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Original Research Article

Evaluation of Pyuria and Gram Stain as a Test for Significant Bacteriuria at Rims Hospital, Raichur

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Abstract

Introduction: Urinary tract infections have been described since ancient times with the documented description in the Eber papyrus dated to C. 1550 BC. It was described by the Egyptians as "Sending forth heat from the bladder". Effective treatment did not occur until the development and availability of the antibiotics in 1930s before which time herbs, blood testing and rest were recommended. *Methodology:* This study was conducted to evaluate the utility of urine microscopy that is pyuria & gram stain in predicting significant bacteriuria. At the same time helping to selectively culture only those samples which are positive for microscopy hence making urine culture a cost effective diagnostic method. Hence with the advantage of greater rapidity and simplicity of urine analysis with the above mentioned methods will help to screen for significant bacteriuria and guide the clinicians to a start an empirical treatment in UTI. If urine samples are not processed immediately the samples were kept in the refrigerator at 4°c till it is processed. All the samples were processed within 6hrs of collection. *Results:* Out of 100 cases, 66 were of females and 34 were of males. The present study indicates that 65 (65%) samples were of patient aged 20-30 years. 20 (20%) samples were of the patient aged 30-40 years. 15 (15%) samples were of patient aged 40-60 years respectively. This shows that maximum patients were aged between 20-30 years. *Conclusion:* Urine analysis by gram stain and microscopic examination as a initial diagnostic tool both in symptomatic and asymptomatic cases has high sensitivity and specificity rates.

Keywords: Urine Analysis; Urinary Tract Infections; Urine Culture.

Introduction

Urinary tract infection have been described since ancient times with the documented description in the Eber papyrus dated to C.1550 BC [1]. It was described by the Egyptians as "Sending forth heat from the bladder" [2]. Effective treatment did not occur until the development and availability of the antibiotics in 1930s before which time herbs, blood testing and rest were recommended [1].

Urinary tract infection is the second most common infection after respiratory tract infection [3]. UTI refers to presences of microbial pathogens within the urinary tract and is the most common

bacterial infection. The spectrum of disease varies from asymptomatic bacteriuria to potentially life threatening pyelonephritis.

UTI occurs more commonly in females than males [4]. The risk factors include female urogenital anatomy, sexual intercourse, family history, diabetes, smoking, BPH, alcoholic.

UTI are divided into uncomplicated and complicated categories [5,6]. Uncomplicated infections may be acute cystitis or pyelonephritis in young women without underlying urinary tract pathology or systemic disease and complicated UTI is with underlying disease or urinary tract abnormality.

The cardinal clinical manifestation is fever with chills; flank pain, dysuria, frequency and urgency are more suggestive of lower UTI. The differentiation from upper and lower UTI is essential because the approach to antimicrobial chemotherapy differs for two conditions.

E. coli is the cause of 80-85% of UTI with staphylococcus saprophyticus being the cause in 5-10% [7]. Other bacterial causes include klebsiella, proteus, pseudomonas and Enterobacter rarely they may be due to viral or fungal infections [8]. These are uncommon and typically related to abnormalities of the urinary system or urinary catheterization [9]. UTI due to staphylococcus aureus typically occurs secondary to blood borne infection [10].

Urine analysis one of the most important diagnostic tool to screen, diagnose the presence of inflammation and once inflammation has seen documented further microbiologic culture is done to determine the etiology of the infection.

Presence of any bacteria of 1/oil immersion field by gram stain and presence of >10⁵ CFU/ml in culture media is defined as significant bacteriuria. Asymptomatic bacteriuria is clinically significant because it may end in to symptomatic UTI. Pyuria is defined as positive of >3WBC/hpf in a centrifuged urine sample.

Methodology

- This was a prospective study done on 100 random urine samples collected from central lab at RIMS Hospital, Raichur. If urine samples are not processed immediately the samples were kept in the refrigerator at 4°C till it is processed. All the samples were processed within 6hrs of collection.

- *Selection of cases*

   Irrespective of age, sex of an individual and presence of urinary tract infection signs and symptoms, the cases were randomly selected.

• Sample collection

   Urine samples were collected by the midstream clean catch method.

• Urine analysis

1. Gram stain was done on un centrifuged urine sample (loop method). Presence of >1micro organism/OIM was considered as significant bacteriuria after observation of at least 10 fields.

2. All samples were cultured irrespective of gram stain results and pyuria, on using blood agar, Mac. Conkey agar and Nutrient agar. It was considered significant growth if colony count is >10⁵/ml of urine. Plates were incubated at 37°C for 24hrs and if no growth after 48hrs it was reported as negative.

3. Microscopic examination of urine, 10ml of urine sample was centrifuged at 2500- 3000 rpm for 5 mins after that supernatant was removed. Then one drop of sediment was placed on the microscopic slide and examined using light microscope under 40x. Any leukocyte >3/hpf in 10 fields were considered as significant pyuria [11].

Observation and Results

A total of 100 cases were studied irrespective of age and sex of an individual. Out of 100 cases, the patient age distribution is as follows.

Table 1: Age Profile

Age	Number	Percentage
20-30	65	65%
30-40	20	20%
40-60	15	15%

Table and chart indicates that 65 (65%) samples were of patient aged 20-30 years. 20 (20%) samples were of the patient aged 30-40 years. 15 (15%) samples were of patient aged 40-60 years respectively. This shows that maximum patients were aged between 20-30 years.

Out of 100 cases, 66 were of females and 34 were of males. In our study most of the positive results were females.
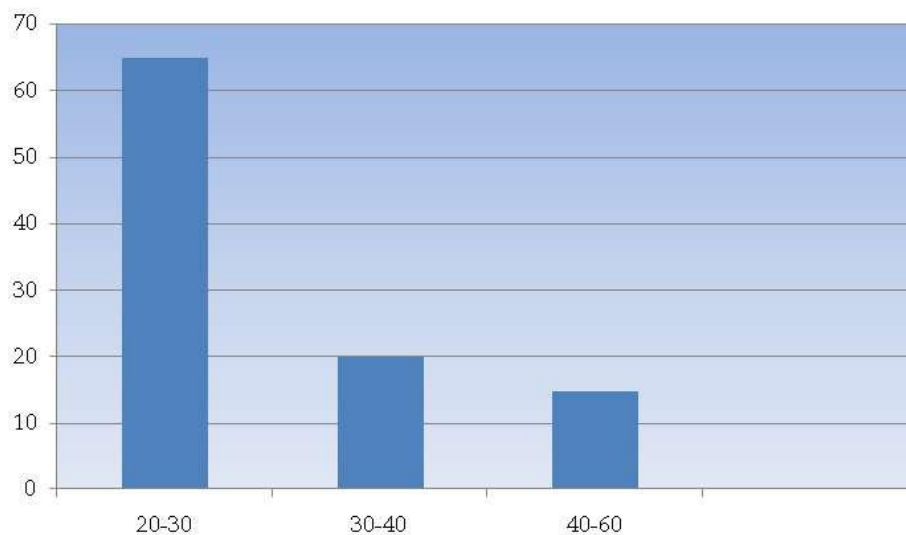
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Fig. 1: Age distribution

Table 2: Sex Profile

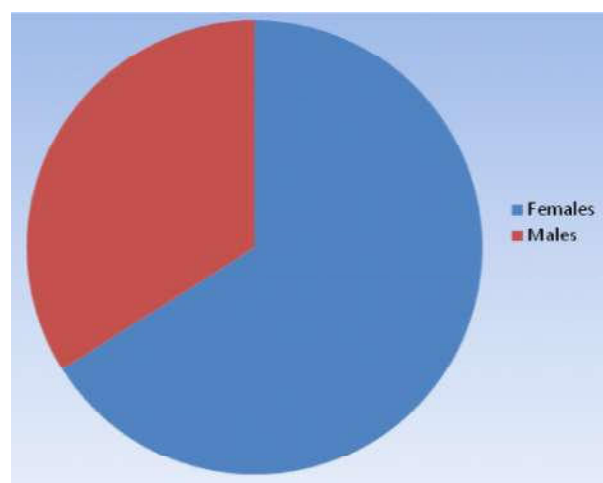| Sex	Number	Percentage
Female	66	66%
Male	34	34%

Fig. 2: Sex Distribution

Table and chart indicates that 66 (66%) out of 100 samples were of female patients and 34 (34%) were of males.

Table 3A: Correlation between Microscopic Examination & Culture (S- Significant & NS- Non- Significant)

Culture Results	Gram Stain Positive (N=44)	Gram Stain Negative (N=56)	Pyuria Positive (29)	Pyuria Negative (71)
Positive (Sg)	10	00	07	03
Negative(Nsg)	34	56	22	68

The gram stain was positive in 44 cases out of them 10 showed significant growth & rest of them showed non significant growth. The reason may be out of 34 cases 16 were gram positive bacilli which may be a lactobacillus which is a normal commensal of female genital tract & rest of them may be because the micro organisms that could not be grown on the blood, nutrient & Mac conkey agar as they may be needing a special culture medias. Whereas 56 cases were negative for gram stain & all of them were negative for culture. Which shows that the gram stain has high sensitivity to rule out UTI as a preliminary test & low specificity may be because of the gram positive bacilli.

The pyuria was positive in 29 cases out of them 7 showed significant growth & 22 were showing no significant growth which may be because of sterile pyuria. This shows that enhanced urine analysis is a simple test but has high specificity & sensitivity to rule out UTI.

Table 3B: Correlation Between Combined Results Of Gram Stain & Pyuria With Culture.

Culture Results	Gram Stain & Pyuria Positive	Gram Stain & Pyuria Negative	Total
Significant Growth	10	00	10
Non Significant Growth	09	81	90
Total	19	81	100

The pyuria & gram stain together showed good correlation in evaluating the UTI cases. In our study pyuria, gram stain negative cases all of them showed no significant growth on culture. This indicates that a combination of microscopy for pyuria & bacteriuria can be used accurately to rule

Table 4: Diagnostic Values of Different Microscopic Tests in Relation to Significant Bacteriuria.(PPV- Positive predictive value & NPV- Negative predictive value)

Urine Analysis Parameters	Sensitivity	Specificity	Ppv	Npv	Chi Square	P Value
Gram Stain	100%	62%	23%	100%	14.14	<0.001
Pyuria	70%	75%	24%	91%	9.07	<0.005
Gram Stain & Pyuria	70%	90%	44%	91.3%	24.11	<0.001

out UTI. Sensitivity, specificity, predictive value (positive & negative), chi-square & P value for the parameters analyzed has predictors of UTI in this study is shown in the table 4.

In our study the gram stain predicting significant bacteriuria was 100% where as for the pyuria it is 70% may be because of technical errors or due to sample collection. But together the specificity increases to 90%. Though gram stain has high sensitivity it has only 62% specificity where as pyuria alone has 75% respectively, by this we can draw a conclusion that enhanced urine analysis is better predictor of UTI rather than a single test.

In our study the negative predictive value is 91% in pyuria & combination of pyuria, gram stain & culture however it was 100% in gram stain this evidences that is strong for diagnostic tests which bring up the property of these tests in determination of UTI. Similarly Moshen. R et al. found 97% of negative predictive value for the above mentioned parameters this is important for health care system in view of cost effectiveness by this we can cut down the number of cases required for urine culture which is costly & time consuming.

In our study out of 44 gram stain positive cases 14 were showing gram positive bacilli they were female patients, none of them showed significant growth on culture. This may indicate presence of normal vaginal flora (most of it being anaerobes), hence have not grown in aerobic cultures. These findings may point towards the fact that gram positive bacilli seen in urine gram stain does not correlate with positive culture hence should not be taken as criteria for positive gram stain, Gram stain positive bacilli are rarely the cause of UTI and hence may be taken as vaginal contaminants.

Discussion

UTI are among the most common bacterial infectious diseases encountered in clinical practice and account for significant morbidity and high medical costs.

If the urine contains significant bacteria but there are no symptoms the condition is known as asymptomatic bacteriuria. In our study out of 100 cases only 5 cases had symptoms of UTI rests were asymptomatic.

Urine analysis is one of the most important tests in clinical laboratories for diagnosis, screening and prevention of UTI and now days it is used as guide for empirical treatment in UTI [12,13].

Yildrim et al for pyurian reported 32%, 93.7%, 83.7% and 58% sensitivity, specificity, positive predictive value and negative predictive value respectively. According to low sensitivity in their study, they suggested that pyuria as a single test could not be able to diagnosis asymptomatic bacteriuria.

In our study negative predictive value is high for a diagnostic test and there by bringing up the property of these tests in determination of urinary tract infection. In other words in urine analysis if no pyuria/bacteriuria was observed which will confirm there is no UTI. This point is important for health care system as a view of cost effectiveness. Because routinely these cultures which being ordered for confirmation of negative tests would be cut down more over than exclusion of infection, establishing of that is critical in health care.

Though today new technological evolution has enabled new diagnostic approaches in urine analysis such as urine flow cytometry and automated microscopic pattern recognition [14] but such approaches needed more studies to evaluate benefits and cost effectiveness aspects of them.

The present study in comparison with others studies showed that the gram stain provided the lowest cost per utility as well as high negative predictive value hence this could alone be used as a screening tool for the presumptive diagnosis of UTI. But as in the most studies one diagnostic tool is not reliable hence in combination with microscopic examination will be the best choice for clinical decision making. The comparative studies are shown in Table 5.

The Table 5 shows the comparison our study with other studies which is showing the sensitivity & specificity for gram stain 80% to 100%, 62% to

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Table 5: Comparison of present study with other studies
(Sn- Sensitivity, Sp- specificity, PPV- Positive Predictive Value, NPV- Negative Predictive Value)

S. No	Authors	Total No cases	Gram stain				Pyuria			
			Sn	Sp	PPV	NPV	Sn	Sp	PPV	NPV
1	Raheela Hussain et al[15]	250	85%	100%	100%	88%	85%	98%	97%	89%
2	Mohsen. R chenari et al	1770	97%	98%	90%	99%	85%	88%	51%	97%
3	Rehmani et al[16]	984	80%	83%	73%	95%	82%	81%	73%	95%
4	Viroj et al[18]	95	96%	93%	94%	95%	65%	74%	75%	64%
5	Present study	100	100%	62%	23%	100%	70%	75%	24%	91%
6	Syed Abdul Mujeeb et al[17]	100	86%	86%	67%	94%	78%	81%	59%	91%

100% respectively. The pyuria shows 65% to 85% of sensitivity & 74% to 98% of specificity.

Conclusion

The following conclusion can be drawn from the present study

1. Urine analysis by gram stain and microscopic examination as a initial diagnostic tool both in symptomatic and asymptomatic cases has high sensitivity and specificity rates.

2. Early diagnosis by enhanced urine analysis will help in therapeutic decision making & thereby preventing further complications.

3. Though there are limitations of above mentioned tests due to various factors like sample collection, technical errors during processing which would influence on results. But it still plays a major role in the primary diagnosis of UTI.

4. Economically the cost effectiveness of both microscopic analysis and gram stain is low as well as less time consuming and less technical skills with high accuracy enabling quick diagnosis.

5. The urine samples negative alone for pyuria and bacteriuria and negative for both needs no further evaluation by culture there by eliminating the costly and time consuming tests in all the cases.

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