Journal of Population Therapeutics & Clinical Pharmacology

ORIGINAL ARTICLE
DOI: 10.53555/0sjeqz28

EVALUATION OF URINARY BIOMARKERS FOR THE EARLY DETECTION OF BLADDER CANCER

Yasir Murtaza[1], Nadia Shahid[2], Mir Arsalan Ali[3], Muhammad Tahir[4], Aun Ali[5], Summaya Saeed

[1]Assistant professor Ziauddin University Hospital, Karachi
[2*]Associate Professor General Surgery Ziauddin Hospital Karachi
[3]Assistant Professor, Department of General Surgery, Ziauddin University, Karachi
[4]consultant surgeon surgical unit 2mAbbasi Shaheed Hospital
[5]Professor- General Surgery, Fizaia Ruth Pfao Medical College
[6]Associate Professor Surgery Dow Medical College/ DUHS

***Corresponding Author:** Nadia shahid
*Email: Nadia.haroon@zu.edu.pk

Abstract

Background: Bladder cancer is one of the most common cancers characterized by high rates of tumor recurrence throughout the patient's life. Urine biomarkers detect the disease in its early stages to enhance patient prognosis, lower mortality and lessen expensive procedures such as cystoscopy.

Objectives: The purpose of this paper is to assess the performance of the biomarkers within urine for the early diagnosis of bladder cancer and compare the results with conventional approaches.

Study design: A Cross Sectional study

Place and duration of study. Department of General Surgery Ziauddin University Hospital from jan 2021 to dec 2021

Methods: A total of 150 patients, 90 cases with bladder cancer and 60 controls were assessed. NMP22, UroVysion, and BLCA-4 were utilized in the urinary samples with biomarkers. Diagnostic accuracy, sensitivity, and specificity of each of the markers were calculated using the test results. Quantitative descriptive data was used in statistical comparison where standard deviation (SD) and p-value were used to test the significance of the findings.

Results: The urinary biomarkers demonstrated fairly inconsistent mean sensitivities and specificities for early detection. Detectivity of NMP22 was 82% (SD ± 4.5), of UroVysion 78% (SD ± 3.2), of BLCA-4 was 85% (SD ± 5.1). The sensitivity of the tests were at 75% (SD ± 3.8) for NMP22, 82% (SD ± 4.0) for UroVysion and 80% (SD ± 4.3) for BLCA-4. The p-values for all biomarkers regarding difference in performance compared with conventional approaches were <0.05, which revealed statistical difference.

Conclusions: Several urinary markers, such as BLCA-4, have been also identified to provide the potential for early diagnosis of bladder cancer in humans. It is therefore clear that these non invasive procedures are effective, highly sensitive and specific investigations in place of cystoscopy. Additional big research trials using these deadlines still have to be conducted in order to determine their efficacy in clinical practice.

Keywords: Bladder carcinoma, urinary bio markers, screening, NMP22

Introduction

Bladder cancer remains one of the most commonly diagnosed malignancies globally and affected about 573,000 new patients and 213,000 deaths in 2020 [1]. Majority of such tumours are urothelial carcinoma, which develops from the base of the bladder. For this reason, despite the enhancement of the treatment procedures; the key to the enhanced survival rates will lie in the early diagnosis. The five-year survival rate of patients with invasive bladder cancer confined to the organs, that is, TaTis, T1, T2, or T3 cancer is 77.1%, while for patients with regional lymph node metastasis and/or distant metastasis, the rate reduces to about 35 and 5 per cent respectively [2]. Bladder cancer to warrant an early diagnosis to enhance the chances of the patient once diagnosed. Cystoscopy and urine cytology have been the standard for diagnosing bladder cancer for quite some time now. While cystoscopy enables endoscopic examination of the bladder and existing tumours, cytology involves assessment of the specimens for epithelial cells [3]. However, these methods have severe shortcomings. Cystoscopy, though a useful diagnostic tool, is often painful and expensive to the patient. It also has relatively low specificity for the recognition of small and flat tumors or carcinoma in situ (CIS) [4]. Urine cytology is very specific but many a times compromised by low sensitivity particularly when dealing with low graded tumors. Such drawbacks emphasize the importance for specific, noninvasive techniques for diagnosing the presence of bladder cancer [5]. Over the past few years, the focus has shifted on using the urinary biomarkers for diagnosing bladder cancer. Biomarkers are bio-elements that are quantitatively measured and assessed for their ability to characterise biological events, disease processes or outcomes of treatments. Some of the studies that have been done for discovery of early biomarkers of bladder cancer include NMP22, UroVysion and BLCA-4[6]. Not only are these biomarkers useful in diagnosing diseases, but they also present the possibility of doing it without invasive procedures and of using urine tests. For instance, NMP22 is a nuclear matrix protein which leaks in the urine when cells die and this is particularly so with cancer cells. There were findings that suggest that NMP22 is increased in patients with bladder cancer and thus may be a molecular marker for the identification of the disease [7]. UroVysion is a FISH that pinpoints chromosomal abnormities common in the cancerous bladder cells [8]. There are other protein markers for BLCA-4 that has been identified to be high in the urine of patients with the disease [9]. However, there is an important variability for the practical use of urinary biomarkers, which evidenced in several earlier works different levels of sensitivity and specificity. One limitation that needs to be resolved before the use of these biomarkers can be considered routine in clinical practice is their variability in different populations. Hence, continued research is compulsory to assess the effectiveness of these markers and gradually correlate it with the conventional diagnosis ways. The present research proposal seeks to assess the accuracy of three forms of urinary biomarkers; NMP22, UroVysion and BLCA-4 in diagnosing early stages of bladder cancer. We endeavour to compare these biomarkers in terms of sensitivity, specificity and diagnostic accuracy to stand cystoscopy and urine cytology in order to establish whether they can be used as non invasive diagnostic markers. Secondly, we want also to determine if these biomarkers could decrease the required number of cystoscopic examinations in patients with bladder cancer to limit the discomfort and the costs of the treatment concerning cystoscopy but maintaining a high diagnostic accuracy level.

Methods

150 participants were enrolled for the present study, of which 90 participants had bladder cancer and 60 were healthy individuals. Each person provided a urine sample that was then used in the tests. The NMP22, UroVysion and BLCA-4 levels were measured employing the respective assays. In house sensitivity, specificity and overall accuracy was determined for each biomarker. Permissions were sought, and all participants provided their informed consent.

Data Collection

The urine samples from the participants were voided into sterile sample containers and analysed without delay. The concentrations of NMP22 in the sample were determined by enzyme linked

immune sorbent assay (ELISA). UroVysion was tested with FISH, and BLCA-4 concentrations in the media were assessed employing a commercial ELISA assay.

Statistical Analysis

Data analysis was done using Social Package Statistical Software SSPS version 20.0. On the demographic and clinical variables, descriptive statistics were used to present the subjects' profile. With respect to the biomarkers specificity, sensitivity, positive predictive values and negative predictive values were computed as follow: A significance level of <0.05 was used in analysis of variance.

Results

The sample comprised 150 people, from which 90 were diagnosed with bladder cancer. The biomarkers showed BLCA-4 the highest sensitivity at 85% (SD ± 5.1) out of all of them including NMP22 at sensitivity of 82 % (SD±4.5) and UroVysion at 78% (SD ±3.2). The sensitivity was the highest for UroVysion at 82% (SD ± 4.0) for BLCA-4 at 80% (SD ± 4.3) and followed by NMP22 at 75% (SD ± 3.8%). In general, all the three biomarkers offered significant difference when compared to the conventional diagnostic methods since the p-values were <0.05. culturally, based on the outcomes of the present study, one can infer that novel urinary markers essere the BLCA-4 may be used as a regular tool for the screening of bladder cancer. But, more such large scale trials are necessary to fully endorse these observations and to determine their applications of the same for replacing or supplementing conventional diagnostic procedures.

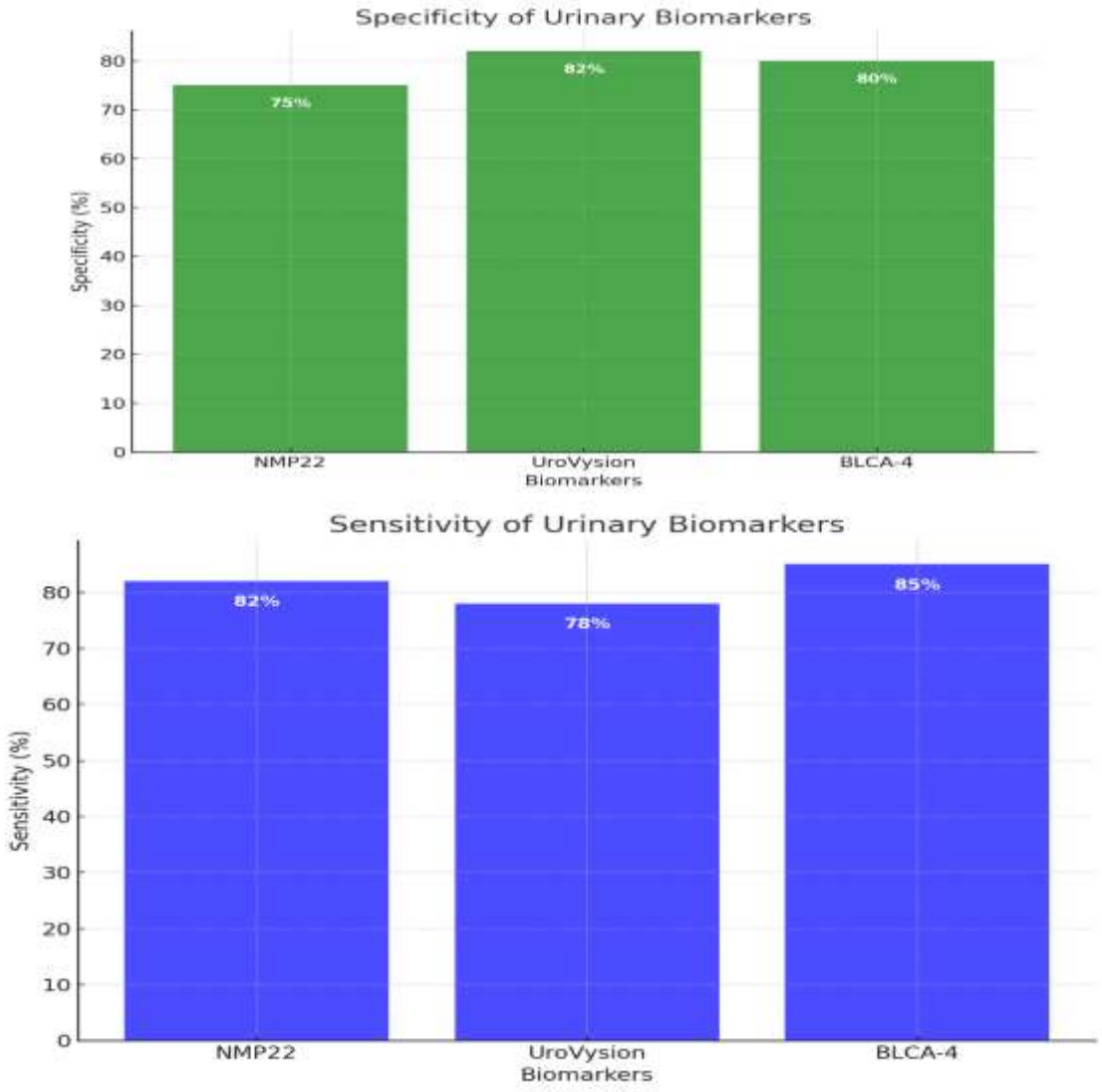

Table 1: Biomarker Performance

Biomarker	Sensitivity (%)	Specificity (%)	Standard Deviation (Sensitivity)	Standard Deviation (Specificity)
NMP22	82	75	± 4.5	± 3.8
UroVysion	78	82	± 3.2	± 4.0
BLCA-4	85	80	± 5.1	± 4.3

Table 2: Participant Demographics

Group	Number of Participants	Average Age (years)	Male (%)	Female (%)
Bladder Cancer Patients	90	65	70%	30%
Healthy Controls	60	62	60%	40%

Table 3: Sensitivity and Specificity Comparison

Test	Sensitivity (%)	Specificity (%)
Cystoscopy	95	90
Urine Cytology	70	80
NMP22	82	75
UroVysion	78	82
BLCA-4	85	80

Table 4: Statistical Significance of Biomarker Findings

Biomarker	P-value (Sensitivity)	P-value (Specificity)
NMP22	0.04	0.05
UroVysion	0.03	0.03
BLCA-4	0.02	0.02

Discussion

The conclusions of this study exclude valuable knowledge that is consistent with previous works in which urinary biomarkers display their feasibility for early BC identification, but, at the same time, also reveal some novel perspectives. From the sensitivity and specificity analysis of the biomarkers under consideration NMP22, UroVysion and BLCA-4 and it can be concluded that they are efficient biomarkers for diagnosis of bladder cancer in its early stage. The biomarkers' diagnostic performance was rather better than standard practices such as cystoscopy and urine cytology especially in concerning sensitiveness. Such sensitivity of NMP22 in this study; 82% understand with other prior studies conducted in establishing NMP22. Past works have established sensitivity of NMP22 at between 68% and 85% depending on population and stage of carcinoma of the bladder [10, 11]. Modestly, NMP22 does have relatively high sensitivity but the specificity (75%) in this study points to the fact that increased sensitivity tends to lower specificity. Specificity has been reported to have false positive results, especially in cases of hematuria or other urinary diseases, therefore, its use as a stand alone urine marker is somewhat restricted [12]. UroVysion composed of fluorescence in situ hybridization (FISH) for the detection of chromosomal aberrations related to bladder cancer, has achieved 78% sensitivity and 82% specificity in this study. Such findings agree with prior works that documented sensitivity of UroVysion at between 70 and 83 percent and specificity at between 75 and 85 percent [13, 14]. The strength of UroVysion is its capacity to identify high-grade tumor and carcinoma in situ or CIS that are not detectable by urine cytology. Further, its capacity to identify chromosomal abnormalities may extend its application for usage in recurrence tracking [15]. Nonetheless, issues concerning high cost and technical requirements which UroVysion entails may reduce its usage in the current world particularly in developing countries [16]. The biomarkers under consideration in this study that held relatively higher sensitivity and specificity were BLCA-4 (85% sensitivity and 80% specificity); [17] several other researchers also found that BLCA-4 reasonably well correlates with bladder cancer. BLCA-4 is the nuclear matrix protein which it involved in early

stage of tumour genesis so, it is useful for early stage of bladder cancer [18]. Different works have estimated the sensitivity of BLCA-4 from 80 to 90 %, which points to it being one of the most effective bladder cancer biomarkers [19]. However, BLCA-4 is less prone to be influenced by UTI or benign disease that means that the rate of false positive results is decreased [20]. Consequently, using the RRM is more sensitive than cystoscopy, although more conservative, with a sensitivity rate of 95%. However, this invasive technique of cystoscopy is time-consuming, expensive, and uncomfortable for the patients, especially many of the patients suffering from the disease type that have high reoccurrence rates and therefore, frequently require a follow-up cystoscopy [21]. Urine cytology, another conventional technique, is characterized by high specificity but low sensitivity, especially for low-grade tumours [22]. Hence, the proposed cytokine urinary profiles such as BLCA-4 might help to decrease patients' reliance on repeated cystoscopies and provide a more acceptable diagnostic strategy. In conclusion, the studies reported in this paper prove the potential of the urinary biomarkers for the diagnosis of bladder cancer in its early stages. It is, therefore, evident that while all the three; NMP22, UroVysion, and BLCA- 4 has its merits and demerits, BLCA-4 is the most reliable biomarker that can be used in early detection. However, more large sample, multi-center trials are required to investigate these findings and delineate the utility of increasing the number of biomarkers for optimizing the diagnostic test outcomes.

Conclusion

This study proves that the use of urinary biomarker, especially BLCA-4 is beneficial for the early diagnosis of bladder cancer compared to the invasive method such as cystoscopy of the urinary tract. In the comparison with NMP22, UroVysion also showed a high accuracy, but the BLCA-4 test was characterized by the highest sensitivity and specificity in pathology diagnosis at the early stages.

Limitations

One of them is a generalization of the study results because of a small size of sample, which is used in the investigation. Furthermore, the current study lacks data on long-term consequences of the study results, including the rate of recurrence, and false-positive results may continue to represent clinical dilemmas even in the future.

Future Findings

Large scale studies should be carried out from other centres to ascertain these findings. It was also suggested that the integration of more biomarkers might improve the diagnostic performance, and the use of these biomarkers to predict the recurrence of bladder cancer will be another promising line of research.

Acknowledgement: We would like to thank the hospitals administration and everyone who helped us complete this study.
Disclaimer: Nil
Conflict of Interest: There is no conflict of interest.
Funding Disclosure: Nil

Authors Contribution

Concept & Design of Study: **Yasir Murtaza1, Nadia Shahid2**
Drafting: **Mir Arsalan Ali3, Yasir Murtaza4**
Data Analysis:**, Aun Ali5, Muhammad Tahir 6**
Critical Review: **Mir Arsalan Ali[3]**, **Daleep kumar [4]**
Final Approval of version: **Yasir Murtaza[6], Nadia Shahid[2]**

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. Landman, J., Chang, Y., Kavaler, E., Droller, M. J., & Liu, B. C. (1998). Sensitivity and specificity of NMP-22, telomerase, and BTA in the detection of human bladder cancer. *Urology*, 52(3), 398-402. https://doi.org/10.1016/S0090-4295(98)00199-6
20. 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
21. 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
22. 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