Exploring Apolipoprotein A1 As A Liquid Biopsy Biomarker For Diagnosis Of Low-Grade Bladder Cancer

Hosam A.A. Al-Issawi* Omar F. Abdul-Rasheed* Mohammed F. Alqanbar** Ali Mohammed Salih Abo Taheen***

ABSTRACT

Bladder cancer is known for its high recurrence rate, necessitating frequent invasive and costly re-examinations. The development of a non-invasive diagnostic method utilizing urinary biomarkers could greatly enhance early detection and monitoring. This study investigates apoA1 as a potential non-invasive marker for diagnosing low-grade bladder cancer. A total of 60 participants were enrolled, including 50 males and 10 females, with a median age of 63. Using fully automated ELISA, urinary ApoA1 levels were assessed, and a cutoff value of 190 ng/ml was determined. ApoA1 exhibited a sensitivity of 89.4% and a specificity of 85% for distinguishing low-grade bladder cancer from high-grade cases. The study concludes that urinary ApoA1 demonstrates high diagnostic accuracy and is a promising liquid biopsy biomarker for early detection of low-grade bladder cancer, offering an alternative to invasive diagnostic methods.

Keywords: Bladder cancer, Urinary biomarker, Apolipoprotein A1, liquid biopsy, Early diagnosis.

INTRODUCTION

It is expected to find bladder tumors in the urology department. These tumors have a very high rate of recurrence compared to other types of tumors. In 2022, it is projected that a total of 81,180 individuals will receive new diagnoses of bladder cancer, with 61,700 cases affecting males and 19,480 affecting females (1). The incidence rate of bladder cancer is four times higher in men than in women (2). Additionally, the risk of developing bladder cancer increases with age, with individuals aged 80 and above, both males and females, facing the highest risk (3). Furthermore, with a significant mortality rate, it is expected that 17,100 individuals die from the disease within the same year. Although urothelial bladder carcinomas are primarily detected in their early, non-muscle invasive stage, approximately one-third of cases progress into aggressive recurrent disease despite this initial identification (4). Bladder cancer diagnosis poses significant challenges for clinicians due to the limited diagnostic tools available (5).

The current gold standard, cystoscopy, while effective, often leads to complications such as pain, urinary tract infections, and hematuria (6). Additionally, urine cytology, a non-invasive method of examining cells from urine samples, is commonly used for diagnosis and surveillance. However, it suffers from limitations, including high specificity (86%) but low sensitivity (48%), resulting in poor interobserver variability, especially in grading urothelial carcinoma (7). Current diagnostic techniques for bladder cancer, such as cystoscopy and cytology, while effective, are invasive, uncomfortable, and costly. Consequently, there is a pressing need for non-invasive diagnostic markers that offer accuracy and patient comfort (8).

In recent years, researchers have increasingly focused on identifying urinary biomarkers for bladder cancer diagnosis. Among these biomarkers, apolipoprotein A1 (apoA1) has emerged as a promising candidate (9). ApoA1, a protein constituent of High-density lipoprotein (HDL), is essential for lipid metabolism and has been implicated in various physiological processes, including inflammation and immune response (10). Studies investigating the capability of apoA1 as a noninvasive marker for bladder cancer analysis have shown encouraging results. ApoA1 has demonstrated high sensitivity and specificity in distinguishing patients with bladder cancer from healthy individuals, suggesting its potential utility in clinical practice (11).
This research aims to delve deeper into the role of apoA1 as a noninvasive marker for diagnosing low-grade bladder cancer.

MATERIALS AND METHODS

Subjects in the study

This cross-sectional study was conducted in Iraq in 2023. All patients enrolled were diagnosed with bladder cancer through histopathological confirmation, and none had received radiation or chemotherapy before their inclusion. Additionally, patients with symptoms of renal failure were excluded from the study. Ethical approval for the study protocol was obtained from the Ethics Committee of Al-Nahrain University (Approval No.1367, Date: 29/2/2023). All participants provided informed consent before their inclusion in the study, ensuring voluntary participation and the protection of their privacy.Urine samples were collected at Al-Saffer Hospital in Karbala, Iraq, in accordance with the guidelines set by the Ethics Committee. The research was conducted at the Biochemistry Department's laboratory, College of Medicine, University of Al-Nahrain. Sample collection spanned from March 1, 2023, to September 30, 2023.

Urinary sample preparation

The samples containing approximately 20 ml were obtained as the first urine of the day (mid-stream) and promptly placed into a sterile container. Subsequently, the sample is subjected to centrifugation to eliminate any particulate matter. After centrifugation, the clarified urine samples were stored at (-20°C) until it was testing.

* Department of Biochemistry, College of Medicine
University of Al-Nahrain, Baghdad/ Iraq.
E. mail: Hosam.a@uokerbala.edu.iq ; omar.rasheed@colmed-alnahrain.edu.iq
** Department of Pathology, College of Medicine, Karbala University, Karbala/ Iraq.
E. mail: mohammed.fawzi@uokerbala.edu.iq
*** Urology Department, Al-Safeer Hospital, Karbala/ Iraq.
E. mail: alimsabutaheen@gmail.com

Enzyme-linked immunosorbent assay (ELISA)

ApoA1 levels were quantified by fully automated ELISA, employing quantitative Immunoassay Kits from (ELK Biotechnology, CHN), following the manufacturer's protocol. The test principle utilized in this kit is the Sandwich enzyme immunoassay. The color change is quantified using spectrophotometry, specifically at a wavelength of 450nm. The concentration of ApoA1 in the samples is determined by correlating the samples' optical density (OD) with the standard curve.

Statistical analysis

The study employed Receiver Operating Curve (ROC) analysis to determine the optimal cutoff point and evaluate the diagnostic performance of urinary ApoA1 as a biomarker. The analysis also considered the 95% confidence interval (CI) for the ROC to ensure accuracy. SPSS statistical package (version 26) was used for all statistical testing. Descriptive statistics were applied to summarize demographic and clinical characteristics of the participants, while ROC analysis was utilized to assess sensitivity, specificity, and the area under the curve (AUC). The statistical significance was set at *P-value < 0.05, and all data were analyzed using SPSS (IBM Corp, Armonk, NY, USA).

RESULT

Demographic features and clinical characteristics of studied groups.

To assess urinary ApoA1 protein levels, the study included 60 bladder cancer patients, consisting of 50 males (83.3%) and 10 females (16.7%). The median age for individuals with bladder tumors was 63. Among the cases, 54 patients (90%) were aged over 50 years. The grade distribution consisted of 48 patients with low-grade and 12 patients with high-grade.

Evaluating the diagnostic effectiveness of urinary ApoA1

The data unequivocally demonstrates that the concentration of ApoA1 in the urine of persons with low grade bladder cancer is significantly elevated compared to that of high-grade bladder cancer ($P < 0.001$). Using a cutoff value of 190 ng/ml, ApoA1 had a sensitivity of 89.4% and a specificity of 85% in differentiating bladder cancer from low-grade and high-grade bladder cancer. The diagnosis was established using the optimal threshold value advised by (ROC) curve, as depicted in Figure 1. The ultimate clinical diagnosis was determined using histological findings.

DISCUSSION

In recent times, there has been a growing anticipation for the use of "liquid biopsy" as a diagnostic tool that does not require invasive procedures (12). Consequently, there have been substantial endeavors to create new urine markers that exhibit better diagnostic capabilities compared to urinary cytology (13). The urine biomarker for diagnosing bladder cancer, which includes bladder tumor antigen (BTA) STAT and BTA TRAK, has been approved by the U.S. FDA. The sensitivities of BTA STAT and BTA TRAK were (64-69%) and (62-71%), respectively, while their specificities were (73-77%), (45-81%) respectively (14).

The current study confirmed the urine level of ApoA1 with sensitivity and specificity of 89.4% and 85%, respectively; ApoA1 seems to be a promising liquid biopsy to diagnosis low-grade bladder cancer, Urinary ApoA1 shows high expression in low-grade bladder cancer cases, while patients with high-grade bladder cancer exhibit lower levels. Our result aligns with nearly several studies conducted among the Chinese and Indians population. (15-18). A meta-analysis of ApoA1 stat reported a specificity of 90.7% and a sensitivity of 90% after reviewing Four studies with 771 participants, which shows high sensitivity and specificity compared with cytology (9). The following investigations have consistently demonstrated the diagnostic importance of ApoA1 in the detection of tumor grades. Studies have consistently discovered increased levels of ApoA1 in ovarian, colorectal, and pancreatic cancers (19).

Salem, Hosni, et al. suggest ApoA1 Urinary Apo a1 has the potential to serve as a non-invasive and extremely sensitive diagnostic and monitoring biomarker for bladder cancer (15). Li, Hongjie, et al. Demonstrate that ApoA1 may serve as a promising biomarker associated with the early detection of bladder cancer (16). Li, Changying, et al. Propose that urine Apo A1 could serve as a supplementary marker for urinary cytology in detecting bladder cancer. (17).

The Receiver Operating Characteristic (ROC) analysis showed that the diagnosis accuracy was 0.901 at a cut-off value of ≤ 190 ng/ml. Despite this, this result was higher than that reported in China (16, 17),

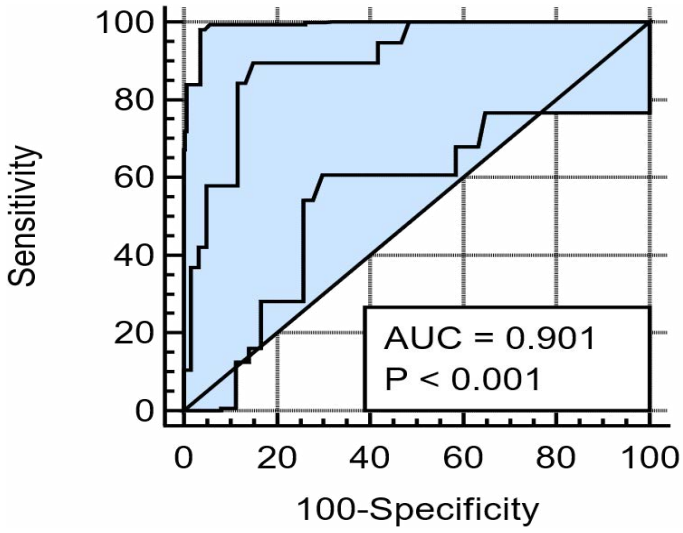

Figure 1. ROC curve of urinary Apo-A1 as a detection marker for bladder cancer grades with 60 urine samples. The optimal cutoff was 190 ng/ml, and the AUC obtained was 0.901 (95% CI 0.813–0.957)

while the result was close to that reported in Egypt (15). Our results demonstrated the presence of ApoA1 in urine, suggesting that ApoA1 could serve as a promising diagnostic biomarker for low-grade bladder cancer.

LIMITATIONS OF THE STUDY

The limited number of participants (n=60) reduces the ability to generalize the findings to larger populations.

FUTURE ACTION PLAN

Conducting studies with larger and more diverse populations to validate the diagnostic accuracy of ApoA1 across different ethnic groups and clinical settings.

CONCLUSION

The high sensitivity and specificity of urinary ApoA1 make it a promising liquid biopsy biomarker for the early diagnosis of low-grade bladder cancer.

Authorship Contribution: All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes

Potential Conflicts of Interest: None

Competing Interest: None

Acceptance Date: 11-09-2024

REFERENCES

1. Alanee S, Alvarado-Cabrero I, Murugan P, Kumar R, Nepple KG, Paner GP, et al. Update of the International Consultation on Urological Diseases on bladder cancer 2018: non-urothelial cancers of the urinary bladder. World J Urol. 2019;37:107-14.
2. Saginala K, Barsouk A, Aluru JS, Rawla P, Padala SA, Barsouk A. Epidemiology of bladder cancer. Med Sci. 2020;8(1):15.
3. Guancial EA, Roussel B, Bergsma DP, Bylund KC, Sahasrabudhe D, Messing E, et al. Bladder cancer in the elderly patient: challenges and solutions. Clin Interv Aging. 2015;10:939-49.
4. Su H, Xue X, Wang Y, Lu Y, Ma C, Ji Z, et al. Competitive Risk Model for Specific Mortality Prediction in Patients with Bladder Cancer: A Population-Based Cohort Study with Machine Learning. J Oncol. 2022;2022.
5. Schmitz-Dräger BJ, Droller M, Lokeshwar VB, Lotan Y, Hudson MLA, Van Rhijn BW, et al. Molecular markers for bladder cancer screening, early diagnosis, and surveillance: the WHO/ICUD consensus. Urol Int. 2015;94(1):1-24.
6. Fankhauser CD, Waisbrod S, Fierz C, Becker AS, Kranzbühler B, Eberli D, et al. Diagnostic accuracy of ultrasonography, computed tomography, cystoscopy and cytology to detect urinary tract malignancies in patients with asymptomatic hematuria. World J Urol. 2021;39:97-103.
7. Lee H-H, Kim SH. Review of non-invasive urinary biomarkers in bladder cancer. Transl Cancer Res. 2020;9(10):6554.
8. Önal B, Han Ü, Yilmaz S, Köybasioglu F, Altuğ U. The use of urinary nuclear matrix protein 22 (NMP22) as a diagnostic adjunct to urine cytology for monitoring of recurrent bladder cancer—institutional experience and review. Diagn Cytopathol. 2015;43(4):307-14.
9. Dardeer KT, Mohammed KA, Hussein TD, Elsheemy MS. Apolipoprotein A1 as a novel urinary biomarker for diagnosis of bladder cancer: a systematic review and meta-analysis. Indian J Urol. 2021;37(3):217-25.
10. Nazir S, Jankowski V, Bender G, Zewinger S, Rye K-A, van der Vorst EP. Interaction between high-density lipoproteins and inflammation: Function matters more than concentration! Adv Drug Deliv Rev. 2020;159:94-119.
11. Tzouanas C, Lim JSY, Wen Y, Thiery JP, Khoo BL. Microdevices for non-invasive detection of bladder cancer. Chemosensors. 2017;5(4):30.
12. Castro-Giner F, Gkountela S, Donato C, Alborelli I, Quagliata L, Ng CK, et al. Cancer diagnosis using a liquid biopsy: challenges and expectations. Diagnostics. 2018;8(2):31.
13. Tomiyama E, Fujita K, Hashimoto M, Uemura H, Nonomura N. Urinary markers for bladder cancer diagnosis: A review of current status and future challenges. Int J Urol. 2024;31(3):208-19.
14. Chakraborty A, Dasari S, Long W, Mohan C. Urine protein biomarkers for the detection, surveillance, and treatment response prediction of bladder cancer. Am J Cancer Res. 2019;9(6):1104.
15. Salem H, Ellakwa DE-S, Fouad H, Hamid MA. APOA1 AND APOA2 proteins as prognostic markers for early detection of urinary bladder cancer. Gene Rep. 2019;16:100463.
16. Li H, Li C, Wu H, Zhang T, Wang J, Wang S, et al. Identification of Apo-A1 as a biomarker for early diagnosis of bladder transitional cell carcinoma. Proteome Sci. 2011;9:1-11.
17. Li C, Li H, Zhang T, Li J, Liu L, Chang J. Discovery of Apo-A1 as a potential bladder cancer biomarker by urine proteomics and analysis. Biochem Biophys Res Commun. 2014;446(4):1047-52.
18. Magray JA, Pandith AA, Qasim I, Khateeb M, Hamid A, Koul A, et al. Significant implications of APOA1 gene sequence variations and its protein expression in bladder cancer. Biomedicines. 2021;9(8):938.
19. Liu X, Zheng W, Wang W, Shen H, Liu L, Lou W, et al. A new panel of pancreatic cancer biomarkers discovered using a mass spectrometry-based pipeline. Br J Cancer. 2017;117(12):1846-54.