Ref: Ro Med J. 2024;71(2)
DOI: 10.37897/RMJ.2024.2.11

Serum and urine apolipoprotien A1 (ApoA1) as biomarkers in bladder cancer

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ABSTRACT

Bladder cancer (BC) is the predominant malignant neoplasm in the urinary system and ranks as the tenth most prevalent malignant tumor worldwide. Compared with females, males displayed a four-fold more common incidence of bladder cancer. It mainly affects men. Bladder cancer is the fourth most prevalent neoplasm in males. The most important protein that makes up high density lipoprotein (HDL), ApoA-I apolipoprotein A1 is essential in regulating the right amount of cholesterol. Multiple inquiries have demonstrated that APOA1 plays a pivotal role in the progression, infiltration, and spread of tumors.

Objectives. The objective of this study was to measure the level of urine to serum apolipoprotein A1 in patients suffering from bladder cancer and investigate the impact of variations to this nanotechnology on the development of the cancer.

Material and methods. The study collected 45 blood and urine samples from individuals diagnosed with bladder cancer at Ghazi Hariri Hospital for Specialized Surgery. The samples included both males and females of various ages (61.47±11.28 years). Additionally, 45 blood and urine samples were collected from individuals without the disease. The samples were analyzed using an ELISA method to measure the levels of apolipoprotein A1 in the serum and urine of both groups, and the data collection period spanned from January 2023 to June 2023.

Result. The average levels of serum and urine apolipoprotein A1 in the patients' group (14.18±2.62ng/ml, 20.04±4.67ng/ml) were significantly higher than the average levels in the control group (8.21±1.35, 8.94±1.74), with p-values of <0.001 and ≤0.001, respectively. The blood concentration of apolipoprotein A1 showed a significant positive moderate connection with the concentration of apolipoprotein A1 in urine (r=0.45, p<0.001).

Conclusion. The mean and SD of serum apolipoprotein A1 in the patients group were higher than control group and urine apolipoprotein A1 in the patients group were higher than control group can be utilized as biomarkers for detecting bladder cancer. However, urine apolipoprotein A1 is a superior biomarker compared to serum apolipoprotein A1 due to its association with several other diseases.

Keywords: bladder cancer, serum apolipoprotein A1, urine apolipoprotein A1, high-density lipoprotein (HDL)

INTRODUCTION

Bladder cancer (BC) is the most prevalent malignancy in the urinary system and ranks as the tenth most prevalent cancer globally. The predicted demographic is expected to be predominantly male, with a male-to-female ratio of 4:1. In 2020, bladder cancer was one of the top 10 most common types of cancer globally, with over 570,000 diagnosed cases [1].

In Iraq, bladder cancer ranks as the eighth most prevalent cancer among women and the fourth most prevalent malignant tumor among males. The majority of newly diagnosed bladder cancer cases, over 90%, occur in individuals aged 55 and above. Bladder cancer can manifest in individuals at a relatively early stage of life. The user's text is enclosed in tags [2].

The two basic types of CA bladders are primary and secondary. Tumors that start within the bladder itself are referred to as primary bladder carcinomas. Secondary malignancies are those that originate in a different organ and then spread to the bladder. Cer-

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Article History:
Received: 8 May 2024
Accepted: 20 June 2024

tain tumors have the capacity to metastasize to the bladder through the circulatory system or lymphatic system or by escaping from an encapsulated organ such as the cervix or prostate. Bladder cancers have a higher prevalence compared to metastatic tumors originating from other sites and spreading to the bladder. There are four different kinds of bladder cancer: adenocarcinoma, mixed carcinoma, carcinoma of the squamous cells (SCC), and transitional cell carcinoma of the bladder (TCC) [3].

Urologists, or urologic surgeons, often employ the term "progression" to describe the likelihood of a malignancy spreading or recurring frequently. The following criteria are used to determine the stage of treatment plans: The International Association of Urologic Pathologists of the World Health Organization, or WHO, has discovered two types of cancer of the bladder. Settlement muscle-invasive and non-invasive bladder cancers are referred to as classifications [4].

Tobacco consumption represents the primary and most significant determinant for the development of bladder cancer. Furthermore, exposure to pollutants at work or in the environment considerably raises the burden of disease [5].

Additionally, male sexuality and advancing years have been associated to cancer of the bladder [6]. To assess the presence of bladder tumors, upper tract imaging, and cystoscopy are employed to examine the visual characteristics of microscopic or gross hematuria, depending on its severity and the likelihood of malignancy [7].

Urine cytology is frequently used alongside cystoscopy to detect cancer cells in urine. This test is particular and serves as a valuable supplement to visual detection. However, it is essential to note that urine cytology it is not a method for examination for bladder or urothelial cancer. However, this is one of the initial evaluations conducted to enhance the diagnostic procedures [8].

A cystoscopy is a procedure in which a local anesthetic is used to examine the bladder wall using an endoscope. It is done on individuals who exhibit visible blood in their urine or who are suspected of having bladder cancer. If a radical cystectomy is expected for a muscle-invasive tumor, a biopsy is performed. Alternatively, a resectoscope is utilized to extract the lesion. Therefore, TURBT serves both as a therapeutic and diagnostic procedure. If the lesion can be effectively excised, it has the potential to be curative, provided that the tumor is non-invasive [9].

There is currently a lack of wholly identified urinary biomarkers that provide both high sensitivity and specificity for bladder cancer. Given that these innovative tests fulfill all of these requirements, further investigation may warrant their use in clinical practice [10].

Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) particles are all measured as part of the lipid profile. The two primary apolipoprotein kinds that have been extensively investigated are ApoA1 (apolipoprotein A1) and ApoB (apolipoprotein B) [11].

An apolipoprotein is the primary protein constituent of HDL responsible for regulating the transportation and metabolism of cholesterol. Human high-density lipoprotein (HDL) consists of 22 unique apolipoprotein components. Due to their amphipathic nature, these apolipoproteins play a vital role in preserving the structural integrity of lipoproteins and enhancing their solubility in water-based environments. Additionally, they possess the capacity to stimulate and suppress lipid metabolic enzymes [12].

The most important protein that makes up high density lipoprotein (HDL), ApoA-I apolipoprotein A1 is essential in regulating the right amount of cholesterol. Research has demonstrated that high-density lipoproteins (HDLs) consist of a wide range of particles that vary significantly in terms of their size and composition. HDL is mainly recognized for its function in the circulatory system of cholesterol inverted mechanism. This process entails the absorption and uptake of cholesterol from peripheral cells by small HDLs or lipid-free Apo A-I, followed by the transfer of cholesterol to the liver for excretion [13].

The liver and thirty percent, respectively, account for seventy percent of APOA1 production It becomes generated, then it puts together into homodimers with and binds different lipids, including phospholipids (PL), cholesterol, sphingolipids, and ceramides, using the ATP-binding cassette A1 (ABCA1). Lecithin-cholesterol-acyltransferase utilizes APOA1 as a coenzyme to convert cholesterol into esters. The ATP-binding cassette G1, ABCA1 transporters, and scavenger receptor type B1 modulate the size, shape, and content of the newly formed high-density lipoprotein [14].

Given their significant connections to cardiovascular disorders, blood lipids and associated proteins have been the subject of enduring research. Nevertheless, subsequent studies have demonstrated a robust correlation between these characteristics and the onset of cancer. Several animal investigations have demonstrated that certain apolipoproteins can modulate tumor development by modifying the activity of immune cells. Furthermore, the assessment of the prognosis of different types of malignancies relies on the measurement of specific apolipoproteins. However, both blood lipid levels and relevant apolipoproteins possess predictive capabilities [15].

Multiple inquiries have demonstrated that APOA1 plays a pivotal role in the progression, infiltration, and spread of tumors. Several investigations have reported on the relationship between APOA1 and tumor chemotherapy [16].

A practical approach in the pursuit of realistic biomarkers for bladder cancer involves analyzing the

urine proteome at the early stages of the disease. Urine's content, quantity, and quality variations may convey information regarding the genesis, development, and prognosis of urinary malignancies as urine comes into direct touch with bladder epithelial cells. Urine collection is a less intrusive and more convenient method compared to obtaining other physiological fluid samples like plasma. Several urine proteins have previously been examined as possible indicators of bladder cancer [17].

It has been determined that their protein expressions can function as indicators for bladder cancer. Proteomic noninvasive biomarkers are still being sought after in an effort to find disease biomarkers in biological fluids that can be assessed for early illness detection at a low cost. Urine is a potentially attractive alternative to blood plasma for identifying disease biomarkers for bladder cancer, as it is immediately exposed to the bladder epithelium [18].

MATERIAL AND METHOD

The University of Baghdad, Iraq's Biochemistry Department, College of Medicine, conducted a case-control study. All patients were selected from the surgical specialties at Bagdad's Ghazi Hariri Hospital between January and June of 2023. For this study, a total of 90 participants were enrolled and divided into two groups. There were forty-five patients in the first group (8 females, 37 males), aged from 47 to 82 years. Approximately 35 patients have smoked for at least five years. Forty-five healthy people (8 females and 37 males) with normal bladder tissue and no prior history of renal systemic diseases made up the second group. Patients with bladder tumors undergo transurethral resection of the bladder tumor while under general or spinal anesthesia. All patients arrived at the hospital complaining of pelvic pain and hematuria. They were first sent for an ultrasound and subsequently a CT scan. Additionally, via a resectoscope, the complete tumor will be removed, and it will be sent for a histopathological examination, which will provide crucial details about the tumor, including its diagnosis, staging, and grading. A venous blood sample of about 10 milliliters was taken from each participant between 9:00 and 1:00 A.M.. After that, the blood was transferred into gel tubes, given time to clot, and centrifuged for ten minutes at 3000 rpm speed at which the serum is extracted. Apolipoprotein A1 (APOAI) levels in the serum and urine were then measured. The serum was then put into labeled Eppendorf tubes and frozen at -80C. Without the need for initial apolipoprotein A1 purification, urine apolipoprotein A1 (APOAI) kits ensure dose-dependent apolipoprotein A1 identification across a range of samples. Apolipoprotein A1 concentration in each sample can be estimated thanks to assay calibration, which enables both relative and absolute quantification.

Estimation of apolipoprotein A1(APOA1) in the serum and urine

The kit is a sandwich enzyme immunoassay designed to quantitatively quantify APOA1 in various human biological samples, which involves, blood, se-

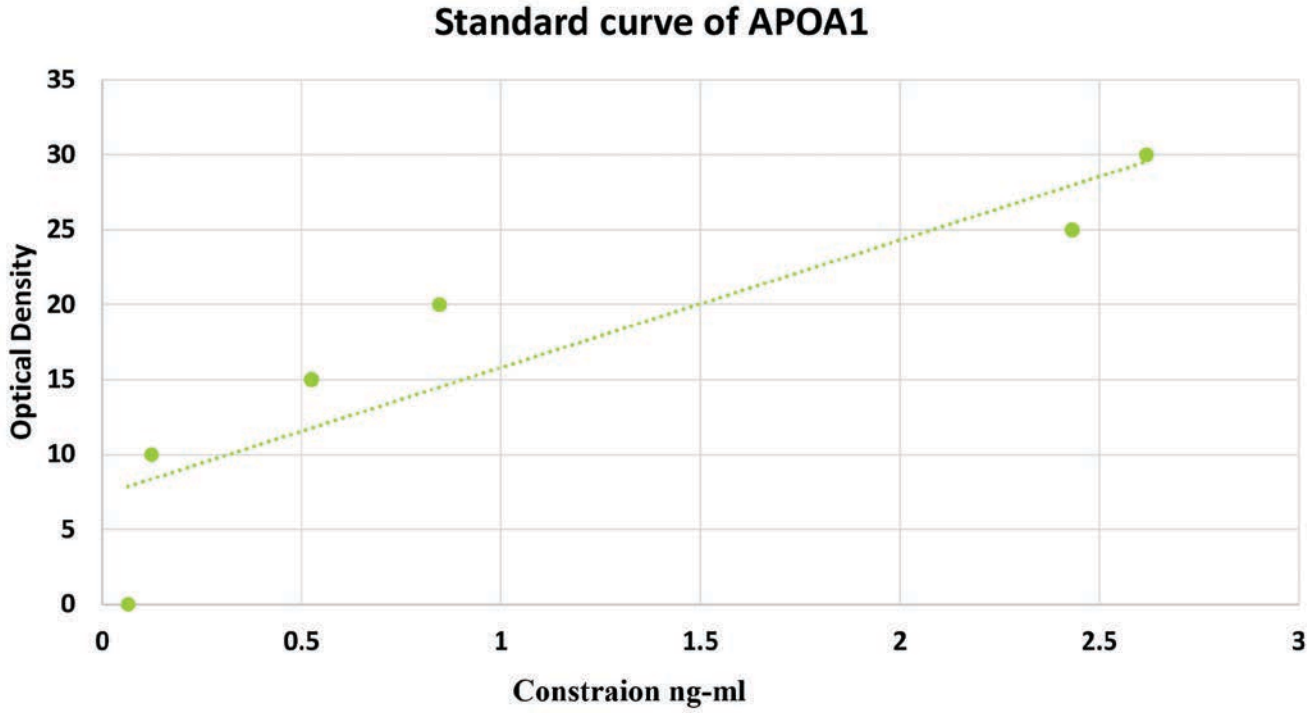

FIGURE 1. Standard curve of APOA1

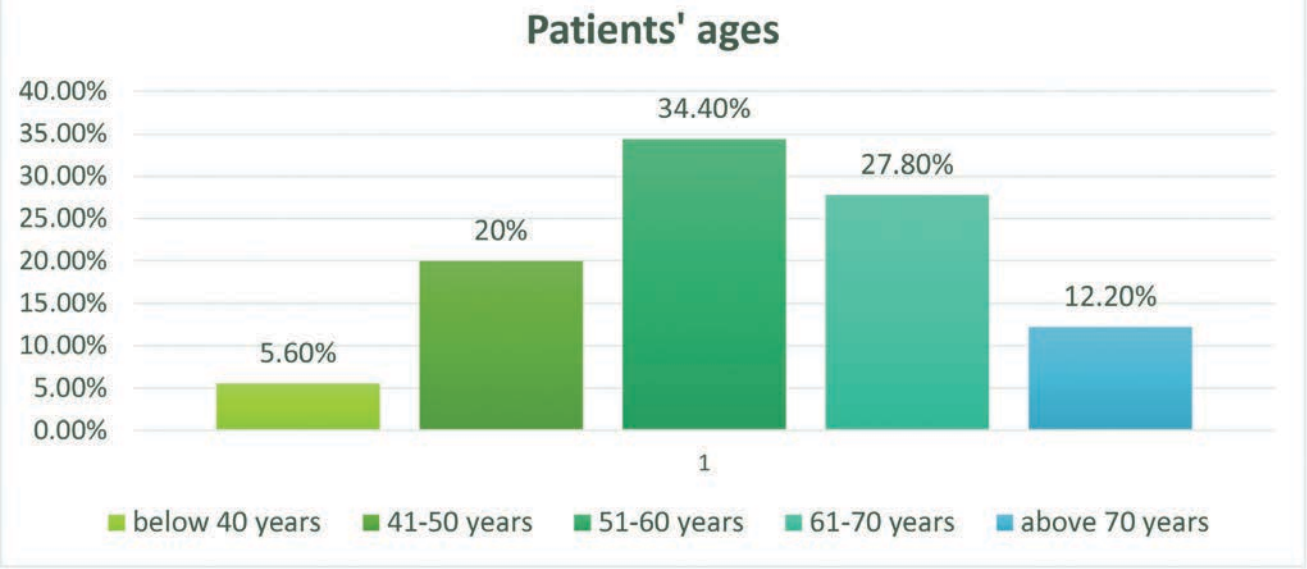

FIGURE 2. Distribution of patients by age group

rum, urine, cultures of cells supernatants, and other physiological fluids.

To create a standard curve, plot the average measured optical density (OD) for each standards on the Y (vertical) axis against the concentration on the X (horizontal) axis. Join the data points on the chart to generate a best-fit curve. The most accurate method for carrying out these computations is the analysis of regression, which is employed by a computer program curve fitting applications in finding the most appropriate regression line (Figure1).

RESULTS

The study included 90 participants divided into two groups equally. Regarding the gender, 16 were females (8 patients and 8 healthy control) and 74 males (37 patients and 37 healthy control), with no significant association between studied group and gender, p-value 1.00 [15]. Regarding the age group, 5.6% were below 40 years, 20% were 41-50 years, 34.4% were 51-60 years, 27.8% were 61-70 years and 12.2% were above 70 years. A significant association was found between older age group (>70 years) and being a patient, and between lower age group (<40 years) and healthy control, p≤0.008. The mean of age in patients' group (61.47±11.28 years) was significantly higher than the control group's mean (58.87±9.41 years), p-≤0.03 (Table 1 and Figure 2).

Regarding the groups (Table 1), a significant association was found between smoking and patients' group (73.3 vs 26.3), p≤0.001.

The average levels of serum and urine apolipoprotein A1 in the patients' group (465.15±157 ng/ml and 904.61±280.63 ng/ml, respectively) were substantially higher than the average levels in the control group (92.60±32.69 ng/ml and 238.72±104.39 ng/ml, respectively). The p-values for the differences were both <0.001 (Table 2).

Serum ApoA1 had significant positive weak correlation with urine ApoA1 (r 0.31, p <0.03) (Table 2).

ROC test showed that serum apolipoprotein A1 had AUC of 0.977, with sensitivity of 91.1% and specificity of 98.9%, and cut off >126.62 urine apolipo-

TABLE 1. Association between demographic data and studied groups

		group		Total	p-value
		patients	control		
Gender	Female	8	8	16	1.00
		17.8%	17.8%	17.8%	
	Male	37	37	74	
			82.2%	82.2%	
Age group	below 40 yrs.	0	5	5	0.008*
		0.0%	11.1%	5.6%	
	41-50 yrs.	10	8	18	
		22.2%	17.8%	20.0%	
	51-60 yrs.	12	19	31	
		26.7%	42.2%	34.4%	
	61-70 yrs	13	12	25	
		28.9%	26.7%	27.8%	
	above 70 yrs.	10	1	11	
		22.2%	2.2%	12.2%	
Age (mean ± SD)		61.47 ± 11.28	58.87 ± 9.41		0.03*
Smoking	Non-smoker	12	40	52	< 0.001*
		26.7%	88.9%	57.8%	
	Smoker	33	5	38	
			11.1%	42.2%	
Total		45	45	90	
		100.0%	100.0%	100.0%	

**p-value is significant*

TABLE 2. Mean and SD of serum and urine apolipoprotein A1

group	stage	Mean SD	SE	p-value
serum apolipoprotein A1 ng/ml	patients	465.15 ± 157.68	0.39	< 0.001*†
	control	92.60 ± 32.69	0.20	
urine apolipoprotein A1ng/ml	patients	904.61 ± 280.63	0.69	< 0.001*†
	control	238.72 ± 104.39	0.26	

TABLE 3. Sensitivity and specificity of serum and urine apolipoprotein A1

Test result variable(s)	Area	Cut off	Sensitivity	Specificity	p-value
serum apolipoprotein A1 ng/ml	0.977	> 126.62	91.1%	98.9%	<0.001
urine apolipoprotein A1ng/ml	0.985	> 403	91.1%	96%	<0.001

protein A1 had AUC 0.985, sensitivity of 91.1% and specificity of 96%, and cut off >403 (Table 3).

DISCUSSION

According to age group (Table 1), several studies such as Noor Ibrahim (Bagdad University) support this result. The mean age of the studied population was 63.5±11.4 years and the most prevalent age was more than 60 years [19].

In adult males >60 years, urinary bladder cancer was the most common type representing 11.94% of cancers in other studies in Basrah University [20].

In Iranian studies, people over 60 years old are the most vulnerable to developing bladder cancer [21].

As stated by the smoking group with reference to Table 1, this is in line with all national and international studies, including BAQER MR. The results of this study, which showed that smoking is the main risk factor for bladder cancer were consistent with the findings of the NCI regarding the effect of smoking on the occurrence of bladder cancer, with 66.7% of the affected patients [22]. Iran's high cigarette smoking rate is associated with a higher risk of bladder cancer, claims Abdolahinia Z [23].

The mean of serum of apolipoprotein A1 (ApoA1) in patients' groups (465.15±157 ng/ml) higher than the mean of control groups (92.60±32.69 ng/ml) p-value ≤0. 001 (Table 3). Serum apolipoprotein A1 is associated with many diseases, especially obesity, high fat liver diseases, cardiovascular diseases, and diabetes, and it cannot be considered as a marker for cancer [24,25].

The mean of urine of apolipoprotein A1 (ApoA1) in patients' group (904.61±280.63 ng/ml) was higher than the mean of control groups (238.72 ng/ml) (Table 2). Therefore, this marker can be used to detect the bladder cancer and follow up with the patient after treatment.

However, urine apolipoprotein A1 is linked to several malignant conditions, as demonstrated in this work and other similar research. The analysis of the urine proteome has emerged as a highly promising method for diagnosing urinary bladder cancer patients. The current study's objective is to evaluate the levels of blood and urine protein expression of Apo-A1 in patients with bladder cancer. The findings demonstrated that the cancerous group's levels were noticeably higher of Apo-A1 in their voided urine when compared to the benign and healthy control groups. High sensitivity and specificity were also demonstrated in this study for patients with bladder cancer [26].

According to a various study, patients with bladder cancer may be diagnosed more easily if their urine has higher levels of ApoA1, which has a significant degree of specificity and sensitivity. [27].

Researchers Yongcheng He et al. discovered that an elevated prior to surgery level of ApoA1 in the urine can improve cancer-specific survival as well as the general survival rate among those suffering from bladder cancer that is not muscle invasive (NMIBC). ApoA1 can be an advantageous predictor for patients who undergo surgery, assisting in choosing of a more appropriate course of treatment [28].

According to Jae-Hak Ahn from Seoul, Korea, kinase activation by Apo-A1 stimulates tumor angiogenesis and is the main protein component of high-density lipoproteins. The study utilised two-dimensional elec-trophoresis and subsequent mass spectrometry to evaluate the potential of Apo-A1 as a biomarker for bladder cancer. The results revealed increased expression of Apo-A1, which was further confirmed by Western blot analysis. This biomarker's sensitivity and specificity were found to be 89.2% and 84.6%, respectively, in a study involving 379 urine samples. 92–95% sensitivity and 85–92% specificity was independently confirmed for Apo-A1 involvement in bladder cancer in other similar studies [27].

CONCLUSION

Based on the results of these studies - high level of serum and urine of APOA1 in the patients group and control group, p-value <0.0001 and the data in Table 3 (high sensitivity and specificity), the conclusions of the aforementioned research it may be inferred that apolipoprotein A1 in urine can serve as a reliable marker for the detection and differentiation of bladder cancer.

Conflict of interest:
The authors declare no conflict of interest.
Financial support: none declared

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Authors' contributions:
All authors made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Acknowledgement:
I extend my thanks and appreciation to everyone who contributed and helped in preparing this manuscript