Utility of Dipstick Test (Nitrite and Leukocyte Esterase) and routine urine microscopy in comparison with urine culture for detecting urinary tract infection in children between 1-5 years of age.

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Abstract
BACKGROUND
UTI causes 5% of the febrile illness in children below 5 years of age. Early diagnosis and treatment is required to prevent long term health hazard like renal scarring and reflux nephropathy.
AIMS AND OBJECTIVES
Whether Nitrite and leukocyte esterase test by dipstick and urine microscopy can give us a clue and start early treatment awaiting urine culture result.
MATERIAL METHOD
50 symptomatic children between 1-5 years of age are compared with 50 asymptomatic ones and tested for dipstick, microscopy and culture. Data analysis was done using Statistical Package for Social Sciences using Chi-Square Pearson coefficient test.
RESULT AND ANALYSIS
Leukocyte esterase has a sensitivity of 80% and specificity of 90%. Nitrite test has a sensitivity of 34% and specificity of 98%. Pyuria has a specificity of 94% and sensitivity of 74%.
CONCLUSION:
The combination of dipstick test along with routine microscopy has a high sensitivity and specificity and can be used for screening and early treatment of urinary tract infection awaiting urine culture results.
KEYWORDS
Urinary tract infection
DIPSTICK examination of urine, Routine urine microscopy

I. Introduction

(UTI) causes 5% of the febrile illness in childhood. Renal scarring leads to hypertension and may lead to reflux nephropathy, chronic kidney disease and end stage renal failure[1]. Therefore rapid diagnosis and an immediate empirical antibiotic therapy is crucial awaiting urine culture results to come, which takes around 48 hours[2]. This study aims at finding the correlation of rapid test like Dipstick test (Leukocyte-esterase and Nitrite test) and urine microscopy awaiting urine culture report[2].

II. Aims And Objectives

To determine the usefulness of Dipstick Test and Routine Urine Microscopy[1] and compare it with urine culture for detecting UTI between 1-5 years of age visiting Ramakrishna Mission Seva Pratisthan and Vivekananda Institute of Medical Sciences , Kolkata.

III. Material And Method

Febrile children between 1-5 years of age attending the out-patient department or admitted to Ramakrishna Mission Seva Pratisthan and Vivekananda Institute of Medical Sciences, Kolkata, over a period of 12 months between 1stMarch 2020 to 28th February 2021 with history of fever greater than 37.8C were included in the study.

Urinary Tract Infection is defined as growth of significant number of organisms of a single species in urine, in the presence of symptoms. Significant bacteriuria is growth of greater than 10^5 per millilitre of a single species in a midstream clean catch urine sample. However, this is difficult in a child below 5 years of age and we often have to rely on either any clean catch sample or bagged urine. Suprapubic aspirate of urine though ideal in these. children,is however too invasive and painful.

Children with symptoms suggestive of UTI were enrolled and were selected using structured questionnaire for UTI. For all symptomatic children we did a dipstick test of leucocyte-esterase test and nitrite test and sent the sample for microscopy while preserving another sample in a boric acid containing tube and sending for culture.

Inclusion criteria:
* Ethical committee approved
* Consent from legal guardian of the child
* All febrile children suspected with UTI between 1-5 years
* Fever- axillary temperature greater than 37.8 C

Exclusion criteria:
* Those not willing to participate
* Children below 1 year or above 5 years
* Any child who received antibiotics within 48 hours prior to evaluation

STUDY DESIGN: CROSS SECTIONAL COMPARATIVE STUDY
Sample size -100 febrile children with suspected UTI between 1-5 years of age.
All these children had
  a) routine urine microscopy of an uncentrifuged urine sample.
  b) urinary dipstick test (Leucocyte-esterase test, Nitrite test)
  c) urine culture

A) ROUTINE URINE MICROSCOPY: Pyuria is the presence of visible leucocytes in   the urine sample as seen in the high power field of a microscope. The only method of assessment of pyuria that correlates tightly with the gold standard leucocyte excretion rate is presence of 10 WBCs/mm3 as detected by haemocytometer analysis of an uncentrifuged urine specimen.

B) LEUCOCYTE ESTERASE: Leucocyte esterase refers to the enzymatic remnants of WBC. It is predominantly found in granules of neutrophils that possess proteins that which react with impregnated reagent to produce a positive result of blue colour . Since neutrophils are labile, leucocyte esterase denotes enzymatic remnants of cells which are visible microscopically. A positive leucocyte esterase thus denotes significant number of neutrophils – either intact or lysed. Leucocyte esterase catalyses hydrolysis reaction to produce respective alcohols and acid components.

False negative results can occur in:
1.Altered specific gravity, protein and glucose.
2.High oxalic acid concentration.
3.Antibiotics like tetracycline and cephalexin.
4.High ascorbic acid content.
False positive results occur in:
1.   Contaminated urine.
2.   An alternative for cellular sources of esterase.
3.   In presence of formalin and oxidizing agents.

C) NITRITE TEST
Gram negative bacteria especially E.Coli reduce dietary nitrates to nitrites. At least a minimum duration of 4 hours of urinary stasis in the bladder is required for action of bacteria to breakdown nitrates. Hence ,though nitrites are less sensitive in detecting UTI due to the above reasons, the presence of nitites in fresh urine sample is highly specific for an underlying UTI.
False positive results:
a.   If there is a delay in testing sample or in case long standing sample.
b.   The reagent impregnated on the nitrite dipstick is highly sensitive in air, so containers should be closed immediately after use. After a week of exposure one third of the strips get false positive results.
c.   High specific gravity
False negative results:

Non nitrate reducing organism or a child who consumed low nitrate in their diet.

D)   URINE CULTURE

Urine culture has remained the gold standard for the diagnosis of urinary tract infection as well as diagnosis of asymptomatic bacteriuria. The urine samples are cultured onto the MacConkey agar and Blood agar plates by the semi quantitative culture technique using a standard calibrated loop having internal diameter of 3mm. The protocol was followed as recommended by WHO.

The results of urinary culture must be however interpreted with caution because these may be altered by:
a.   Technique of urine collection
b.   Prior use of antibiotic
c.   Improper or faulty laboratory techniques

Technique of urine collection: An early morning of fresh sample is ideal although it is not always practically possible.

There are various methods of urine collection:
1.   Bag method
2.   Midstream clean catch
3.   Suprapubic aspiration
4.   Catherized sample

The preferred method out of all the above methods is suprapubic aspiration among the invasive methods since there is increased risk of introduction of bacteria by catheterization. Among the non-invasive methods, mid-stream clean catch urine is the preferred method of urine collection for culture. However, this is not routinely preferred as there is high risk of contamination with per urethral flora especially in males. Bag method is not reliable in detection of UTI due to the high risk of contamination in bagged specimens and thereby high rates of false positive results. It is useful in ruling out a negative sample, but not useful in documenting UTI in the absence of clinical supportive evidence.

Recommendations by various guidelines on method of urine collection

NICE guidelines suggest that non invasive method of urine collection is practically acceptable. Although midstream is the preferred method of urine collection, methods such as urine collection pads and bag methods are also acceptable according to NICE guidelines. Invasive methods are used only if the non-invasive method of urine collection fails.

The preferred method of collection of urine for culture by AAP is suprapubic aspiration and catheterized methods. Bag and midstream clean catch method is not recommended by AAP.

COLLECTION OF URINE SAMPLE

Trained research assistants (nurses) participated in the collection of urine samples. 2 urine specimens were collected from each patient, one for dipstick and microscopy and the other for culture. Urine samples were obtained under strict aseptic precautions for both urine analysis and urine culture in 2 different containers. For all symptomatic children, Dipstick test(leukocyte esterase test and nitrite test) was done and the uncentrifuged urine was sent for microscopy while preserving another sample in boric acid containing tube and sending for culture. The methods of urine collection adopted for the study were catheterized and mid-stream clean catch sample. The samples for urine analysis and urine culture were sent to clinical pathology lab and microbiology lab respectively within 2 hours of collection.

INTERPRETATION AND STANDARDS USED

1.   Pyuria- The presence of 10 white blood cells (WBCs)/hpf an uncentrifuged urine specimen.

2.   Nitrite (Siemens Multistix 10SG used) was reported as positive or negative based on presence or absence.

3. Leukocyte esterase (Siemens Multistix 10 SG used) was interpreted as negative, trace, 1+,2+ and 3+.
1+ or more is positive.

Significant Bacteriuria

Criteria for UTI diagnosis based on the bacterial colony count in urine culture.

GUIDELINES	Method of collection	Criteria Colony

		Count(CFUs/ml)
2011 AAP	Suprapubic aspiration or catheterization	$>5x10^4$
2006CCHMC	Suprapubic aspiration	$>10^3$
	Catheterization	$>10^4$
	Clean catch midstream	$>10^5$
2012 Italian Society of Paediatric Nephrology	Catheterization	$>10^5$
	Clean Voided Urine	$>10^5$
	Urine Bag	Any growth
2014 Canadian Pediatric Society	Suprapubic aspiration	$\geq 5x10^4$
	Catheterization	$\geq 10^5$
	Midstream (clean catch)	Any growth
2015EAU/ESPU guidelines	Suprapubic aspiration	
	Catheterization	$\geq 10^3 - 5x10^4$
	Midstream	$\geq 10^4$ with symptoms Or
		$\geq 10^5$ without Symptoms
	Urinalysis	Pyuria+/- bacteriuria
	Suprapubic aspiration	Any growth
	Catheterization	$>5x10^4$
	Midstream	$>10^5$

CCMHC: Cincinnati Children's Hospital Medical Centre
UAA/AAUS: Urological Association of Asia/Asia Association of UTI & STI

IV.   Data Analysis

The data collected from the patients were formatted into Microsoft Excel sheets to generate master charts. Tables and graphs, diagrammatic representation was used to depict significant clinical data from patients with culture proven UTI. The sensitivity, specificity, negative predictive value and positive predictive values for the three screening methods were calculated against the urine culture (reference group) for the diagnosis of UTI. Overall accuracy was calculated and compared.

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)

ANALYSIS OF DATA
Data was analyzed by using statistical package for social sciences (SPSS). Correlation of parameters was assessed using chi-square Pearson co-efficient test.
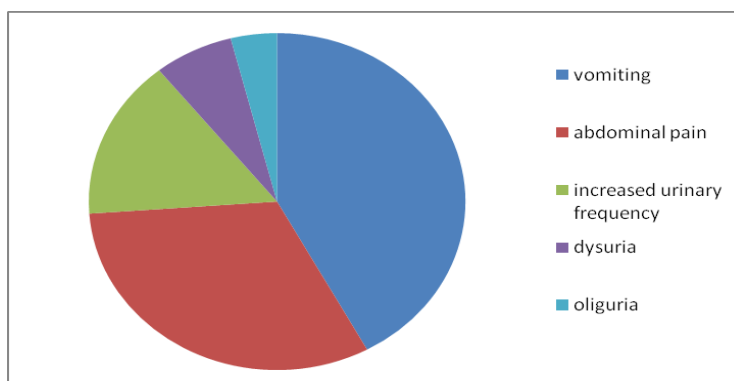
V.   Results And Analysis
100 children with suspected urinary tract infection were enrolled in the study,
50 children with culture proven UTI and 50 patients with sterile urine cultures.
The most common symptoms of UTI in our study were vomiting (74%), abdominal pain (56%), increase urinary frequency (27%), Dysuria (12%) and oliguria (7%).
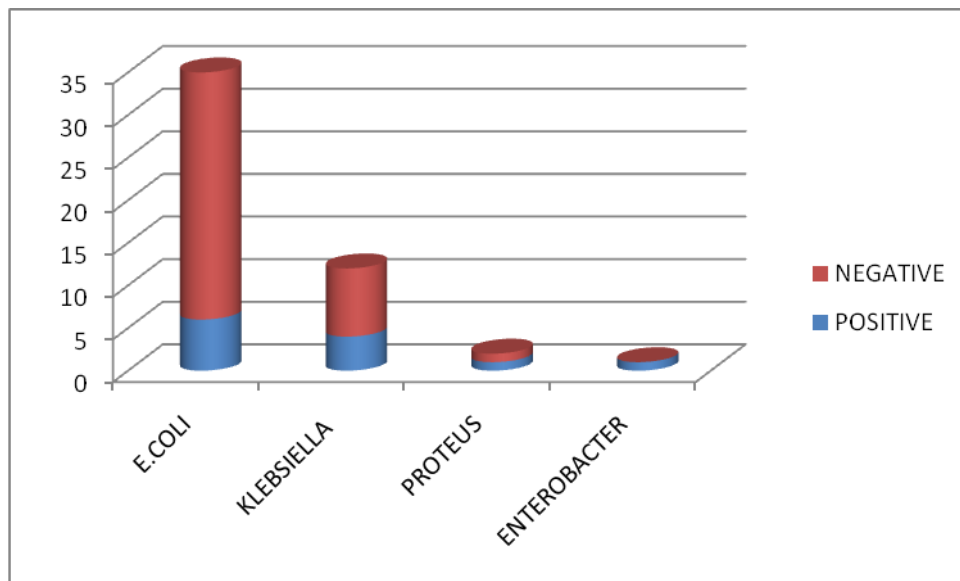
SYMPTOMS

Out of this 50 positive cases, 35 were E.coli (75%) followed by 12 cases of Klebsilla (24%), 2 cases of Proteus (4%) and one case of Enterobacter (2%).
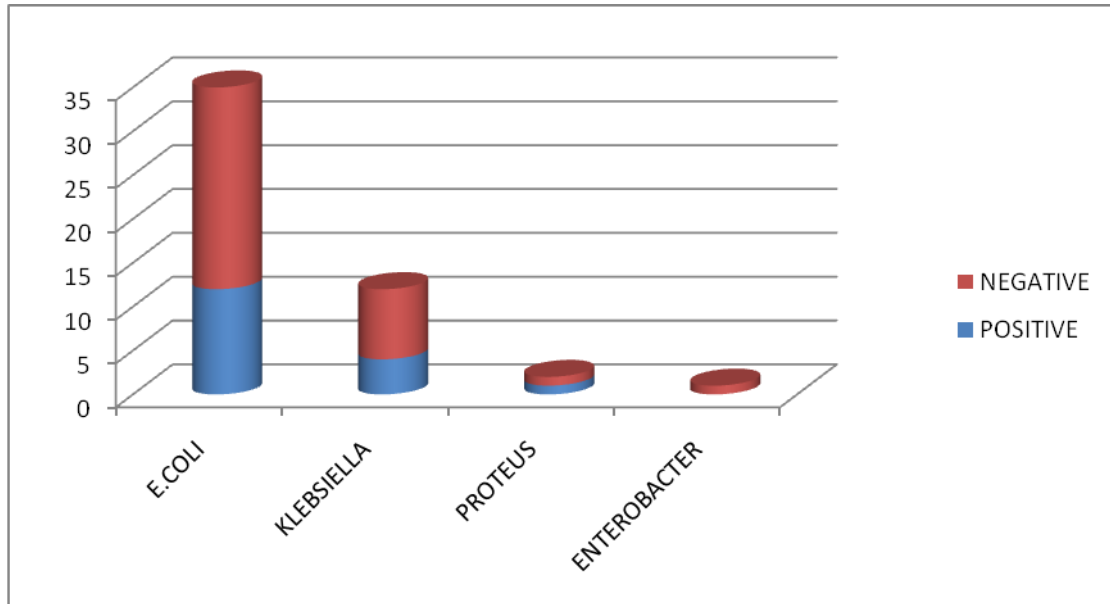
ORGANISM
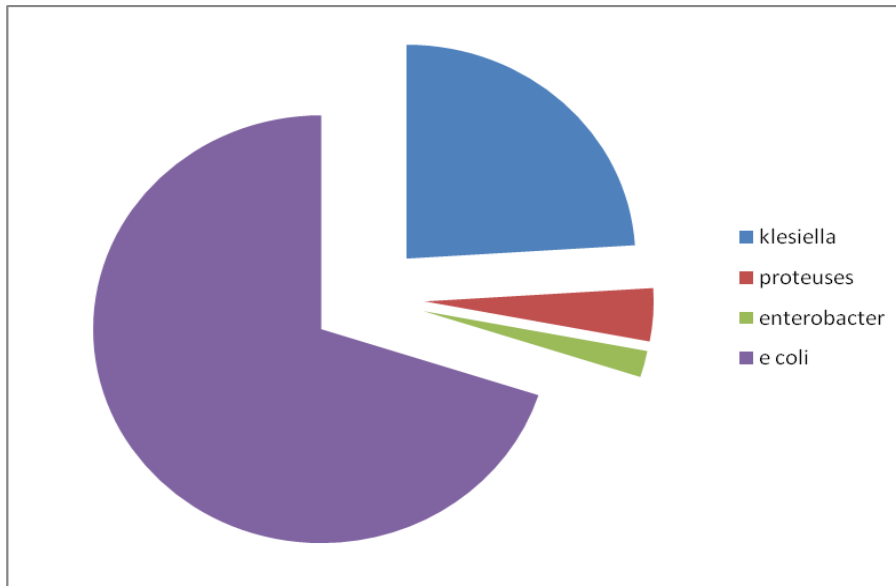
LEUKOCYTE ESTERASE

Leukocyte esterase identified 29 samples out of 35 E.Coli positive (82%), 9 samples out of 12 klebsiella positive (75%).
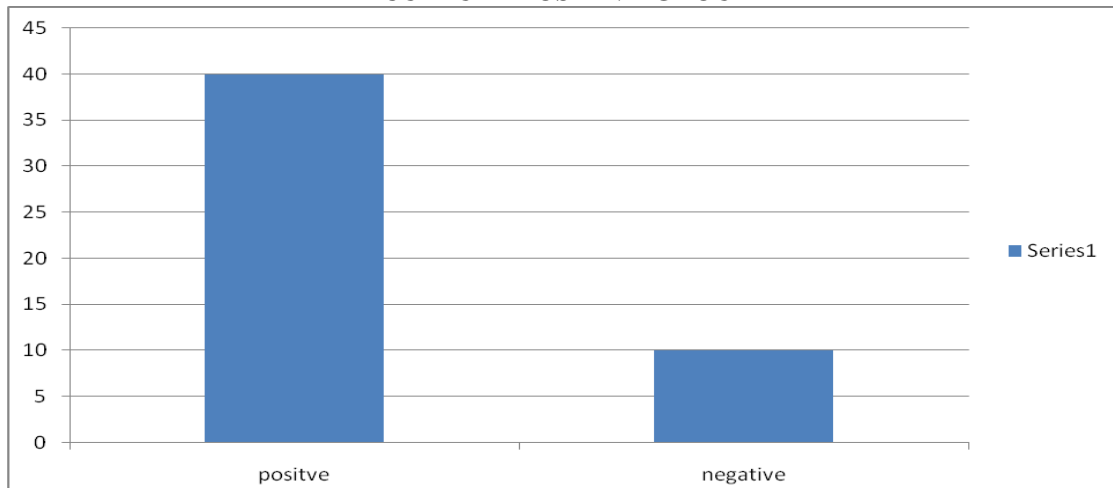

NITRITE

Out of 35 E.Coli positive samples, nitrite test detected only 12 samples, with the positive rate of 34.2%. The similarly positive rate of nitrite test for Klebsiella was 33%.
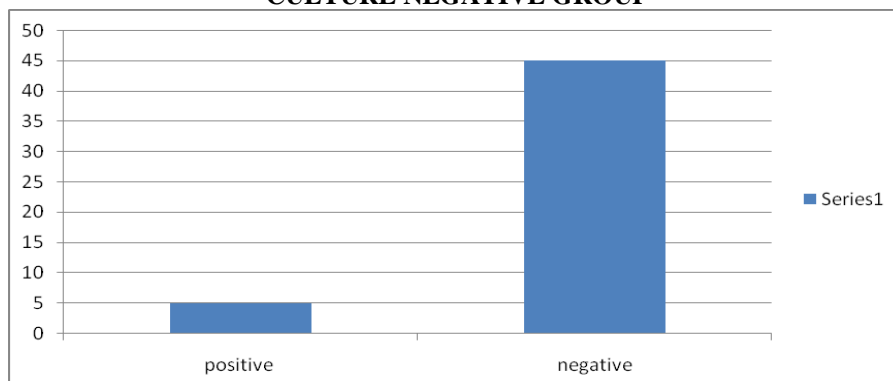
LEUKOCYTE ESTERASE:
In the culture proven UTI, Leukocyte esterase was positive in 40 cases and negative in 10 cases.

CULTURE POSITIVE GROUP

Leukocyte esterase was positive in 5 cases and negative in 45 cases in sterile culture group.
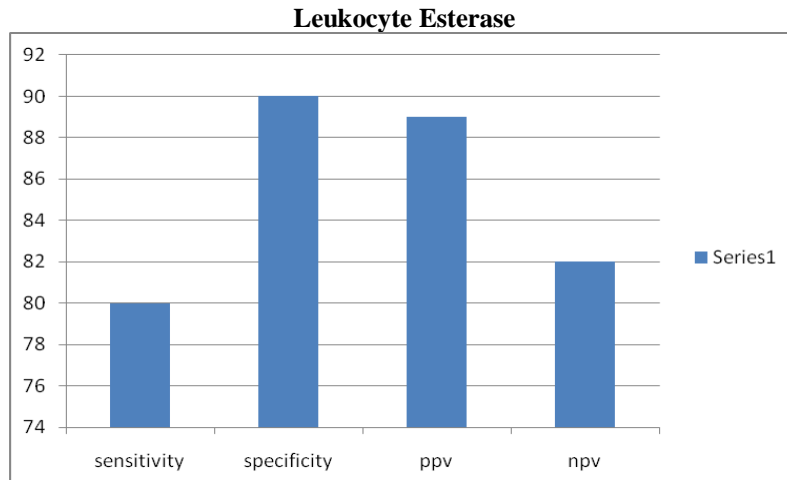
CULTURE NEGATIVE GROUP

CHI-SQUARE TEST VALUE= 49.49 D.F. =1, P<0.0001.
The significant p-value confirms that the presence of Leukocyte esterase was higher in the culture positive group as compared to the sterile culture group.

Sensitivity, Specificity, Negative Predictive Value and Positive Predictive value.

Leukocyte Esterase

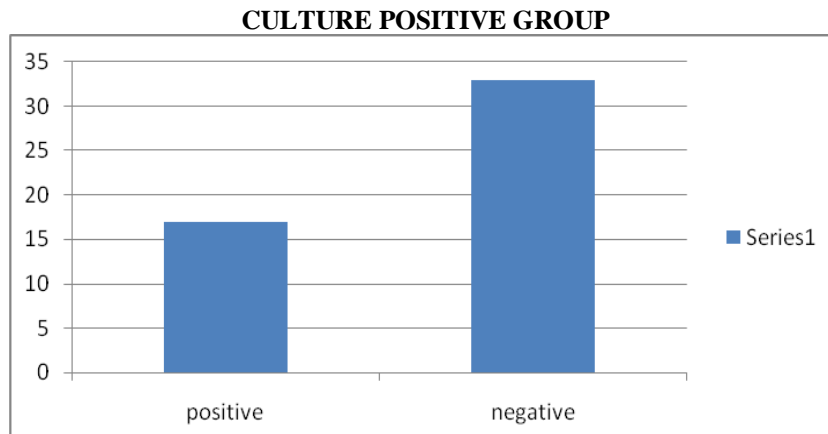| LEUKOCYTE ESTERASE	CULTURE		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	40	5	
NEGATIVE	10	45	
TOTAL	50	50	100

Sensitivity = 40/(40+10)=80%
SPECIFICITY = 45/(45+5)=90%
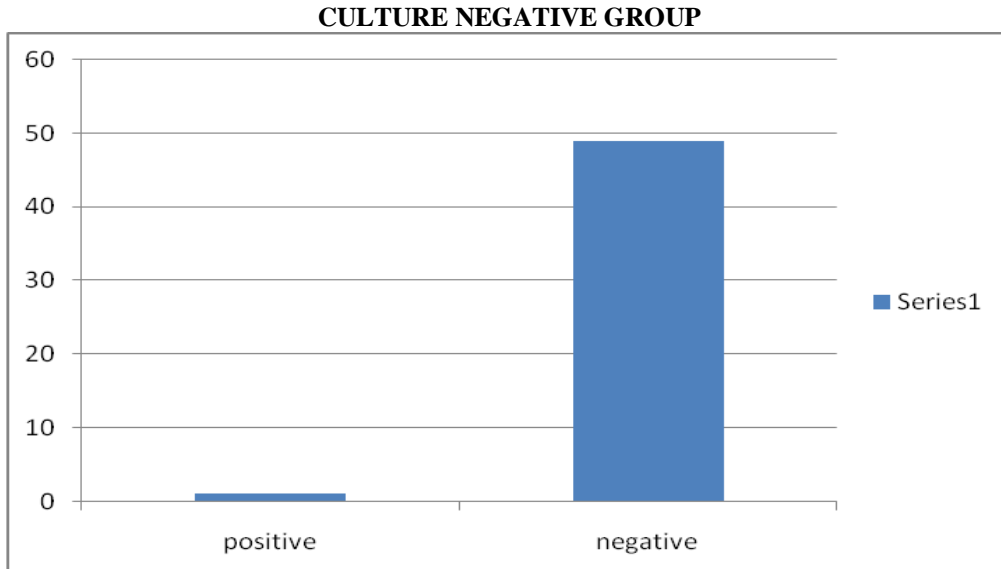POSITIVE PREDICTIVE VALUE = 40/(40+5)=89%
NEGATIVE PREDICTIVE VALUE = 45/(45+10)=82%

NITRITES :
Among the culture proven UTI cases, nitrites were positive in 17 cases and negative in 33 cases.

CULTURE POSITIVE GROUP

Among the sterile culture groups, nitrites were positive in 1 case and negative in 49 cases.

CULTURE NEGATIVE GROUP

CHI-SQUARE TEST VALUE = 17.34 D.F. =1, P<0.0001.
The significant p-value infers that presence of nitrites has been higher among urine culture positive children compared to urine culture negative children.
Sensitivity, Specificity, Negative Predictive value and Positive Predictive value
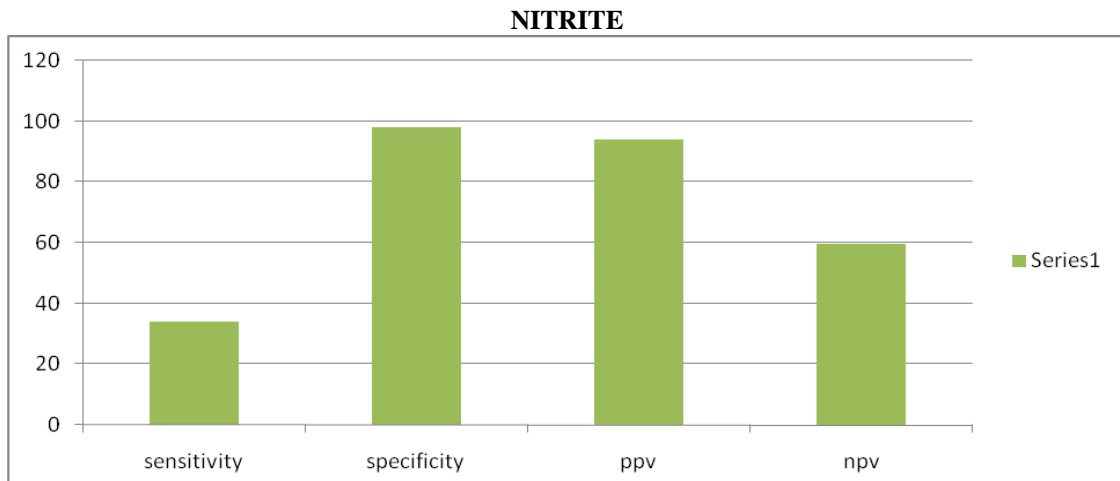
NITRITE

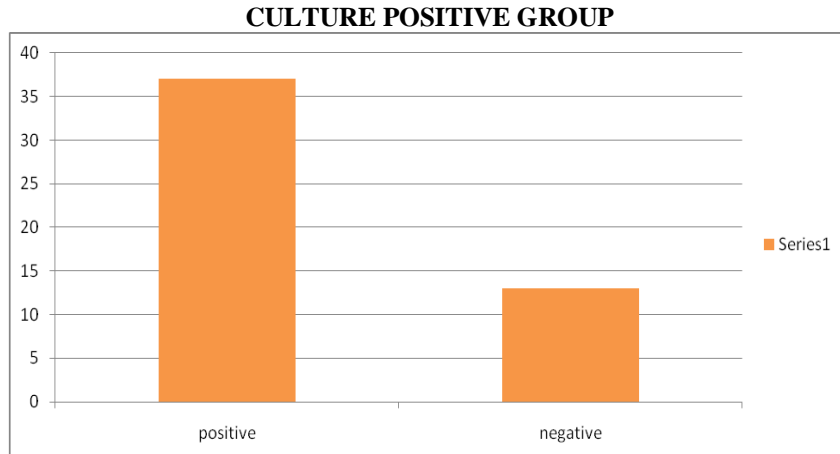| NITRITE	CULTURE		TOTAL
	POSITIVE	**NEGATIVE**	
POSITIVE	17	1	
NEGATIVE	33	49	
TOTAL	50	50	100

SENSITIVITY = 17/(17+33)=34%
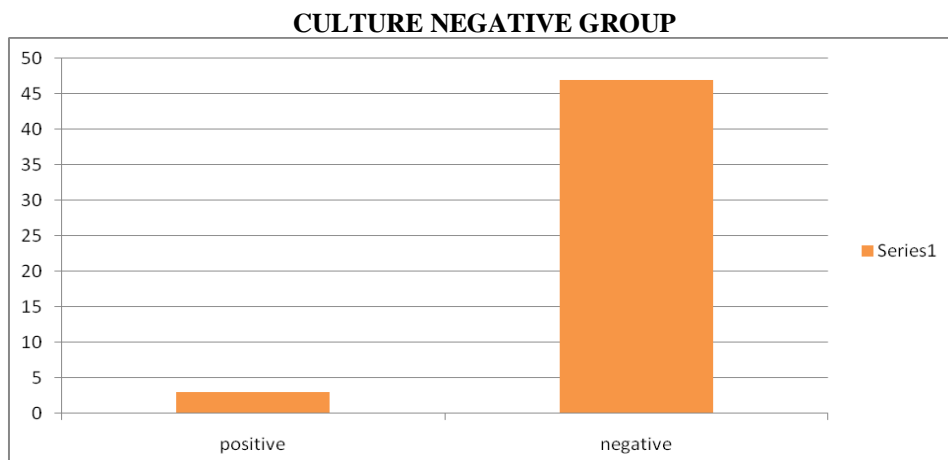SPECIFICITY + 49/(49+1)=98%
POSITIVE PREDICTIVE VALUE= 17/(17+1)=94%
NEGATIVE PREDICTIVE VALUE=49/(33+49)=59.75%

PYURIA
Among the culture prove UTI cases, pyuria was positive in 37 cases and negative in 13 cases.

CULTURE POSITIVE GROUP

Among the sterile culture group, pyuria was positive in 3 and negative in 47 cases.

CULTURE NEGATIVE GROUP

CHI-SQUARE TEST VALUE=48.17 D.F. =1,P<0.0001 Chi square test was applied to look at pyuria in both culture positive and sterile culture group. P value suggests that pyuria was significantly higher in the culture positive group when compared to the sterile group.

Sensitivity, Specificity, Negative Predictive Value and Positive Predictive Value.
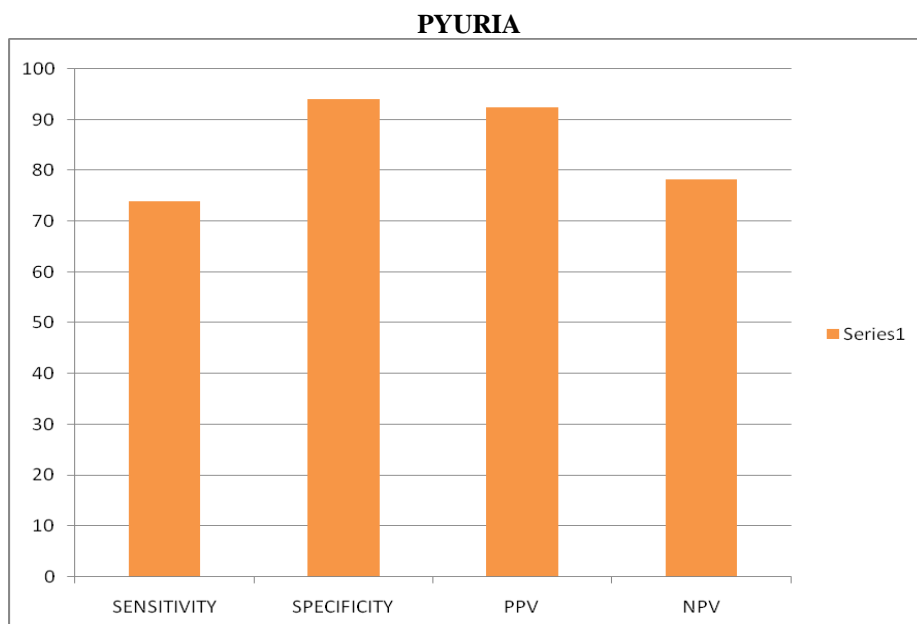
PYURIA

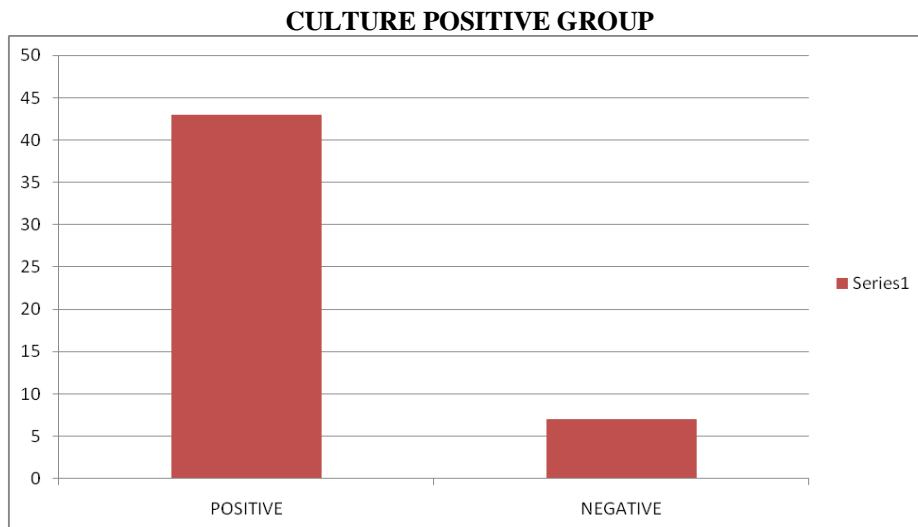| PYURIA	CULTURE		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	37	3	
NEGATIVE	13	47	
TOTAL	50	50	100

SENSITIVITY=37/(13+37)=74%
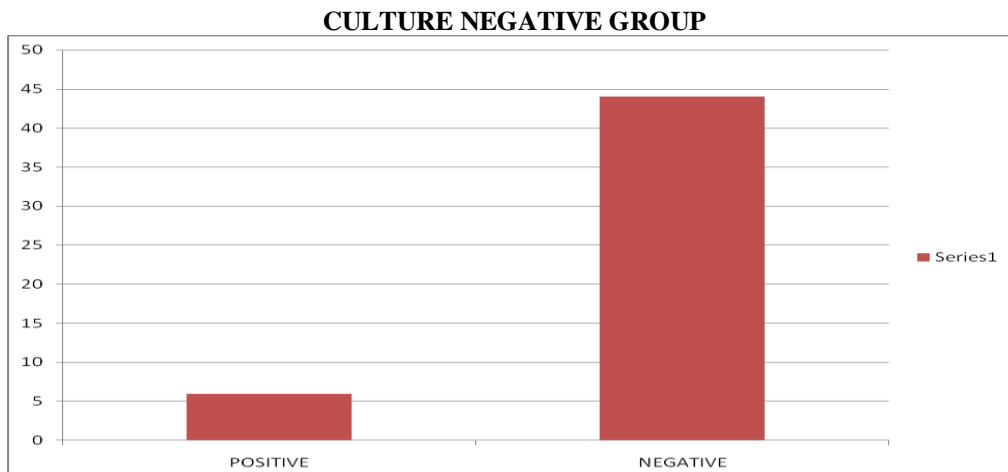SPECIFICITY = 47 /(47+3)=94%
POSITIVE PREDICTIVE VALUE=37/(37+3)=92.5%
NEGATIVE PREDICTIVE VALUE= 47/(47+13)=78.3%

LEUKOCYTE ESTERASE AND NITRITE:
At least one positive in 43 cases and both negative in 7 cases among the culture proven UTI group.

Leukocyte esterase and nitrite-positive (at least 1 positive) in 6 cases and negative (both) in 44 cases among sterile culture group.

CHI-SQUARE TEST VALUE=54.78 D.F. =1, P<0.0001. The significant p-value confirms that the presence of Leukocyte Esterase and Nitrite was higher in the culture positive group as compared to the sterile culture group. Sensitivity, Specificity, Negative Predictive Value and Positive Predictive Value.

L.E & NITRITE
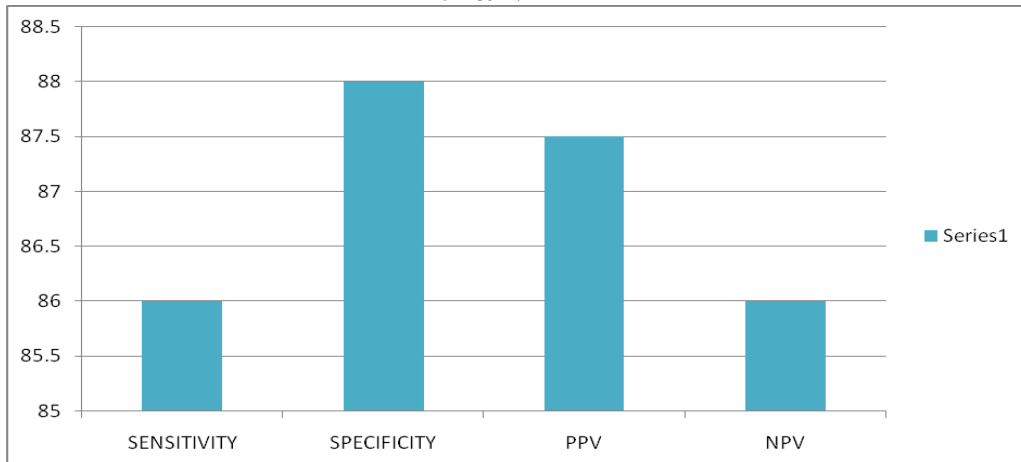
L.E & NITRITE	CULTURE		TOTAL
	POSITIVE	**NEGATIVE**	
POSITIVE	43	6	
NEGATIVE	7	44	
TOTAL	50	50	100

Sensitivity= 43/(43+7)=86%

Specificity = 44(44+6)=88%

Positive Predictive Value=43/(43+6)=87.5%

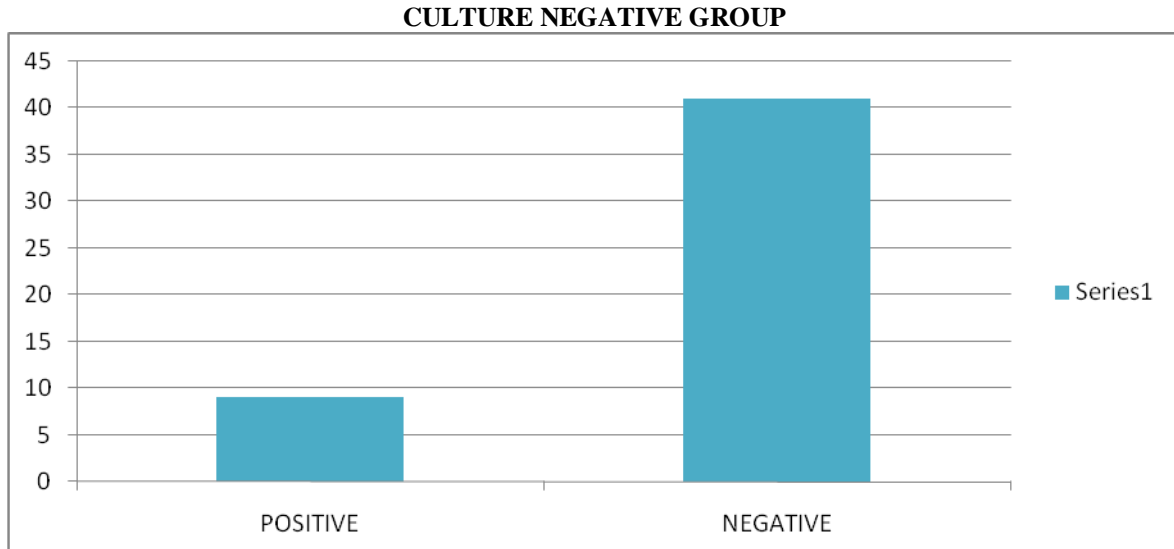Negative Predictive value= 44/(44+7)=86%

URINE ANALYSIS (AT LEAST ONE POSITIVE AMONG NITRITE, L.E AND PYURIA):
- was positive in 44 cases and all 3 negative in 6 cases among the positive culture groups.

CULTURE POSITIVE GROUP
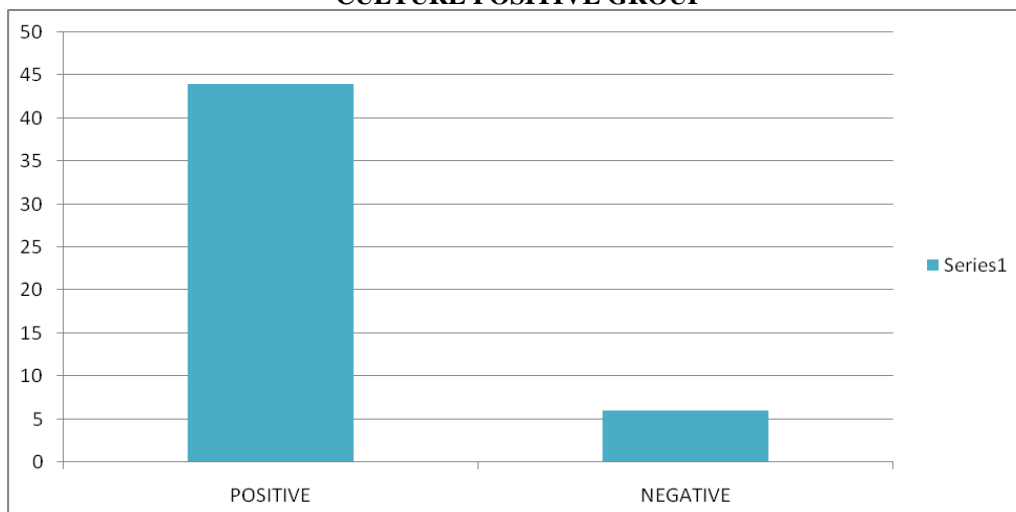
Urine analysiswas positive in 9 (atleast one positive among Nitrite, L.E and Pyuria) cases and negative (all 3 negative) in 41 cases in the sterile culture group.

CULTURE NEGATIVE GROUP

CHI-SQUARE TEST VALUE=49.18 D.F. =1, P<0.0001
The significant p-value confirms that the urine analysis was higher in the culture positive group as compared to the sterile culture group.
Sensitivity, Specificity, Negative Predictive Value and Positive Predictive Value.
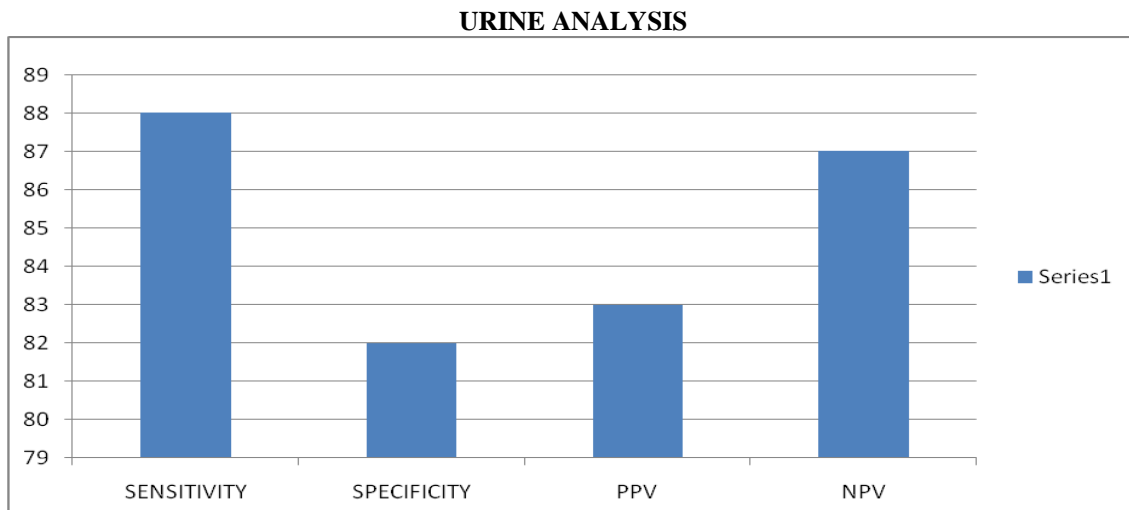
URINE ANALYSIS

URINE ANALYSIS	CULTURE		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	44	9	
NEGATIVE	6	41	
TOTAL	50	50	100

Sensitivity= 44/(44+6)=88%
Specificity=41/(41+9)=82%
Positive Predictive Value=44/(44+9)=83%
Negative Predictive Value=41/(41+6)=87%

VI.   Discussion

In the present study, the sensitivity, specificity, negative and positive predictive values for leukocyte esterase, pyuria and nitrites were analyzed by comparing the test results with the gold standard norms (culture proven UTI cases and sterile culture cases).

This was done by identifyimg the true negative, true positive, false positive and false negative values for each variable and substituing it by the standard formula. Each parameter was compared in both the groups to see if these parameters were significantly higher in the culture proven UTI group as compared to the sterile

culture group. Further combination of parameters was compared to findout the maximum sensitivity and specificity. Chi-square was computed. Graphical illustrations were also done.

Comparison of validity and reliability parameters of nitrite test, leukocyte esterase test and urine microscopy in present study.

Screening Test	Sensitivity (True +ve)%	Specificity (True –ve)%	PPV %	NPV%
LeukocyteEsterase	80%	90%	89%	82%
Nitrite	34%	98%	94%	59.75%
Urine Routine microscopy	74%	94%	92.5%	78.3%
LE and /or Nitrite	86%	88%	87.5%	86%
Leukocyte esterase and Nitrite and Routine microscopy	88%	82%	83%	87%

It was observed that Leukocyte esterase had a sensitivity of 80% i.e among those who had the infection leukocyte esterase rightly detected UTI in 80%. Specificity was observed to be 90% i.e leukocyte esterase had the ability to rightly rule out UTI in 90% of children. Positive predictive value was 89%, i.e if the test was reported as positive, the likely chances that the patient has the infection was 89%. Negative Predictive value was 82% i.e if a child was tested as negative the probability that the child does not have the infection was 82%.

Sensitivity was consistent with studies [1,2,3,] where the leukocyte sensitivity ranged from 75-85% and specificity ranged from 85-95%.

It was observed that Nitrites had a senstivity of 34% i.e among those who had the infection Nitrite rightly detected UTI in 34% cases. Nitrites had a specificity of 98% i.e it has the ability to rule out UTI in 98% cases. The positive predictive was observed to be 94% i.e the chances that child has an actual urinary infection was 97% when tested positive. The negative predictive value was the ability of the test to rule out UTI, when tested was 59.75% which was low. Walter LJM et.al [4] in his study stated that nitrites had a low sensitivity (45-60%) and higher specificities (85-98%).

Standard norms laid by AAP for the sensitivity and specificity of nitrites ranged from 15-82% and 90-100% respectively which was at par with our study findings. Although specificity of nitrites in our study was consistent with the other studies, the expected sensitivity was a little low as compared to other studies. Lejeune et al [6] in their study found sensitivity of 16.2% and specificity of 97.6%. Muna et al [7] reported a sensitivity of 27.3% and a specificity of 100%. Although speficity of nitrites in our study were consistent with the other studies, the expected sensitivity was either in lower limit compared to other studies. This could be explained by the varied sample sizes used in different studies.

Comparison of validity and reliability parameters of nitrite test.

SCREENING TEST (LEUKOCYTE ESTERASE)	PRESENT STUDY	AL DAGHISTANI et.al[8]	Rehmani R et.al[9]	Williams GJ et al[4]
Sensitivity (True +ve)	34%	59%	81%	49%
Specificity (True _ve)	98%	78%	87%	98%
PPV	94%	60%		
NPV	59.75%	86.2%		

The sensitivity of pyuria to rightly diagnose UTI was observed to be 74% and specificity was 94% i.e the ability of the parameter to rightly ruling out UTI was found to be 94%. Positive Predictice Value i.e when tested positive , the likely chances that the patient has UTI was 92.5%. Negative Predictive Value i.e when tested negative the likely chances the patient doesn't have UTI was 78.3%. The sensitivity and specificity norms laid by AAP[2] for pyuria were 73% and 45-98%. Findings were consistent with study by Hoberman et.al [10] where sensitivity and specificity for pyuria were 54 and 96% respectively. Although specificity was at par with studies done [11,12,13], the expected sensitivity were above 80% which can be explained by the clarge sample size adopted in the study.

In the present study, sensitivity of Leukocyte esterase and /or Nitrite (either of two positive or both positive) is 86%, which is more than nitrite (34%) or Leukocyte esterase (80%)alone.

Specificity of both tests (either of two positive or both positive)is 88%, which is lower than Leukocyte esterase (90%) OR NITRITE (98%) alone. Positive predictive value is 87.5%, which is lower than the Nitrite (94%) & LeukocyteEsterase (89%). Negative Predictive Value of Leukocyte Esterase and/or Nitrite, Nitrite and Leukocyte Esterase are 86%, 59.75% and 82% respectively.Taneja et.al[1] showed that combined sensitivity and Negative predictive value of Leukocyte Esterase and Nitrite test were 79.6% and 90.9% respectively. He concluded that for faster diagnosis of UTI, dipstick tests for leukocyte esterase and Nitrite test should be added

in routine laboratory practises. Another study conducted by Sundvall and Gunnarsson[14] showed that if both Nitrite and Leukocyte Esterase were negative, it was less likely that culture results were positive.

Urine analysis (at least one parameter present) had a maximum sensitivity (88%) and Negative Predictive Value (87%). This was consistent with other studies. One study found[15] sensitivity of urine analysis by dipstick and microscopy to be 83% in children aged 1 month -16 years. Richard Bacher et.al[16] found sensitivity of urine analysis to be 82% in children. Shaw et.al reported sensitivity of combined urine dipstick and microscopy to be 83%.

The combination of leukocyte esterase with pyuria and nitrites had the maximum sensitivity in this study. One study [17] concluded that the sensitivity of leukocyte esterase, nitrites and pyuria were 75%. The drawbacks of this study were relatively small sample size and lack of standarization of urine collection due to practical difficulties. A recent study shows dipstick may be an adequate screening test for UTI with negative predictive value of 98.7%. Adding microscopy increases the negative predictive value to 99.2% [18].

E. Coli was the commonest organism found in our study followed by Klebsiella, Proteus and Enterobacter, which is comparable to other studies [18]. Rate of isolation of E. Coli was found to be 70% which is comparable to other studies (47-89%) [15]. Rate of isolation of Klebsiella was high (24%) as compared to other studies (3.7-10%) [15]. Rate of isolation of Proteus and Enterobacter was almost comparable to other studies [15].

TABLE [19,20,21,22]:

STUDIES	PRESENT STUDY	PAHL et al	Pape L et al	Sakran et al	Qureshi et al	McLoughlin et al
E.Coli	70%	75%	47%	87%	71%	89%
Klebsiella	24%	10%	4%	4%	13%	3.7%
Proteus	4%	15%	8%	4%	11%	1.2%
Enterobacter	2%					3.7%

VII.   Summary And Conclusion

Urine culture is the "Gold Standard Test" for detecting Urinary Tract Infection. However simple biochemical tests may be useful for rapid diagnosis when the urine culture report is pending.

Nitrite test alone cannot be used as a rapid diagnostic test in childhood urinary tract infection in spite of its high specificity, because of its very low sensitivity.

The leukocyte esterase test may be used as a rapid diagnostic test in childhood because of its high predictive value of a positive test and a negative test.

The combination of leukocyte esterase and nitrites had a very high sensitivity and specificity so it can be used as a screening test and also high negative predictive value in the present study which is indicative of the fact that the dipstick test could be used at bedside; if the LE and the nitrite are not detectable as it favours a sterile culture more predictively.

Future Scope

From this study, we suggest that a combination of leukocyte esterase and nitrite and pyuria is reliable parameters in predicting urinary tract infection in children. This will help in early diagnosis and help bring down health care costs in developing nations.

LIMITATIONS:

Limitation of this study was infants were not included in this study as the collection of urine samples with proper aseptic precaution was difficult. Further studies on large scale with inclusion of infants are required for better results and comparison.

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