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Gram Staining As a Predictor of Urinary Tract Infection.

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Abstract: ***Introduction:*** *Urinary tract infection is one of the most common bacterial infection encountered in clinical practices both in community as well as in hospital settings. UTIs are the second most frequently occurring infections in the general populations after upper respiratory infections. Gram staining of the uncentrifuged urine is a useful guide tool to avoid undue delay for starting empiric therapy for suspected patients of urinary tract infection thus helping to decrease the burden of morbidity caused by chronic UTIs.* ***Material and Methods:*** *This prospective study was conducted in bacteriology section of the Department of Microbiology, Rajendra Institute of Medical Sciences, (RIMS) Ranchi, from September 2017 to January 2018. After taking permission from ethical committee from RIMS, Ranchi a total of 100 uncentrifuged mid-stream urine samples within one hour of collection, were processed for wet mount direct microscopy, gram staining and subsequently semi quantitative culture.* ***Results:*** *During the study period a total of 100 urine samples were submitted to the bacteriology section and processed In the present study, gram staining PPV was 100% and NPV 83.20% which proves that the GS is very reliable in interpretation of urine cultures and superior to wet mount direct microscopy.* ***Discussion and Conclusion:*** *Due to its nonspecific clinical presentation, early diagnosis is important to prevent the complications of UTI. Urine culture and sensitivity is the gold standard test for detecting Urinary tract infection. Gram stain of uncentrifuged urine is a very sensitive and specific screening test for diagnosis of UTI. Presence of pus cells in urine gives the clue and supportive evidence of UTI. GS is a quick and reliable substitute to culture report and superior to direct urine microscopy.*

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I. Introduction

Urinary tract infection is one of the most common bacterial infection encountered in clinical practices both in community as well as in hospital settings. Urinary tract infection is due to pathogenic invasion of the urinary tract from renal cortex of the kidney to the external urethral opening .UTIs are the second most frequently occurring infections in the general populations after upper respiratory infections [1].

Urine analysis for presence of pus cells, bacteria and culture are important in the adequate management of UTIs [2].

A gram stain of urine is an easy, inexpensive means to provide an immediate information about the causative organism of the urinary tract infection. More often, patients are treated by empirical therapy as per local sensitivity pattern of the microorganism. Gram staining of the uncentrifuged urine is a useful guide tool to avoid undue delay for starting empiric therapy for suspected patients of urinary tract infection thus helping to decrease the burden of morbidity caused by chronic UTIs.

II. Material and Methods

This prospective study was conducted in bacteriology section of the Department of Microbiology, Rajendra Institute of Medical Sciences, (RIMS) Ranchi, from September 2017 to January 2018.After taking permission from ethical committee from RIMS, Ranchi a total of 100 uncentrifuged mid-stream urine samples within one hour of collection, were processed for wet mount direct microscopy, gram staining and subsequently semi quantitative culture.

- **Direct microscopy or wet mount preparation:** 0.05ml of well mixed uncentrifuged urine samples was placed on a clean, grease free, glass slide and covered with 20 mm X 20 mm coverslip. The wet mount preparation was then examined under a high power magnification (40X) of a microscope for presence of pus cells. The presence of >1 pus cell / 7 high power fields was considered significant pyuria[3].
- **Gram staining:** 0.05ml of well mixed urine was poured on a clean glass slide, left for air drying, heat fixed and then gram stained [4]. At least 20 fields of the smear were examined under oil immersion objectives (100X).

Presence of more than or equal to 1 bacteria per oil immersion field, which corresponds to 100,000 organisms / ml of urine, was considered significant[5].

- Gram staining and wet mount findings were evaluated for its validity as a screening method by calculating sensitivity, specificity, positive predictive value and negative predictive values respectively.

Formulae and abbreviation:

- **UTI:** Urinary Tract Infection.
- **GS:** Gram staining
- **True positive (TP):** GS and culture both positive.
- **False positive (FP):** Positive GS finding and negative culture.
- **True negative (TN):** GS and culture both negative.
- **False negative (FN):** GS negative and culture positive.
- **Sensitivity = TP/ (TP+FN):** Probability that GS will be positive in patients with UTIs (positive culture).
- **Specificity = TN/ (TN+FP):** Probability that GS will be negative in patients without UTIs (negative culture).
- **Positive predictive value (PPV):** TP/ (TP+FP): Probability that a UTI is present when GS is positive.
- **Negative predictive value (NPV):** TN/ (TN+FN): Probability that a UTI is not present when GS is negative.

III. Results

During the study period a total of 100 urine samples were submitted to the bacteriology section and processed. Majority of the patients were age group of 15-45 years. Male patients were 46% and females. The test results of urine direct microscopy, and Gram staining were compared with urine culture (Table-1) and findings of gram staining and direct urine microscopy were statistically calculated in terms of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) as shown in table-2 and figure-1.

Table-1.Comparative table of Wet mount, GS and urine culture findings:

Urine microscopy	**Culture positive**	**Culture negative**
Positive	34 (TP)	06 (FP)
Negative	46 (FN)	14 (TN)
Gram staining		
Positive	76 (TP)	00(FP)
Negative	04 (FN)	20 (TN)

Table-2. Comparisionof sensitivity, specificity, positive(PPV) and negative predictive value (NPV) of direct microscopy and gram staining of urine.

Parameters	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Direct microscopy	42%	70%	85%	23%
Gram staining	95%	100%	100%	83.20%

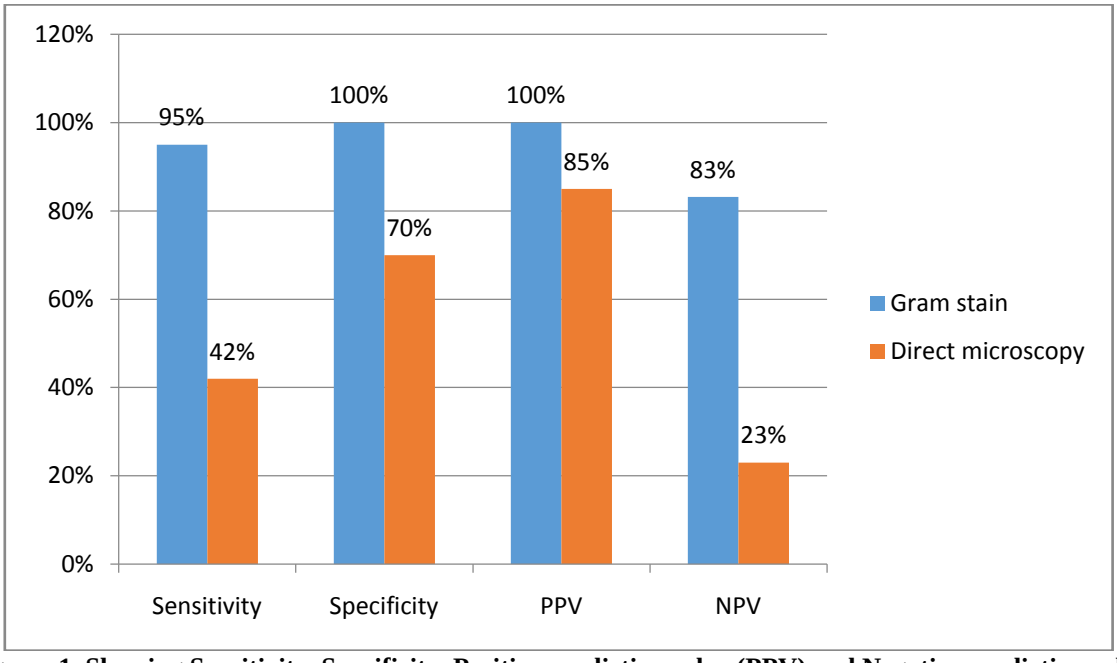

Figure- 1. Showing Sensitivity, Specificity, Positive predictive value (PPV) and Negative predictive value (NPV) of Gram staining and direct microscopy of urine.

IV. Discussion

UTI is defined as a disease caused by microbial invasion of the genitourinary tract that extends from the renal cortex of the kidney to the external urethral meatus[6]. UTIs are the second most infection after respiratory tract infection. Due to its nonspecific clinical presentation, early diagnosis is important to prevent the complications of UTI. Urine culture and sensitivity is the gold standard test for detecting Urinary tract infection. Although, several tests have been proposed for screening and rapid diagnostic methods of UTI, our study aimed at evaluating Sensitivity, Specificity, Positive predictive value and negative predictive value for direct urine microscopy and urine gram staining in diagnosing urinary tract infection. As ideal screening test for significant bacteria must be rapid, inexpensive, simple to use and sensitive and specific.

During the study period a total of 100 urine samples were submitted to the bacteriology section and processed. Majority of the patients were age group of 15-45 years. Male patients were 46% and females 54%.This is inconsonance with the increased prevalence of UTI in women; the main reason being the basic anatomical and physiological differences between the two sexes[7]. Of 100 urinary samples, 80 yielded growth (**≥10^5 or 10^4 to 10^5**) and 20 did not grow any pathogen. Sensitivity of gram staining was calculated to be as sensitive 95%, specificity 100%, positive predictive value (PPV) 100%, negative predictive value (NPV) 83.20% which is comparable with the study done by Satish SP et al[8](2011) reported as sensitivity 89.1%, specificity 86%, positive predictive value (PPV) 85.4%, and negative predictive value (NPV) 89.6% respectively. A study by Matias L et al[9], found that this test had sensitivity of 92.7%, specificity of 88.7%, PPV 68.5% and NPV of 97.9%.

Wet mount examination of uncentrifuged urine was used to detect pyuria as it has been reported that wet mount of well mixed uncentrifuged urine is more reliable than that of centrifuged urine. In ideal condition of the wet film examinations the finding of 1 leucocytes per 7 high power fields corresponds with 10^4 leucocytes per ml and the finding of clearly larger numbers than this indicates significant pyuria[10]. Significant pyuria, in the absence of significant bacteriuria in a symptomatic patient (e.g. acute urethral syndrome) is an indication for treatment and hence the importance of wet film examination. In our study sensitivity of wet mount microscopy was 42%, specificity 70%, PPV85% and NPV 23% respectively. A study by Fatima A et al [11](2017) showed sensitivity to be 85.85%, specific 72.89 %, PPV 64.84% and NPV 89.55%.

Most of the usual causative agents were gram negative bacteria followed by coagulase negative staphylococcus. In the present study, gram staining PPV was 100% and NPV 83.20% which proves that the GS is very reliable in interpretation of urine cultures and superior to wet mount direct microscopy. Here false positive (FP) was recorded zero and 4 false negative (FN) results in our study, false positives could be due to bacteria which failed to grow in culture either because of pre-treatment with antibiotics or they were fastidious organisms. False negatives could be due to lesser numbers of bacteria in the urine that could not be detected by GS but grown in culture. As we know that threshold of detection for microscopy is 10^5 organisms / ml and for culture is 10^3 organisms / ml of urine .Our study had a few limitations. Study was conducted in teaching hospital which receives patients referred from various hospitals in the region. Hence, the patients who received antibiotic when they reached the hospital could not be identified. However, gold standard for diagnosis of UTI is quantitative urine culture for specific bacteria. As one diagnostic test is not reliable for confirmation of UTI, so researchers consider a combination of tests as the best choice for clinical decision making[7]. The advantages to urine microscopy are that leucocytes, casts, and other cellular elements are observed directly[12]. Gram staining has added advantage of guiding the antibiotic therapy by observing the morphology and staining property of the organisms. However, clinician should consider local sensitivity patterns of the possible pathogens.

V. Conclusions

Gram stain of uncentrifuged urine is a very sensitive and specific screening test for diagnosis of UTI. Presence of pus cells in urine gives the clue and supportive evidence of UTI. GS is a quick and reliable substitute to culture report and superior to direct urine microscopy. Besides, the results of gram staining can be used in selection of antibiotics for empirical therapy. However, the treating clinicians must look into the local sensitivity pattern of the probable causative agents. In institutions one must follow the recommendation laid by the board of antimicrobial policy.

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