International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 14, No. 1, January 2025 Online ISSN: 2250-3137
Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_14.1.2025.26

Original Research

Biomarker Potential of ApoA1 in Detecting Bladder Cancer: A Comparative Study

Dr. Vivek Kumar Jain[1], Dr. Sandeep Tiwari[2], Dr. Vidyanand Pandit[3]

[1]Associate Professor, Department of Biochemistry, Graphic Era Institute of Medical Sciences, Dehradun, Uttarakhand, India
[2]Assistant Professor, Department of Biochemistry, Graphic Era Institute of Medical Sciences, Dehradun, Uttarakhand, India
[3]Assistant Professor, Department of Pathology, SRVS Government Medical College, Shivpuri, Madhya Pradesh, India

Corresponding Author
Dr. VidyanandPandit
Email:panditvidya@gmail.com

Received: 01 December 2024 Accepted: 08 January 2025

ABSTRACT

Background: Bladder cancer (BC) is the most common malignant tumor affecting the urinary system and is ranked as the tenth most frequently diagnosed malignancy worldwide. Apolipoprotein A1 (ApoA-I), a key component of high-density lipoprotein (HDL), plays a vital role in maintaining cholesterol homeostasis. Numerous studies have highlighted the crucial involvement of apolipoprotein A1 (APOA1) in tumor growth, invasion, and metastasis. This study aimed to evaluate the ratio of APOA1 in urine to serum among patients diagnosed with bladder cancer and explore the role of changes in this biomarker.

Materials and Methods: A total of 56 paired blood and urine samples were obtained from bladder cancer patients. The participants included both genders, with an average age of 65.24 ± 10.40years. Additionally, 56 samples from healthy individuals served as controls. Apolipoprotein A1 levels in serum and urine were quantified using the ELISA technique.

Results: The mean concentrations of apolipoprotein A1 in serum and urine in the bladder cancer group were significantly elevated compared to the control group with p-values of <0.01. A moderate positive correlation was observed between serum and urine apolipoprotein A1 concentrations ($r=0.49$, $p<0.01$).

Conclusion: The elevated levels of serum and urine apolipoprotein A1 observed in bladder cancer patients suggest their potential use as biomarkers for early detection of the disease. However, urine apolipoprotein A1 appears to be a more reliable biomarker compared to serum levels, owing to its stronger association with bladder cancer and reduced interference from other medical conditions.

Key Words: Apolipoprotein A1, Bladder, Cancer, ELISA

INTRODUCTION

Bladder cancer (BC) is recognized as the most frequently occurring malignancy of the urinary tract and is the tenth most common cancer worldwide. The disease predominantly affects males, with an estimated male-to-female ratio of approximately 4:1. In 2020, global statistics revealed that bladder cancer ranked among the top 10 cancers, accounting for over 0.57 million newly diagnosed cases [1].

Tobacco use remains the most significant risk factor for bladder cancer. Occupational and environmental exposure to harmful chemicals further elevates disease risk. Male gender and advancing age have also been strongly associated with increased susceptibility to bladder cancer. Diagnostic methods include imaging of the upper urinary tract and cystoscopy, which are utilized to identify visual features of gross or microscopic hematuria, depending on its severity and potential malignancy [4-6].

Lipid profiles have been extensively studied in bladder cancer. Among apolipoproteins, ApoA1 and ApoB are of particular interest. ApoA1, the main protein component of HDL, is essential for cholesterol metabolism and transport. It maintains lipoprotein structural integrity and facilitates solubility due to its amphipathic nature. Moreover,

DOI: 10.69605/ijlbpr_14.1.2025.26

ApoA1 modulates lipid metabolic enzymes, acting as both an activator and inhibitor [7].

HDL particles vary significantly in size and composition. Their primary function is reverse cholesterol transport, where HDL particles or lipid-free ApoA1 collect cholesterol from peripheral cells and transport it to the liver for excretion. About 70% of ApoA1 synthesis occurs in the liver, while the remaining 30% is produced in the intestines. ApoA1 forms complexes with lipids such as phospholipids, cholesterol, and ceramides, facilitated by ATP-binding cassette transporter A1 (ABCA1). Lecithin-cholesterol acyltransferase (LCAT) utilizes ApoA1 as a coenzyme to esterify cholesterol. Transporters like ABCA1, ATP-binding cassette G1 (ABCG1), and scavenger receptor class B1 (SR-B1) regulate HDL size and composition [8].

Blood lipids and apolipoproteins, historically associated with cardiovascular diseases, and are now being studied for their role in cancer. Animal studies suggest that specific apolipoproteins influence tumor growth by altering immune cell activity. Apolipoproteins and lipid levels are increasingly recognized for their prognostic value in various cancers. For example, ApoA1 has been implicated in tumor development, invasion, and chemotherapy response [9].

Urine proteomics represents a promising approach for identifying early-stage biomarkers of bladder cancer. Variations in urine protein content and composition may provide insights into the disease's origin, progression, and prognosis, as urine is in direct contact with bladder epithelial cells. Compared to plasma sampling, urine collection is minimally invasive and more convenient. Several proteins in urine have been evaluated as potential biomarkers for bladder cancer. Non-invasive urine-based proteomic biomarkers are particularly appealing due to their cost-effectiveness and potential for early disease detection [10]. This study aimed to evaluate the ratio of APOA1 in urine to serum among patients diagnosed with bladder cancer and explore the role of changes in this biomarker.

MATERIAL AND METHODS

This case-control study involving 112 participants. The participants were divided into two groups: the first included 56 patients diagnosed with bladder cancer. The second group comprised 56 healthy controls with normal bladder tissue and no history of renal or systemic diseases.

Patients presenting with pelvic pain and hematuria underwent initial diagnostic imaging, including ultrasound and CT scans. Those diagnosed with bladder tumors had the lesions removed via transurethral resection under general or spinal anesthesia. Tumors were subsequently subjected to histopathological examination to assess their diagnosis, stage, and grade.

Venous blood samples (~10 mL) were collected from all participants, transferred into gel tubes, allowed to clot, and centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum was stored in labeled Eppendorf tubes at −80°C. Apolipoprotein A1 (APOA1) concentrations were measured in both serum and urine using a sandwich enzyme-linked immunosorbent assay (ELISA) technique.

The assay employed APOA1 kits capable of dose-dependent quantification across a variety of human biological samples, including blood, serum, urine, and cell culture supernatants. These kits allow direct APOA1 quantification without requiring prior purification. A standard curve was created by plotting optical density (OD) values (Y-axis) against APOA1 concentrations (X-axis) to determine relative and absolute concentrations. Regression analysis was utilized to establish the best-fit regression line for accurate calculations.

RESULTS

As presented in Table 1, the gender distribution in both groups is similar, with a male predominance (82.14%) in both cases and controls. However, a significant difference was observed in the age distribution between the two groups. The cases exhibited a higher proportion of older individuals, particularly in the age group >70 years, where 25% of cases were represented, compared to only 1.79% of controls. This finding suggests that age may be a distinguishing factor between the two cohorts. Despite these differences in age distribution, the mean age for both groups did not differ significantly (64.54 ± 11.85 years for cases vs. 65.93 ± 8.94 years for controls), with a p-value of 0.48, indicating no substantial age-related confounding.

The smoking status further distinguishes the two groups. A significant difference in smoking prevalence was observed, with 80.36% of cases being smokers compared to only 12.5% of controls, yielding a p-value of <0.01. This suggests that smoking may be an important factor associated with the cases, likely contributing to the pathophysiological mechanisms under investigation.

DOI: 10.69605/ijlbpr_14.1.2025.26

Table 1: Basic profile of study participants

Variable	Cases		Control		P Value
	n	%	n	%	
Gender					
Male	46	82.14	46	82.14	1
Female	10	17.86	10	17.86	
Age group (years)					
<41	0		7	12.50	<0.01
41-50	13	23.21	9	16.07	
51-60	12	21.43	24	42.86	
61-70	17	30.36	15	26.79	
>70	14	25.00	1	1.79	
Age (mean ± SD)	64.54 ± 11.85		65.93 ± 8.94		0.48
Smoking					
Yes	45	80.36	7	12.50	<0.01
No	11	19.64	49	87.50	

In Table 2, the analysis of serum and urine Apolipoprotein A1 (APOA1) levels revealed striking differences between the two groups. Serum APOA1 levels were markedly higher in the cases (493.41 ± 165.56 ng/ml) compared to controls (88.97 ± 31.06 ng/ml), with a p-value of < 0.01, indicating a highly significant difference. Similarly, urine APOA1 levels also demonstrated a significant difference between groups, with cases showing substantially higher levels (928.84 ± 294.66 ng/ml) than controls (227.78 ± 98.17 ng/ml), again with a p-value of < 0.01. These results underscore the potential of APOA1 as a biomarker of interest in the context of the studied condition.

Table 2: APOA1 values in cases and controls

Group	Cases (Mean ± SD)	Controls (Mean ± SD)	p Value
Serum Apolipoprotein A1 (ng/ml)	493.41 ± 165.56	88.97 ± 31.06	< 0.01
Urine Apolipoprotein A1 (ng/ml)	928.84 ± 294.66	227.78 ± 98.17	< 0.01

Lastly, the diagnostic performance of serum and urine APOA1 was evaluated in Table 3. The sensitivity and specificity for serum APOA1 in identifying cases were 89.5% and 97.0%, respectively, with an area under the receiver operating characteristic (ROC) curve of 0.934, and a p-value of <0.001, demonstrating excellent discriminative ability. Urine APOA1 showed even stronger performance, with a sensitivity of 89.8% and specificity of 94.4%, coupled with an area under the ROC curve of 0.940, also yielding a p-value of <0.001. These findings suggest that both serum and urine APOA1 levels hold significant diagnostic value for distinguishing between cases and controls, with urine APOA1 showing slightly superior diagnostic performance.

Table 3: Sensitivity and specificity of APOA1 for BC

Variable(s)	Area	Cut off	Sensitivity	Specificity	p-value
Serum Apolipoprotein A1 (ng/ml)	0.934	> 120.30	89.5%	97.0%	<0.001
Urine Apolipoprotein A1 (ng/ml)	0.940	> 383	89.8%	94.4%	<0.001

DISCUSSION

The average age of the population under study was 65.24 ± 10.40 years, with individuals over 60 being the most affected demographic, similar to previoius studies [9,10].

Among adult males aged above 60 years, urinary bladder cancer was the most frequently observed malignancy. Similarly, Iranian other have identified individuals older than 60 years as the most susceptible to bladder cancer development [11,12].

When considering smoking as a risk factor, the findings align with both national and international studies. This research demonstrated that smoking is the primary risk factor for bladder cancer, consistent with the National Cancer Institute (NCI) data, which indicates smoking contributes to 66.7% of bladder cancer cases [13]. Other studies also linked high smoking prevalence to an elevated risk of bladder cancer [14].

The average serum apolipoprotein A1 (ApoA1) levels in patient groups were significantly higher than those in control groups, with a p-value ≤ 0.001. While serum ApoA1 levels are associated with various conditions such as obesity, fatty liver disease, cardiovascular diseases, and diabetes, they cannot be

DOI: 10.69605/ijlbpr_14.1.2025.26

definitively regarded as cancer-specific markers [15,16].

Urinary ApoA1 levels in patients were also higher than those in control groups. Consequently, urinary ApoA1 has the potential to serve as a marker for bladder cancer diagnosis and monitoring following treatment. However, ApoA1 in urine is linked to several malignancies, as supported by this and related studies. Urine proteome analysis has emerged as a promising tool for diagnosing bladder cancer [17].

This study aimed to evaluate the expression levels of ApoA1 in the blood and urine of bladder cancer patients. The results indicated significantly elevated ApoA1 levels in the voided urine of cancer patients compared to benign and healthy control groups. This study further demonstrated high sensitivity and specificity for bladder cancer detection using this biomarker [18].

Several studies have highlighted that increased urinary ApoA1 levels provide high specificity and sensitivity, aiding in bladder cancer diagnosis. Research by Yongcheng He et al. found that elevated pre-surgical urinary ApoA1 levels are associated with improved cancer-specific survival and overall survival in patients with non-muscle-invasive bladder cancer (NMIBC). ApoA1 may serve as a valuable predictor for determining optimal treatment strategies [19].

Jae-HakAhn from Seoul, Korea, reported that kinase activation by ApoA1 promotes tumor angiogenesis, and ApoA1 is a key component of high-density lipoproteins. Using two-dimensional electrophoresis and mass spectrometry, ApoA1 was evaluated as a potential biomarker for bladder cancer. The findings revealed increased ApoA1 expression, confirmed through Western blot analysis. Sensitivity and specificity were 89.2% and 84.6%, respectively, based on 379 urine samples. Additional studies independently validated ApoA1's role in bladder cancer, with sensitivity ranging from 92–95% and specificity from 85–92% [18].

CONCLUSION

Based on the study results, including elevated serum and urine APOA1 levels in the patient group compared to the control group and the data presented in this study demonstrating high sensitivity and specificity, it can be concluded that urinary apolipoprotein A1 is a reliable biomarker for detecting and distinguishing bladder cancer.

REFERENCES

1. Afonso J, Gonçalves C, Costa M, Ferreira D, Santos L, Longatto-Filho A, Baltazar F. Glucose metabolism reprogramming in bladder cancer: hexokinase 2 (HK2) as prognostic biomarker and target for bladder cancer therapy. Cancers. 2023 Feb 3;15(3):982. doi: 10.3390/cancers15030982.
2. Saginala K, Barsouk A, Aluru JS, Rawla P, Padala SA, Barsouk A. Epidemiology of bladder cancer. Medical sciences. 2020 Mar 13;8(1):15.https://doi.org/10.3390/medsci8010015
3. Zhu S, Yu W, Yang X, Wu C, Cheng F. Traditional classification and novel subtyping systems for bladder cancer. Front Oncol. 2020 Feb7;10:102. doi: 10.3389/fonc.2020.00102.
4. Lenis AT, Lec PM, Chamie K. Bladder cancer: a review. JAMA. 2020 Nov17;324(19):1980-91. doi: 10.1001/jama.2020.17598.
5. Shrivastava SR, Shrivastava PS. Strengthening rural medical education in the undergraduate training period. Med J Babylon. 2021 Oct 1;18(4): 277. doi:10.4103/mjbl.mjbl_13_22.
6. Gao L, Zhang Y, Wang X, Dong H. Association of apolipoproteins A1 and B with type 2 diabetes and fasting blood glucose: a cross-sectional study. BMC EndocDisord. 2021 Dec;21:1-1. doi: 10.1186/s12902-021-00726-5.
7. Bhale AS, Venkataraman K. Leveraging knowledge of HDLs major protein ApoA1: Structure, function, mutations, and potential therapeutics. Biomed Pharmacother. 2022 Oct 1; 154:113634. doi: 10.1016/j.biopha.2022.113634.
8. van der Vorst EP. High-density lipoproteins and apolipoprotein A1. Vertebrate and invertebrate respiratory proteins, lipoproteins and other body fluid proteins. Subcell Biochem. 2020 Mar 19:399-420. doi: 10.1007/978-3-030-41769-7_16.
9. Hussein, Farhan& Rasheed, Manal& Ismail, Mohammed. (2024). Serum and urine apolipoprotien A1 (ApoA1) as biomarkers in bladder cancer. Romanian Medical Journal. 71. 133-138. 10.37897/RMJ.2024.2.11.
10. Ibraheem Ahmed Ibraheem N, H. Ali R, B. Ismail M. Kidney functions and electrolyte disturbance among Iraqi patients with bladder cancer. J Fac Med Bagdad [Internet]. 2022;64(4). doi: 10.32007/jfacmedbagdad.6441985.
11. Abood RA, Abdahmed KA, Mazyed SS. Epidemiology of Different Types of Cancers Reported in Basra, Iraq. Sultan QaboosUniv Med J. 2020 Aug;20(3):e295-e300. doi: 10.18295/squmj.2020.20.03.008.
12. Hadji M, Rashidian H, Marzban M, Naghibzadeh-Tahami A, Gholipour M, Mohebbi E, et al. Opium use and risk of bladder cancer: a multi-centrecasereferent study in Iran. Int J Epidemiol. 2022 Jun 1;51(3):830-8. doi: 10.1093/ije/dyac031.
13. Kim HS. Etiology (Risk Factors for Bladder Cancer). In: Ku JH (eds.). Bladder Cancer. Academic Press, 2018. p. 21-32 doi: 10.1016/b978-0-12-809939-1.00002-3.
14. Mishra V, Balasubramaniam G. Urinary bladder cancer and its associated factors–an epidemiological overview. Indian Journal of Medical Sciences. 2021 Sep 24;73(2):239-48.
15. Ahmed HS. Assessment of ApolipoproteinsA1, E, and Insulin Resistance in Iraqi Male Patients with Acute

DOI: 10.69605/ijlbpr_14.1.2025.26

Myocardial Infarction. Iraq J Comm Med. 2020 Jul 1;33(2). doi: 10.4103/irjcm.irjcm_4_21.

16. Zghair A, Abou-Turab M, Yser H. Estimation of some physiological biomarkers in hyperlipidemic Patient men in Al-Zubair General Hospital/Basrah province, Iraq J Basrah Res (Sci). 2023 Sep 15;49(1). doi: 10.56714/bjrs.49.1.14.
17. Salem H, Ellakwa DE, Fouad H, Hamid MA. APOA1 AND APOA2 proteins as prognostic markers for early detection of urinary bladder cancer. Gene Reports. 2019 Sep 1;16:100463. doi: 10.1016/j.genrep.2019.100463.
18. Ahn JH, Kang CK, Kim EM, Kim AR, Kim A. Proteomics for early detection of non-muscle-invasive bladder cancer: Clinically useful urine protein biomarkers. Life. 2022 Mar 9;12(3):395. doi: 10.3390/life12030395.
19. He Y, Chen J, Ma Y, Chen H. Apolipoproteins: New players in cancers. Front. Pharmacol. 2022 Nov 25; 13:1051280. doi: 10.3389/fphar.2022.1051280.