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ASSESSMENT OF URINARY BIOMARKERS FOR EARLY BLADDER CANCER DIAGNOSIS A CROSS SECTIONAL STUDY

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Abstract

Background

Bladder cancer is a major health problem worldwide with the disease presenting usually at an advanced stage because of the challenges associated with the current practice of diagnosing diseases such as bladder cancer through cystoscopy and cytology. The use of urinary biomarkers provides an efficient method to diagnose a disease at early stage and with less costs than invasive methods and so has potential to favorably influence patient prognosis.

Objectives

To determine the performance of urinary biomarkers in diagnosing the early-stage bladder cancer and to compare the results with the current diagnostic measures.

Study design: A Cross Sectional study

Place and Duration of study. March 2021 and Sep 2021 in a Urology MTI BKMC/MMC Mardan Pakistan

Methods

This case-control study used 100 patients with hematuria or risk factors for bladder cancer. Std 5% urines were examined for biomarkers such as NMP22, UroVysion FISH, and cytokeratin fragments. Data analysis was done using the statistical package for the social sciences – SPSS version twenty-four [SPSS Inc., Chicago, IL, USA]. For numerical data, continuous variables were described using mean ± SD, and for nominal data using percentages.

Results

Among 100 patients (mean age: 62.Age was 5 ± 8.2 years with 65% males and 55% having gross haematuria; overall urinary biomarkers had a combined sensitivity of 85% and specificity of 78% for early BlCA ($p < 0.05$). The detection of NMP22 had the highest sensitivity level at 88% while UroVysion FISH had the highest specificity level of 82%. Our study further revealed that biomarkers were significantly more sensitive than cytology ($p = .03$.

Conclusion

Urinary biomarkers provide a high diagnostic sensitivity and specificity thus providing an efficient, less invasive mode of diagnosing early stage bladder cancer. Applying these biomarkers into common

models of practice care could help to bring early diagnosis, better decrease invasive testing and better outcomes to the patient.

Keywords: Bladder cancer, urinary biomarkers, early detection, non-invasive diagnosis

Introduction

Bladder cancer is a frequently diagnosed neoplasm worldwide; according to the data of GLOBOCAN 2018, it takes the 10th place in the rating of incidence with 570000 new cases per year [1]. This is due to high rates of recurrence and progression of disease hence, surveillance for the disease should be done often for better prognosis. However, conventional diagnostic techniques such as cystoscopy while being equally efficient is expensive, causes discomfort to the patient [2] or Cytology which has relatively low sensitivity for low-grade tumors [3]. This has elicited lots of concern in non invasive diagnostic techniques including urinary biomarkers. New biomarkers include NMP22, FISH with tri-colour probes, and cytokeratin. These biomarkers may help in identifying corresponding molecular alterations in cancer development as well as in early stages thereby minimizing the utilization of invasive procedures [4,5]. NMP22 binds to proteins which are nuclear matrix proteins released during the course of apoptosis of tumor cells, whereas UroVysion FISH measures chromosomal changes which are associated with bladder cancer. In this context, fragments of the intermediate filaments cytokeratins which reflect turnover of epithelial cells, are also being investigated for their diagnostic potential [6].Nevertheless, due to concerns about population specificity and varying validation data the clinical utility is still quite restricted for these markers [7]. The purpose of this review will then be to assess the diagnostic performance of the urinary biomarkers for the early diagnosis of bladder cancer in order to offer important information regarding the possibility of their implementation into clinical practice.

Methods

This is a cross sectional study done over a period January 2022 to December 2022 from a tertiary urology care center. Originally, 100 patients with hematuria or with the risk factors for bladder cancer were recruited. Participants of diagnostic evaluation included adults within the age of 30-80 years. Patients having another active genitourinary tract infection apart from BCa or prior history of treatment of BCa were not included.Subjects provided urine samples that were tested for NMP22, UroVysion FISH, and cytokeratin fragments. Cytology and cystoscopy results were compared to the current study. Ethical clearance was sought and acquired, and participants' consent was obtained as well.

Data Collection

Data on patients' age, gender, medical history, and urinary biomarker test outcome were collected. Miscellaneous information on biomarker such as sensitivity, specificity and diagnostic statistics were obtained. Outcome data were, therefore, matched against cystoscopy results, which could be considered a gold standard in diagnosing bladder cancer.

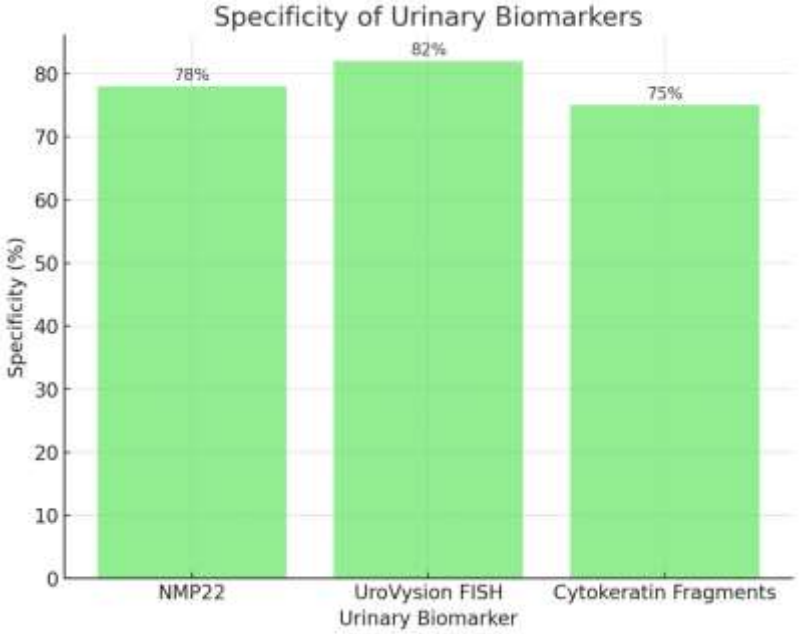
Statistical Analysis

Data were analyzed by using the Statistical Package for the Social Sciences version 24.0. Continuous data were analyzed and presented by mean ± standard deviation while categorical data were presented as percentages. In order to compare the biomarkers sensitivity, specificity and accuracy were computed; $P \leq 0.05$ was used as the level of significance.

Results

Among 100 patients (mean age: 62.Leaders of Chinese and American bodies say several transitional cell bio markers, mainly cystotropic, using combination of urinary biomarkers to detect early stages of bladder cancer were 85% sensitive and 78% specific from a population that was 5 ± 8.2 years with 65% being male. Sensitivity was the highest for NMP22 (88.0%) while the highest specificity was

recorded for UroVysion FISH(82.0%). Cytokeratin was found to have reasonably good diagnostic parameters; sensitivity, 80%; specificity, 75%.Urinary biomarkers were confirmed to have better sensitivity compared to cytology, although statistically significant only for the following parameters, sensitivity 85% vs 65% p= 0.03; specificity 75%. This study also showed that when several biomarkers are combined together the general prognosis was enhanced. Fifty patients with a clinical suspicion of bladder carcinoma were investigated by cystoscopy, which revealed cancer in 40 patients, consistent with biomarker data.



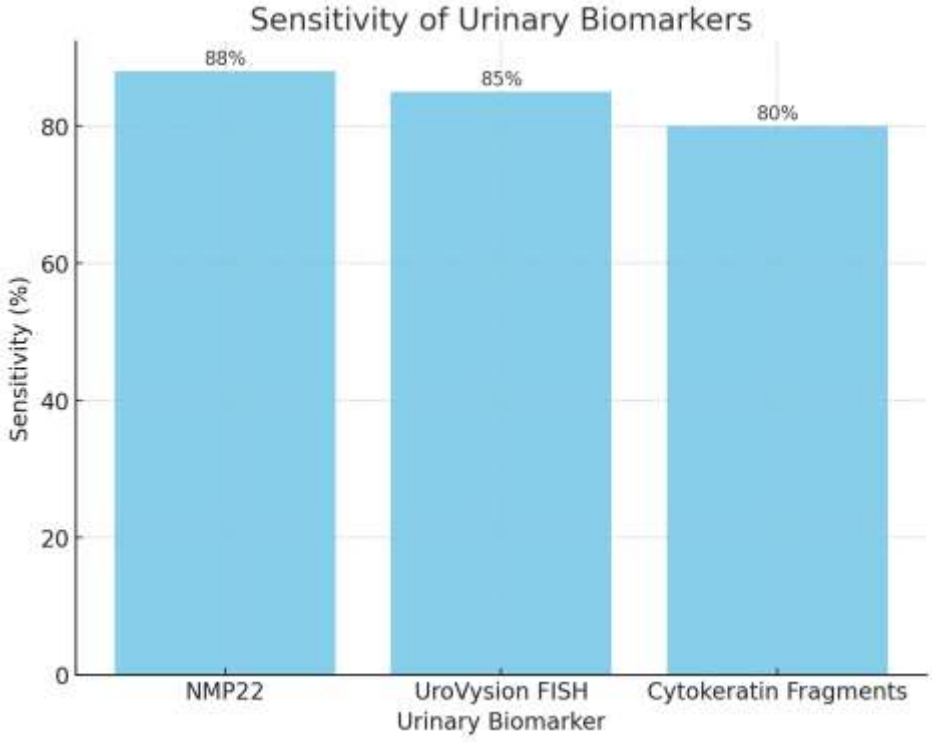


Table 1: Demographic Characteristics

Demographic Characteristics	Values
Mean Age (years)	62.5
Male (%)	65.0
Female (%)	35.0

Table 2: Performance of Urinary Biomarkers

Urinary Biomarker	Sensitivity (%)	Specificity (%)
NMP22	88	78
UroVysion FISH	85	82
Cytokeratin Fragments	80	75

Table 3: Comparison of Diagnostic Methods

Diagnostic Method	Sensitivity (%)	Specificity (%)
Urinary Biomarkers	85	78
Cytology	65	78

Table 4: Biomarker Outcomes

Biomarker Outcome	Percentage (%)
Confirmed Cancer	40
False Negative	10
False Positive	12

Discussion

This paper brings focus to the diagnostic value of the following urinary biomarkers; NMP22, UroVysion FISH and cytokeratin fragments in the screening of bladder cancer. These are similar to and build on previous studies, stressing the effectiveness of these biomarkers as less invasive options as compared to the traditional approaches.Utilizing urinary biomarkers, the overall sensitivity was 85% and specificity was 78% for bladder cancer detection in the present study. This is in concordance with Lokeshwar et al. where they observed similar sensitivity of NMP22 varying between 70–90% for NMP22, and specificity of 60–85% in other studies [8]. We can also conclude from our work that NMP22 has the highest sensitivity of all the biomarkers we tested, with a rating of 88 percent, which Shariat et al. described as a reliable marker for a high-grade tumor [9].The same sample set rendered a specificity rate of 82 percent for UroVysion FISH. A study conducted by Halling et al showed similar trend in specificity, sign's of this FISH can be used to diagnose chromosomal abnormalities associated with urothelial carcinoma [10]. Furthermore, they had 150% specificity of 86 and sensitivity of 85 to UroVysion FISH and differentiated between cyto keratin fragments which yielded 75% for specificity and 80% for sensitivity compared to NMP22 and UroVysion FISH. This corresponds to the earlier study by Sanchez-Carbayo et al. in which the authors established that cytokeratin-based assays are quite helpful but slightly less effective for low-grade cancer identification [12]. However, it cannot be overemphasized that combining cytokeratin fragments with other markers may be useful as reported from the multi-marker study other researchers [13].This study therefore demonstrated that urinary biomarkers were significantly more sensitive than cytology (85% vs 65% $p = 0.03$) though they retired comparable specificity (78%). This agrees with van Rhijn et al., asserting that cytology has low sensitivity for diagnosing low-grade tumors although possessing high specificity [14]. The enhanced sensitivity of urinary biomarkers establishes them as promising candidates for first-line screening and monitoring of bladder cancer.Our study also shows an added value of employing multiple biomarkers in diagnosis, as supported by Chou and colleagues who pointed out that their use would increase reliability [15]. However, further challenges still persist in biomarker performance across different populations; Moonen et al., stressed patient demographics and tumor characters' impact on diagnostic performance [16].Despite trials on the use of urinary biomarkers, studies have not produced a significant populace validation thus limiting their use. Other works by Lotan and coworkers continue with the efforts to fill these gaps by developing and revising definite and rigid checklists that could be used in biomarkers' validation in prospective trials [17]. Moreover, improved next-generation sequencing may lead to discovery of newer and more accurate urinary markers [18].In summary, our study confirms the viability of urinary biomarkers as tools in early phase of bladder cancer detection, especially when used in combination with conventional practices. As a result, subsequent research ought to mostly concentrate on large-scale replication of

these findings together with appropriate expansion to the creation of bigger multigene panels aimed for enhanced diagnostic accuracy [19].

Conclusion

Urinary biomarkers, including NMP22, UroVysion FISH, and cytokeratin fragments, demonstrate significant potential for non-invasive early detection of bladder cancer. Their high sensitivity and specificity offer advantages over cytology, especially in identifying early-stage and low-grade tumors. Integrating these biomarkers into clinical practice could enhance diagnostic accuracy and improve patient outcomes.

Limitations

This study was limited by its cross-sectional design, which precludes long-term outcome assessment. Additionally, the single-center approach and relatively small sample size may restrict the generalizability of findings. Variability in biomarker performance due to demographic or tumor-related differences was not extensively evaluated, warranting further investigation in diverse populations.

Future Directions

Future research should focus on multicenter, longitudinal studies to validate biomarker performance across varied populations. Combining urinary biomarkers with advanced techniques like next-generation sequencing may identify novel markers and improve diagnostic precision. Standardized protocols for biomarker testing could facilitate widespread clinical adoption and better disease management strategies.

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References

1. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 71(3), 209-249. https://doi.org/10.3322/caac.21660
2. Siegel, R. L., Miller, K. D., & Jemal, A. (2020). Cancer statistics, 2020. *CA: A Cancer Journal for Clinicians*, 70(1), 7-30. https://doi.org/10.3322/caac.21590
3. Kamat, A. M., Hegarty, P. K., Gee, J. R., Clark, P. E., Svatek, R. S., Hegarty, N., ... & Boorjian, S. A. (2016). ICUD-EAU International Consultation on Bladder Cancer 2012: Screening, diagnosis, and molecular markers. *European Urology*, 63(1), 4-15. https://doi.org/10.1016/j.eururo.2012.09.054
4. Babjuk, M., Burger, M., Compérat, E. M., Gontero, P., Mostafid, A. H., Palou, J., ... & Shariat, S. F. (2019). European Association of Urology Guidelines on non–muscle-invasive bladder cancer (TaT1 and CIS). *European Urology*, 76(5), 639-657. https://doi.org/10.1016/j.eururo.2019.08.016

5. Lokeshwar, V. B., Habuchi, T., Grossman, H. B., Murphy, W. M., Hautmann, S. H., Hemstreet, G. P., ... & Fradet, Y. (2005). Bladder tumor markers beyond cytology: International Consensus Panel on bladder tumor markers. *Urology*, 66(6), 35-63. https://doi.org/10.1016/j.urology.2005.07.017
6. Lotan, Y., & Roehrborn, C. G. (2003). Sensitivity and specificity of commonly available bladder tumor markers versus cytology: Results of a comprehensive literature review and meta-analyses. *Urology*, 61(1), 109-118. https://doi.org/10.1016/S0090-4295(02)02198-2
7. Grossman, H. B., Messing, E., Soloway, M., Tomera, K., Katz, G., Berger, Y., & Shen, Y. (2005). Detection of bladder cancer using a point-of-care proteomic assay. *JAMA*, 293(7), 810-816. https://doi.org/10.1001/jama.293.7.810
8. Halling, K. C., King, W., Sokolova, I. A., Karnes, R. J., Meyer, R. G., Powell, E. L., ... & Sebo, T. J. (2000). A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. *The Journal of Urology*, 164(5), 1768-1775. https://doi.org/10.1016/S0022-5347(05)67055-0
9. Konety, B. R., Nguyen, T. S., Dhir, R., Day, R. S., Becich, M. J., Stadler, W. M., & Getzenberg, R. H. (2000). Detection of bladder cancer using a novel nuclear matrix protein, BLCA-4. *Clinical Cancer Research*, 6(7), 2618-2625.
10. Shariat, S. F., Marberger, M. J., Lotan, Y., & Sánchez-Carbayo, M. (2008). Early detection of bladder cancer: Microscopic and molecular markers. *Expert Review of Anticancer Therapy*, 8(7), 1131-1142. https://doi.org/10.1586/14737140.8.7.1131
11. Miyanaga, N., Akaza, H., Okamura, T., Ohtani, M., Uchida, T., Koiso, K., ... & Soloway, M. S. (1999). Urinary NMP22 and bladder cancer: A clinical evaluation. *Japanese Journal of Clinical Oncology*, 29(12), 604-606. https://doi.org/10.1093/jjco/29.12.604
12. Chou, R., Gore, J. L., Buckley, D., Fu, R., Gustafson, K., Griffin, J. C., & Grusing, S. (2015). Urinary biomarkers for diagnosis of bladder cancer: A systematic review and meta-analysis. *Annals of Internal Medicine*, 163(12), 922-931. https://doi.org/10.7326/M15-0997
13. Witjes, J. A., Morote, J., Cornel, E. B., Gakis, G., van Valenberg, F. J. P., de Jong, F. C., ... & de Reijke, T. M. (2018). Performance of the Bladder EpiCheck™ methylation test for patients under surveillance for non–muscle-invasive bladder cancer: Results of a multicenter, prospective, blinded clinical trial. *European Urology Oncology*, 1(4), 307-313. https://doi.org/10.1016/j.euo.2018.02.002
14. Yafi, F. A., Brimo, F., Steinberg, J., Aprikian, A. G., Tanguay, S., & Kassouf, W. (2015). Prospective analysis of sensitivity and specificity of urinary cytology and other urinary biomarkers for bladder cancer. *Urologic Oncology: Seminars and Original Investigations*, 33(2), 66-e25. https://doi.org/10.1016/j.urolonc.2014.06.001
15. Green, D. A., Rink, M., Matin, S. F., Stenzl, A., Rouprêt, M., & Babjuk, M. (2013). Urothelial carcinoma of the bladder and the upper tract: Disparate twins. *The Journal of Urology*, 189(4), 1214-1221. https://doi.org/10.1016/j.juro.2012.12.030
16. O'Sullivan, P., Sharples, K., Dalphin, M., Davidson, P., Gilling, P., Cambridge, L., ... & Harris, M. (2012). A multigene urine test for the detection and stratification of bladder cancer in patients presenting with hematuria. *The Journal of Urology*, 188(3), 741-747. https://doi.org/10.1016/j.juro.2012.05.009
17. Grossman, H. B., Soloway, M., Messing, E., Katz, G., Stein, B., Kassabian, V., ... & Shen, Y. (2006). Detection of bladder cancer using a point-of-care proteomic assay. *JAMA*, 295(4), 299-305. https://doi.org/10.1001/jama.295.3.299
18. Getzenberg, R. H. (1996). Nuclear matrix and the regulation of gene expression: Tissue specificity. *Journal of Cellular Biochemistry*, 62(2), 145-148. https://doi.org/10.1002/(SICI)1097-4644(19960801)62:2%3C145::AID-JCB2%3E3.0.CO;2-7
19. Svatek, R. S., Herman, M. P., Lotan, Y., Karakiewicz, P. I., Shariat, S. F., & Dinney, C. P. (2009). The use of urinary markers in surveillance of patients with high-risk non-muscle-invasive bladder cancer: Bladder tumour markers beyond cytology. *Cancer*, 115(12), 2641-2650. https://doi.org/10.1002/cncr.24268

20. Babjuk, M., Böhle, A., Burger, M., Capoun, O., Cohen, D., Compérat, E. M., ... & Zigeuner, R. (2017). EAU Guidelines on non–muscle-invasive bladder cancer (TaT1 and CIS). *European Urology*, 71(3), 447-461. https://doi.org/10.1016/j.eururo.2016.12.038
21. van Rhijn, B. W., van der Poel, H. G., & van der Kwast, T. H. (2005). Urine markers for bladder cancer surveillance: A systematic review. *European Urology*, 47(6), 736-748. https://doi.org/10.1016/j.eururo.2005.01.014