyte esterase and nitrite consists of inert plastic strip with two test papers attached by nylon mesh. One paper contains an indoxylcarbonic acid ester that was hydrolyzed to indoxyl which reacts with diazonium to produce purple color, indigo (LE). The second paper was impregnated with an aromatic amine that couples to nitrite to give a diazonium salt that in turn produces a red —violet azo dye with sulfanilamide (Nitrite). The reagent strip was placed in urine for 1 second, tapped on the edge of container to shake off excess urine and observed for color reaction. Nitrite was considered as positive if there is change in color of dipstick from colourless to pink in 30 seconds.[11] Leucocyte esterase was considered as positive if there is change in color of dipstick from off white to purple within 2 minutes, positive reactions for leucocyte esterase and nitrite was considered as positive test by reagent strip. Urine culture was done by a lab technician, under the supervision of microbiologist. The result obtained from urine dipsticks were compared with urine culture.

The results were divided into two groups, culture proven UTI and the sterile culture group. The true positive, true negative, false positive, false negative values were obtained and specificity, specificity, positive and negative predictive value was calculated.

Leukocyte esterase was interpreted as negative,1+,2+,3+.Any result >or =1+ was considered as positive. Nitrites were reported as positive or negative based on color change

Culture Based Diagnosis: Urine was sent to the microbiology department of Government Medical college Patiala. To isolate the causative organism and antibiotic susceptibility pattern

Method:

Accurate diagnosis of UTI was based on culture characters on the inoculated plates and biochemical reactions. After specimen collection, specimen was inoculated on different media: Blood agar medium and MacConkey agar medium and in some cases where there was no growth on these 2 media, then the special medium was used. The plate was inoculated in the incubator for 24 hours (overnight). The causative organism was identified by colony characters, Grams staining, motility, and biochemical reactions, and any other special test for confirmation of the particular organism. After identification of the organism, antibiotic sensitivity test was done by Kirby- Bauer disc diffusion method [12] which used the antibiotic disc to test the extent to which bacteria were affected by those antibiotics. In this test wafers containing antibiotics were placed and the plate was left to incubate. Where Antibiotics stopped the bacteria from growing or killed the bacteria there was an area around the wafer where the bacteria have not grown enough to be visible. This area was called as Zone of Inhibition.Drugs of Penicillin, Cephalosporins, Fluoroquinolones,Glycopeptide, Carbepenem group were tested. The results of urine dipstick and urine culture were compared and the sensitivity, specificity, positive predictive value, and negative predictive values were calculated in both the culture-positive group and the sterile group.

SAMPLE SIZE: Sample Size Estimation Was Done Using The Formula:-

4 * p *q/d2

=4*75*25/100

(p=85- average sensitivity and specificity of all 3 parameters calculated from

the Pilot study [which was done before study to calculate sample size])

(q=100-P=100-75= 25)

(d-precision=10)

=7500 /100=75

The sample size was taken as 75 patients in each group, for a better outcome and considering the average number of patients, diagnosed with 1st episode of UTI per month in our hospital (~ 4-6 patients/ month).

The data collected from the patients were formatted into Microsoft Excel sheets to generate master charts, Tables, and graphs. The Sensitivity, specificity, negative predictive value, and positive predictive values were calculated using the standard formulas.

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)

SPSS software was used to analyze data. The correlation of parameters with age was assessed using chi-square Pearson co-efficient test. After calculating the sensitivity and specificity, Chi-square test has been used to find the significance of study parameters on a categorical scale between two groups. P-value: < 0.05 have been considered statistically significant.

Results:

The present study included 150 children.Distribution of cases according to age and sex was as shown in Table 1. The difference in number of male and female children was statistically insignificant(p>0.05). Most common symptom was fever in 132 cases (88%) followed by abdominal pain in 57 cases (38%), vomiting in 30 cases (20%), increased frequency in 21 (14%) cases, dysuria in 17 (11%) cases, urgency in 12 (8%)cases, refusal to feed in 11(7.3%) cases, constipation in 8 (5.3%) cases, poor weight gain in 8(5.3%) cases and lethargy in 6(4%) cases. Out of the 150 cases, 75 were culture positive. Among the culture positive group, In 2-5 y age group, there were 44 cases (58.6%), in 6-10y, there were 19 cases (25.3%) and 11-18 y, there were 12 cases (16%). It was observed that there were more percentage of children in the younger age group.

Among study , in males, 29 cases and in females, 46 cases were culture positive. In the present study most common organism was Esch.coli (52%), followed by Klebsiella pneumonia (25%), then proteus species(8%), then pseudomonas (5.1%), among gram-positive organism, staphylococcus species(5.1%) followed by Citrobacter (2.4%) and Acinetobacter(2.4%).

Among culture proven UTI cases, nitrite test was positive in 26 cases (34.6%) and negative in 49 cases (65.3%).Among culture sterile group, nitrite test was positive in 1 case (1.3%) and negative in 74 cases(98.6%). The sensitivity and specificity of test was 34.6% and 98.6% respectively.The PPV and NPV was 97.2% and 60% respectively(Table 2). P value <0.05 [The significant p-value infers that presence of nitrites has been higher among urine culture positive children compared to culture negative children]. The positive Likelihood Ratio was 24.7 and negative Likelihood Ratio was 0.64.

In the culture proven UTI, leucocyte esterase(LE) was positive in 60 cases and negative in 15 cases. LE was positive in 8 cases and negative in 67 cases in sterile culture group. The sensitivity of LE test was 80% that is among those who had infection, LE test rightly detected UTI in 80% and specificity was 89.3% that it could rightly rule out UTI in 89.3% of children.The PPV of test was 88.2% meaning if test was reported as positive , likely chance that the patient had infection was 88.2%.The NPV of LE test was 81.7% so if test report was negative , probability that child does not have infection was 81.7%(Table 3)(P value <0.05) [the significant p value infers that presence of LE has been higher in Culture positive children compared to culture negative children. The positive Likelihood Ratio was 7.4 and negative Likelihood Ratio was 0.22.

In the present study in age 2-5y, 8 cases (29.6%) were Nitrite positive, in 6-10yr, 11 cases (40.7%) were nitrite positive and in 11-18y, 8 cases (29.7%) were nitrite positive. A Chi-square test was applied to look for the correlation of nitrites with age. Further chi-square trend was applied to see if nitrites positivity increased with age. The results showed a statistically significant correlation with age and as age increases, there was a trend towards increasing nitrites positivity. Among the culture positive cases , combined dipstick was positive in 72 cases (96%)

and negative in 3 cases (4). Among culture the 75 negative cases, combined dipsticks was positive in 1 cases (1.3%) and negative in 74 cases (98.7%). Sensitivity and specificity was 96%and 98.6% respectively.Whereas PPV and NPV was 98.6% and 96% respectively.[The p value obtained by chi square test was <0.05 and was found to be statistically significant](Graph 2).

Discussion:

In present study , UTI was observed to be most common in 2-5 y group (58.6%) followed by 6- 10 y (25.3%) and 11-18 y group(16%). In studies by different authors UTI was more common in 5-12 y age (13,14) and even above 16 y(15). Male : Female ratio in present study was 1:1.5. Male :Female ratio has been observed to vary from 1:1.3(15) to even 1.5:1 (13) and 2.2:1(16). Fever was the most common complaint followed by dysuria in studies [13,15,17] as also observed in present study.

Most common organism was E coli followed by Klebsiella, Proteus and Pseudomonas as were the findings by other authors [1,18].However Nowell et al [19], found that most common organism was coagulase negative staphylococcus.

Nitirte test had sensitivity of 34.6% and specificity of 98.6% in our study .Same were findings by Buzayan et al [20] and Williams et al [21].But sensitivity was higher (69.04%) in study done by Baral et al[22] though specificity was 89.4% in their study . In present study, UTI was more in children below 5 years of age. In younger children breakdown of nitrates to nitrites by gram negative bacteria does not occur due to frequent voiding of urine. The process of breakdown to nitrities requires a minimum of 4 hours of urine retention in bladder, this explains low sensitivity and high specificity in this age group, which has been seen in this study. PPV and NPV was 97.2% and 60% in present study ,Thayyil et al[17] had PPV of 29.8% and NPV of 92.4%in their study.

The differences in above test between the studies might be related to different high risk population ,gender and age , use of different brand strips as well as technique of collection and transportation of sample. The improper technique and transportation of sample allows the colonizing bacteria to multiply which allows test to be false positive[23,24].The

early morning samples give better yield as because bladder should incubate organism for at least 4 hours to convert nitrates to nitrites, so random specimens collected at any time or urine from draining catherters do not show good correlation between significant bacteriuria and nitrite test.

The sensitivity and specificity of LE test was 80% and 89% respectively in present study which was consistent with studies by Adeleke et al[25] and Coulthard et al [26]. PPV and NPV was 88.2% and 81.7% respectively in study group.Sensitivity and PPV was less as repoted by Nayak et al [13] and Bagga et al [27] in comparison to present study.Nayak et al also had low specificity(25%) and NPV(19%) in their study. Both above studies had small sample size.The difference between studies might be related to degree of leucocytes in urine, enzyme cotent of immature leucocytes or both[28].

Sensitivity and specificty of combined LE and Nitrite test was 96% and 98.6% in present study,Glissmeyer et al [29] had sensitivity of 90.8%

and specificity of 93.8% which is similar to present study.Nayak et al[13] , Harsh et al[14] and Bagga et al [27] had sensitivity and specificity low as compared to our study.These studies were having small sample size. PPV and NPV was more than most of studies except of Glissmeyer et al[29]who had NPV of 98.7% close to the present study.

It can be concluded from present study that The LE test is more sensitive in diagnosis of UTI whereas Nitrite test is more specific so helps in excluding UTI . Both test done together have both high sensitivity and specificity.Positive Predictive value of Nitrite test is more than LE test, close to Positive Predictive value of both test together.

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TABLES

Table 1: "Sex distribution of study group according to age"

| AGE(y)	NUMBER	MALE		FEMALE	
		NUMBER	PERCENTAGE	NUMBER	PERCENTAGE
2-5	71	26	17.3	45	30
6-10	38	20	13.3	18	12
11-16	41	11	7.4	30	20
	150	57	38	93	62

Table 2: "Sensitivity,specificity,positive predictive value(PPV)and negative predictive value(NPV) of nitrite test in culture positive and culture negative group"

| NITRITE TEST	CULTURE POSITIVE	CULTURE NEGATIVE	TOTAL
Positive	26	1	27
Negative	49	74	123
Total	75	75	150
Sensitivity	26/49+26=34.6%		
Specificity	74/74+1=98.6%		
PPV	26/26+1=97.2%		

NPV	74/49+74=60%	
P value	<0.00001	

Table 3: "Sensitivity, specificity, positive predictive value (PPV) and negative predictive value(NPV) of leucocyte estrase(LE) test in culture positive and culture negative group"

LEUCOCYTE ESTRASE TEST	CULTURE POSITIVE	CULTURE NEGATIVE	TOTAL
Positive	60	8	68
Negative	15	67	82
Total	75	75	150
Sensitivity	60/60+15=80%		
Specificity	67/8+67=89.3%		
PPV	60/60+8=88.2%		
NPV	67/15+67=81.70%		
P value	<0.00001		
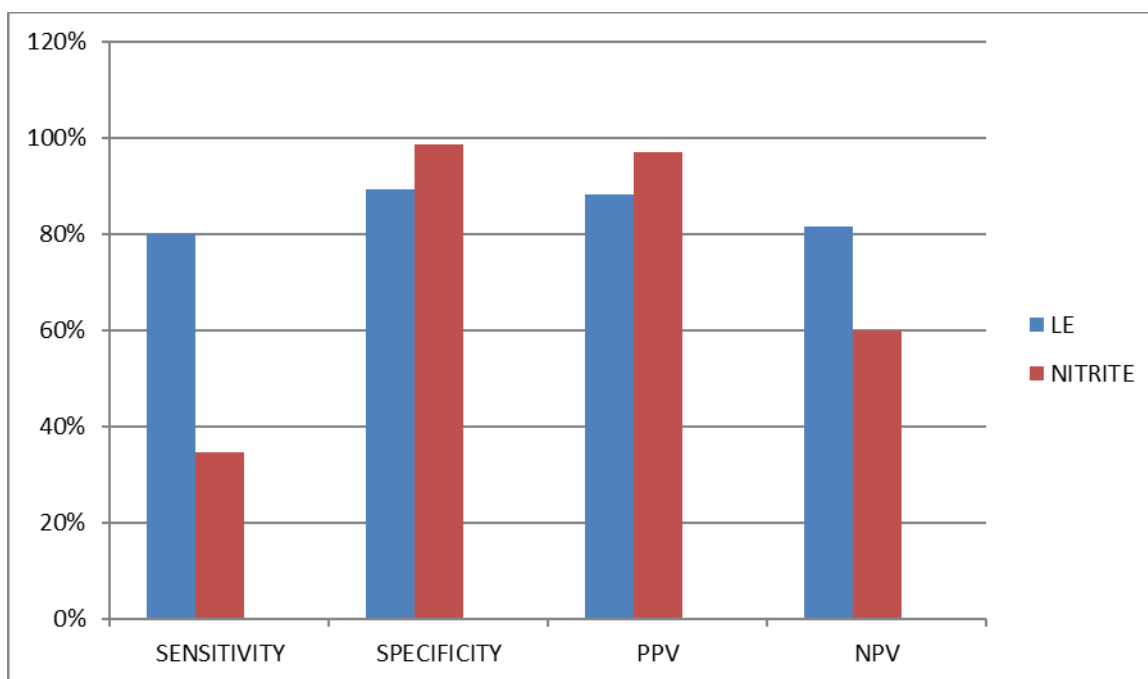
Graph 1: "Sensitivity, specificity, positive predictive value(PPV) and negative value(NPV) of leucocyte estrase(LE) and nitrite test"
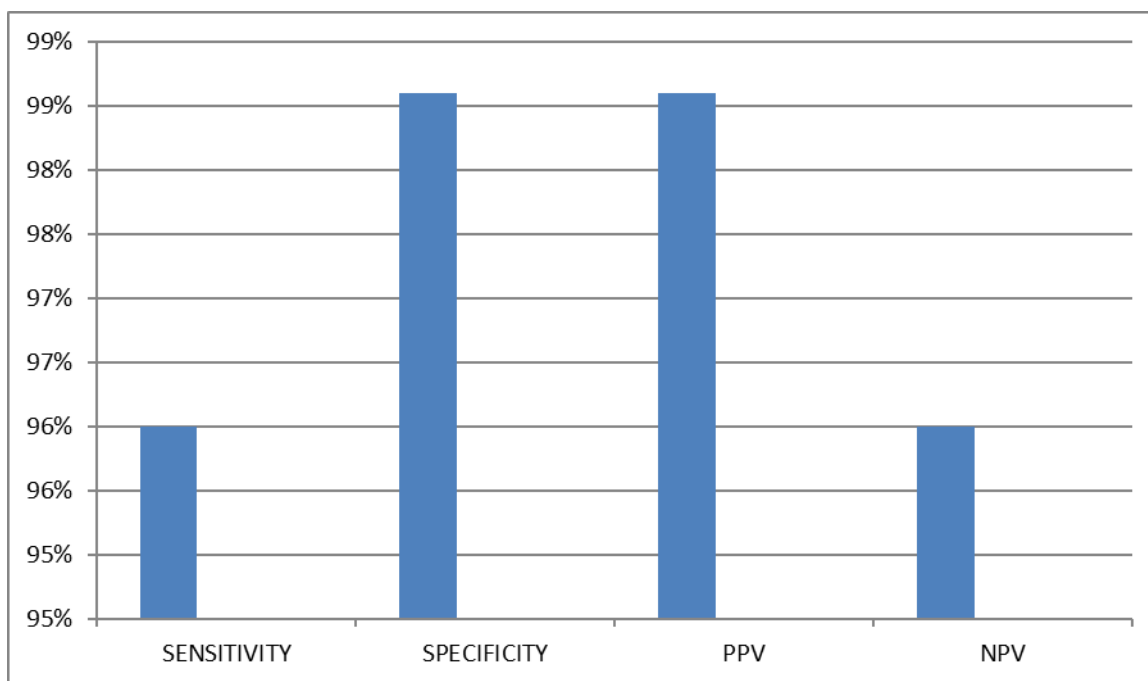
Graph 2: "Sensitivity, specificity, positive predictive value (PPV) and negative value (NPV) of leucocyte estrase(LE) and nitrite test combined"

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