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To Determine the Diagnostic Accuracy of Urinary Nuclear Matrix Protein 22 in Detection of Recurrence of TCC Bladder Among Patients Who are on Active Surveillance by Taking Cystoscopic Biopsy as a Gold Standard

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ABSTRACT

Objective: To determine the diagnostic accuracy of urinary nuclear matrix protein 22 in detection of recurrence of TCC bladder among patients who are on active surveillance by taking cystoscopic biopsy as a gold standard.
Study Design: Cross-sectional study.
Place and Duration of Study: Departments of Urology, Histopathology and Molecular Biology: Sindh Institute of Urology and Transplantation (SIUT), Karachi Pakistan, from Jan 2021 to Jun 2021.
Methodology: Total 83 patients previously biopsy proven bladder carcinoma who are on active surveillance whether or not they have received intravesical chemotherapy were included in this study. All the information were noted into proforma,
Results: The average age of the patients was 48.80±11.86 years. There were 46(55.42%) male and 37(44.58%) female. Sensitivity, specificity, PPV, NPV and accuracy of urinary nuclear matrix protein 22 was 88.9%, 80%, 93.3%, 69.6% and 86.7% resepctively. Practical implication: This study will help us to diagnose TCC bladder in early stages and to manage it in time sothat morbidity and mortality may be reduced.
Conclusion: Measuring NMP22 level in the blood is a very important tool for detecting Tcc of the urinary bladder as it is much specific and sensitive test.

Keywords: Bladder cancer, Urinary nuclear matrix protein 22, Transitional Cell Carcinoma, Urinary NMP22.

How to Cite This Article: *Pyarali S, Kumar S, Saleem R, Jabeen M, Hashmi A, Sultan G. To Determine the Diagnostic Accuracy of Urinary Nuclear Matrix Protein 22 in Detection of Recurrence of TCC Bladder Among Patients Who are on Active Surveillance by Taking Cystoscopic Biopsy as a Gold Standard. Pak Armed Forces Med J 2023; 73(5): 1488-1492. https://doi.org/10.51253/pafmj.v73iSUPPL-1.11156.*

INTRODUCTION

Among the most common cancers worldwide bladder carcinoma is on seventh number with the frequency of 50,000 new cases in Europe and USA.[1] It is the second most common cancer of the urinary tract and increases seven percent of new cancer cases in male and two percent new cancer cases in female.[2] Several risk factors are associated with its development and include modifiable as well as unmodifiable factors. The latter include age, gender, race and ethnicity, chronic bladder irritation by stone or Foley's catheter and Schistosomiasis infections, bladder birth defects like urachus, genetics and family history, and prior chemotherapy and/or radiotherapy. Modifiable causes encompass smoking, workplace exposure or chemicals, certain medicines and herbal supplements, arsenic in drinking water and an inadequate fluid intake.

Clinical presentation of bladder carcinoma include painless hematuria, irritative bladder symptoms like increase urinary frequency, urgency and history of weight loss, bone pain, flank pain and palpable abdominal mass only in cases of advanced metastasis.

It has been estimated that 60% of patients with diagnosed bladder carcinoma are at the greatest risk of recurrent disease especially within the 5 years of treatment.[3] Thus, all patients with TCC are considered at high risk of recurrent Disease.[4] Currently, cystoscopy is considered as gold standard diagnostic way for bladder carcinoma[5] but there are limitations and pitfall related to these investigations. It has good sensitivity for detecting high-grade urothelial cancer, but decrea-sed sensitivity for detection of low-grade tumors like 4-31%.[17] The subtle cytomorphologic changes of low grade TCC with considerable inter-observer variability is the main leading diagnostic issue.[3]

These cytomorphic changes are also associated with benign pathologies such as stones, infections, effects of radiation and chemotherapy, and trauma to the bladder mucosa resulting from repeated catheterizations.[6] Also, interpretation depends upon the skill of the examiner.[5] On the other hand, the sensitivity of

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Received: 04 Jul 2021; revision received: 21 Sep 2021; accepted: 27 Sep 2021

cystoscopy is limited by the visualization of tumors where flat in situ carcinomas are often missed. Further-more, follow-up cystoscopies also have financial impact and discomfort for the patients.[7] Hence, there is an imperative need to have investigations which are not only cost effective but are least invasive and easy to follow up.

Today, the focus has turned to the identification of molecular markers for diagnostic purposes as well as surveillance including protein based and DNA based assays. Tumor markers used for bladder cancer detec-tion NMP 22 and telomerase.[8]

Urinary NMP22 has recently been introduced at SIUT. The NMP2 is a complexed and fragmented forms of the nuclear mitotic apparatus protein involved in DNA replication, RNA transcription and gene expres-sion is found in voided urine.[9] Bladder cancer cell lines contain 25 fold higher concentration of these mitotic apparatus protein compared to normal cellular linings.[10] The sensitivity of NMP22 is 48-90% while specificity is 85-87%.[11]

To perform NMP22 test in different series, supports the view that frequency of cystoscopic examinations can be reduced in selected patients and can be replaced with NMP22 in detecting recurrence of superficial urothelial carcinoma, reducing not only recurrent early intervention but also logistic burden of cystoscopies.

The present study aimed to determine the diagnostic accuracy of urinary NMP 22 in detection of recurrence of TCC bladder among patients who are on active surveillance by taking cystoscopic biopsy as a gold standard.

METHODOLOGY

This cross sectional study was conducted in departments of Uro-oncology, Histopathology and Molecular Biology: Sindh Institute of Urology and Transplantation, Karachi, Pakistan, from January to June 2018. Sample was selected using non probability consecutive. Sample size was calculated by taking sensitivity as 85.4% and specificity as 76.5% prevalence 70%, margin of error for sensitivity as 10% and margin of error for specificity as 14%, the calculated sample size was as 83.15. Approval of the study was taken from the SIUT's Ethics Review Committee (ERC) and Research Evaluation Unit (REU), CPSP, inform consent was taken from the patient and reports was verified by the Head of Department Microbiology.

Inclusion Criteria: Adult patients from age group 30-70 years of age of either sex, with previously biopsy proven bladder cancer who are on active surveillance whether or not they have received intravesical chemotherapy

Exclusion Criteria: The exlusion criteria were Who are still under investigation and who were not previously diagnosed with an urothelial carcinoma, With Urolithiasis, Urinary Tract Infections, Prostate pathologies like enlargement, carcinoma, Non bladder genitourinary malignancies, Ileal conduits, Recent history of foreign body like stents.

Data was analyzed using SPSS version 22. Mean and SD was calculated for age. Frequency and percentage was calculated for gender, Histopathology and NMP22. A 2x2 table was used to calculate sensitivity, specificity, positive predictive value and negative predictive value and diagnostic accuracy, taking Histopathology as gold standard. Age and gender was addressed through stratification. Post stratification 2x2 table was used to calculate sensitivity, specificity, positive predictive value and negative predictive value and diagnostic accuracy.

RESULTS

Total 83 patients previously biopsy proven bladder carcinoma who are on active surveillance whether or not they have received intravesical chemotherapy were included in this study The average age of the patients was 48.80±11.86 years as shown in Table-I. There were 46 (55.42%) male and 37 (44.58%) female. Recurrence TCC bladder was in 75.9% cases.

Table-I: Descriptive Statistics of Age

Descriptive Statistics		Age (Years)
Mean ± SD		48.80 ± 11.86
95% Confidence Interval for Mean	Lower limit	46.21
	Upper limit	51.38
Median (IQR)		50(20)
Minimum		30
Maximum		70

Diagnostic accuracy of urinary NMP22 in detection of recurrent TCC bladder among patients who are on active surveillance is shown in Table-II. Sensitivity, specificity, PPV, NPV and accuracy of urinary nuclear matrix protein (NMP) 22 was 88.9%, 80%, 93.3%, 69.6% and 86.7% resepctively.

Accuracy of urinary NMP22 in detection of recurrent TCC bladder for below and equal to 50 and above 50 years of age was 79.1% and 95% as shown in

table-III & IV respectively. Simialrly with resepct to gender, urinary NMP22 in detection of recurrent TCC bladder for male and feamle was 89.1% and 83.4% respectively.

Table-II: Diagnostic Accuracy of Urinary NMP 22 in Detection of Recurrent TCC of Bladder Bladder among Patients who are on Active Surveillance by Taking Cystoscopy Biopsy as a Gold Standard.

Urinary Nuclear Matrix Protein 22 (NMP22)	Histopathology (Recurrence of TCC Bladder)		Total
	Yes	No	
≥ 6.4	56 (TP)	4 (FP)	60 (72.3%)
<6.4	7 (FN)	16 (TN)	23 (27.7%)
Total	63 (75.9%)	20 (24.1%)	83

Sensitivity: 88.9%, Specificity: 80%, Positive predictive Value: 93.3%, Negative predictive value: 69.6%, Accuracy: 86.7%

Table-III: Diagnostic Accuracy of Urinary NMP 22 in Detection of recurrent TCC Bladder among Patients Who Are On Active Surveillance by Taking Cystoscopy Biopsy as a Gold Standard for ≤50 years of Age.

Urinary Nuclear Matrix Protein 22 (NMP22)	Histopathology (Recurrence of TCC Bladder)		Total
	Yes	No	
≥6.4	24	4	28
<6.4	5	10	15
Total	29	14	43

Sensitivity: 82.8%, Specificity: 71.4%, PPV: 85.7%, NPV: 66.7%, Accuracy: 79.1%

Table-IV: Diagnostic Accuracy of Urinary NMP 22 in Detecting Recurrent TCC of Bladder among Patients Who Are On Active Surveillance by Taking Cystoscopy Biopsy as a Gold Standard for 51-70 years of Age.

Urinary Nuclear Matrix Protein 22 (NMP22)	Histopathology (Recurrence of TCC Bladder)		Total
	Yes	No	
≥6.4	32	0	32
<6.4	2	6	8
Total	34	6	40

Sensitivity: 94.1%, Specificity: 100%, PPV: 100%, NPV: 75%, Accuracy: 95%

Table-V: Diagnostic Accuracy of Urinary NMP 22 in Detecting Recurrent TCC Bladder among Patients Who Are On Active Surveillance by Taking Cystoscopy Biopsy as a Gold Standard for Male.

Urinary Nuclear Matrix Protein 22 (NMP22)	Histopathology (Recurrence of TCC Bladder)		Total
	Yes	No	
≥6.4	30	2	32
<6.4	3	11	14
Total	33	13	46

Sensitivity: 90.9%, Specificity: 84.6%, PPV: 93.8%, NPV: 78.6%, Accuracy: 89.1%

Table-VI: Diagnostic Accuracy of Urinary Nmp 22 in Detecting Recurrent TCC Bladder Among Patients Who Are On Active Surveillance By Taking Cystoscopic Biopsy As A Gold Standard for Female

Urinary Nuclear Matrix Protein 22 (NMP22)	Histopathology (Recurrence of TCC Bladder)		Total
	Yes	No	
≥6.4	26	2	28
<6.4	4	5	9
Total	30	7	37

Sensitivity: 86.7%, Specificity: 71.4%, PPV: 92.9%, NPV: 55.6%, Accuracy: 83.8%

DISCUSSION

Urinary bladder carcinoma was mentioned in the literature for the first time in the seventeenth century when it was described by a lithotomist at the time of stone retrieval from the bladder.[14]. While in Middle East, non-urothelial histologies are more frequent due to schistosomiasis.

The Nuclear matrix protein 22 (NMP22) assay has lower sensitivity for Ta tumors (42 to 76%) compared with muscle-invasive tumors (50-98%).[15-20] Studies also evaluated the sensitivities of NMP22 in surveillance of both TCC and Schistosomial bladder cancers. Sensitivities were 91.3% and 95.6% respectively suggesting the role of NMP22 in surveillance of Schistosomial bladder cancer also.[16] The potential role of this assay has been evaluated in several trials.

The Nuclear matrix protein 22 (NMP22) assay and cytology were compared in 1331 individuals at high risk for bladder cancer. In all diagnostic cystoscopy done. Bladder cancer was diagnosed in 79 patients via cystoscopy. The NMP22 assay detected 44 of these (56%), whereas cytology detected 12 (16%). The Nuclear matrix protein 22 (NMP22) detected a bladder cancer in four cases that were missed on initial cystoscopy.[22]

The NMP22 assay, cystoscopy and cytology were done in 668 cases undergoing surveillance for bladder cancer. The NMP22 assay and cystoscopy combined are more effective and sensitive than any alone investigation.[23]

A prospective analysis of four commercially avail-able urine marker tests and urine cytology for bladder cancer surveillance reported that the combination of NMP22 and cytology seemed to increase the sensitivity for detecting high-grade tumors compared with single markers and other combinations.[19] The general use of the NMP22 assay for screening high-risk populations (smokers, high-

risk occupations) rather than for surveillance is more problematic because of the low prevalence of urothelial neoplasia in these populations.

To find out the significance of urinary NMP22 in detection of recurrence of (TCC) bladder, a total of 83 patients, from age group 30-70 years of either sex, previously biopsy proven bladder cancer who are on active surveillance whether or not they have received intraesical chemotherapy were included in this study.

In our study, age bracket ranges from 32-70 years with the mean age of 48±11.86 years. Majority of our studied population is younger i.e,. less than 40 years 28(33.73%). The next most frequent group comprise age ranges from 51-60 years 22(26.51%). The remainder constitute age groups of 61-70 years and 41-50 years that makes up 18(21.69%), and 15(18.07%) of the stu-died population respectively. According to one study, total 12 cases of TCC bladder reported in patients less than 21 years of age. These tumors were of low grade and stage and were associated with excellent prog-nosis.[20] On the contrary, different studies have proved that it is a disease of the elderly, with 80% of cases fall in the age group of 50-79 year with peak incidence in seventh decade.[21] And a median age at diagnosis is 71 for transitional cell carcinoma.[22]

One epidemiological study conducted at the Memorial Sloan Kettering Cancer Centre emphasized that the differential behavior has related to sex steroids and their receptors.[23] Another study has showed that occurrence of TCC bladder is most common in post-menopausal women than men.[24] In our study out of 83 patients, there were 46 (55.42%) males and 37 (44.58%) female of which 95% were postmenopausal.

In our study, Diagnostic accuracy of urinary NMP 22 in detecting recurrent TCC urinay bladder among patients who are on active surveillance were 88.9% (Sensitivity), 80% (specificity), 93.3% (PPV), 69.6% (NPV) and 86.7 (accuracy). Of 83, subjects those having NMP22 values greater than 6.4 U/ml, approximately 60 (72.3%) were found to have recurrent TCC bladder diagnosed on histopathology. Our results are suppor-ted by the study from North America. This study emphasized that NMP22 has a high negative predictive value of 86% and sensitivity to detect malignancy (100% for invasive disease and 70% overall).[25] An Egyptian study showed sensitivity of 91.3% and specificity of 87.5% in detection of malignancy in patience for active surveillance. Shariat *et al* found 50% sensitivity and positive predictive value of 81%. Zippe *et al* found the specificity of 85% and sensitivity of 100%. In a study of 2222 patients with history of non-muscle-invasive blad-der cancer, NMP22 levels were significantly associated with disease recurrence and progression. In our study, the accuracy of NMP22 in detecting recurrent TCC for below and equal to 50 and above 50 years of age was 79.1% and 95% respectively. Similarly, with resepct to gender, NMP 22 in detecting of recurrent of TCC bladder for male and female was 89.1% and 83.4%. Those patients with higher NMP22 values i.e,. (>6.4 U/ml), follow-up cystoscopy with biopsy will be done after three months. On the contrary, with low values (<6.4 U/ml) will have their cystoscopies ommited at three months. Both group will have their cystoscopies done in six month.[11]

CONCLUSION

Urinary NMP22 level is an important test to diagnose TCC bladder in early stages. It is highly specific and sensitive non-invasive test. In our study this test showed high positive predictive value in diagnosing superficial TCC bladder. This test helps us to avoid frequent cystoscopies in follow up cases of TCC for detecting recurrent carcinoma hence reducing burden on the health system.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

SP & SK: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

RS & MJ: Data acquisition, data analysis, approval of the final version to be published.

AH & GS: Critical review, concept, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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