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Research Article

Assessment of Urine and Serum Exosomes as Biomarkers for the Diagnosis of Bladder Cancer

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Abstract

Background: Bladder cancer (BC) is the most common malignant tumor in the urinary tract and the tenth most common malignancy worldwide. Exosomes are 40–100 nm-diameter nanovesicles that are either released straight from the plasma membrane during budding or merged with the plasma membrane by multivesicular bodies. ***Objectives***: To assess the proportion of serum and urinary Exosome levels in urinary bladder cancer patients, as well as their impact on the disease. ***Methods***: From January 2023 to June 2023, a total of 45 samples of blood and urine were collected from individuals diagnosed with bladder cancer at the Ghazi Hariri Hospital for Specialized Surgery. They included 45 male and female patients, varying in age, as well as 45 healthy controls. The samples were analyzed for exosome levels using the ELISA method. ***Results***: The mean serum and urine exosome levels in the patients' group (14.18 and 20.04) were significantly higher than in the control group (8.21 and 8.94), respectively. Serum exosomes exhibited a significant positive and moderate correlation with urine exosome levels. ***Conclusions***: Serum and urine levels of exosomes can be used as biomarkers to detect bladder cancer, and the urine level seems to be preferential.

Keywords: Bladder cancer, Extracellular vesicle, Serum exosome, Urine exosome.

تقييم مستوى الإكسوسومات في البول والمصل كمؤشرات حيوية لتشخيص سرطان المثانة

الخلاصة

الخلفية: سرطان المثانة (BC) هو الورم الخبيث الأكثر شيوعا في المسالك البولية وعاشر أكثر الأورام الخبيثة شيوعا في جميع أنحاء العالم. لأكسوسومات Exosomes هي حويصلات نانوية قطرها 40-100 نانومتر يتم إطلاقها مباشرة من غشاء البلازما أثناء مهدها أو دمجها مع غشاء البلازما بواسطة أجسام متعددة الحويصلات. **الأهداف**: تقييم نسبة مستويات الإكسوسوم المصلي والبولي لدى مرضى سرطان المثانة البولية، وكذلك تأثيرها على المرض. الطريقة: من يناير 2023 إلى يونيو 2023 ، تم جمع ما مجموعه 45 عينة من الدم والبول من الأفراد الذين تم تشخيص إصابتهم بسرطان المثانة في مستشفى غازي الحريري للجراحة التخصصية. وشملت 45 مريضا من الذكور والإناث متفاوتين في العمر، بالإضافة إلى 45 من الأشخاص الأصحاء كمجموعة ضابطة. تم تحليل العينات لمستويات الإكسوسوم باستخدام طريقة ELISA. **النتائج**: كان متوسط مستويات إكسوسوم المصل والبول في مجموعة المرضى (14.18 و 20.04) أعلى بكثير من المجموعة الضابطة (8.21 و 8.94) ، على التوالي. أظهرت إكسوسومات المصل ارتباطا إيجابيا ومعتدلا كبيرا بمستويات الإكسوسوم في البول. **الاستنتاجات**: يمكن استخدام مستويات المصل والبول من exosomes كمؤشرات حيوية للكشف عن سرطان المثانة، ويبدو أن مستوى البول تفضيلي في هذه الحالة.

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INTRODUCTION

Urinary bladder cancer, the most common malignant tumor in the urinary tract, ranks tenth globally in terms of malignant tumor frequency (BC). A 4:1 male-to-female ratio is anticipated, with a preponderance of males. In 2020, about 570,000 people were diagnosed with bladder cancer, making it one of the top 10 malignant tumors globally [1]. In Iraq, bladder cancer ranks as the fourth most common malignant tumor in men and the eighth most common cancer in women. Those over the age of 55 account for more than 90% of newly diagnosed cases of urinary bladder cancer. Bladder cancer can strike people at a young age [2]. Bladder cancer comes in two main varieties: primary and secondary. CA bladders classified as primary are those that start inside the bladder. Subsequent cancers begin in one organ and spread to the bladder. Certain types of tumors can travel to the bladder through the lymphatic or circulatory systems, or they can break free from a closed organ such as the prostate or cervix. Bladder cancers occur more frequently compared to

cancers that metastasize to the bladder. The bladder cancers classified as urothelial CA include transitional cell carcinoma (TCC), squamous cell carcinoma (SCC), adenocarcinoma, and mixed carcinoma type [3]. A cancer's "progression" is often defined by urologists and urologic surgeons as the chance that the tumor will spread or regrow. The following categories' stages are frequently used to inform treatment plans: Bladder cancer can be classified as either muscle-invasive or non-invasive, according to the International Society of Urologic Pathology Consensus Classification developed by the World Health Organization (WHO) [4]. Tobacco use is the biggest risk factor for urinary bladder cancer. Furthermore, exposure to environmental or occupational pollutants increases the burden of disease significantly [5]. Bladder cancer is also associated with male sex and advanced age [6]. Hematuria, whether microscopic or gross, can be a sign of bladder tumors. Upper tract imaging, cystoscopy and transurethral resection of the tumor are used to assess the condition, depending on the severity of the hematuria and the likelihood of malignancy [7]. In addition to upper tract imaging, cystoscopy and transurethral resection of the tumor, urine cytology is often used as an adjunct to cystoscopy and transurethral resection of the tumor and while visional detection of cancer cells in urine is a very specific test with high Urine cytology is not a screening test for bladder or urothelial malignancy. But it is one of the oldest evaluations done to supplement the diagnostic process [8]. When a patient exhibits macroscopic hematuria or is suspected of having bladder cancer, they are put through a cystoscopy and transurethral resection of the tumor, which is an endoscopic examination of the bladder wall under local anesthesia. A resectoscope is used to remove the lesion, or a biopsy is performed if a tumor invades muscle and a radical cystectomy is predicted. Thus, TURBT is a diagnostic and therapeutic procedure. In the event that the tumor is not invasive, a successful removal of the entire lesion may be therapeutic [9]. Bladder cancer urinary biomarkers that are both very sensitive and specific have not yet reached their full potential. Future studies may support the use of these innovative urine biomarker assays in clinical practice because they appear to meet these criteria [10]. Extracellular vehicles (EVs) secreted by different human cells include ectosomes, also known as microvesicles and exosomes, as well as apoptotic bodies. Because these EVs carry proteins and nucleic acids, they are crucial for cellular communications. In the past few decades, they have come to be recognized as novel mediators of tumor progression. They may also prevent extracellular space enzymes from breaking down their component parts. Exosomes are 40–100 nm-diameter nanovesicles that are either released straight from the plasma membrane during budding or merged with the plasma membrane by multivesicular bodies. The majority of biological fluids, including tears, Broncho alveolar lavage, amniotic fluid, blood, urine, and ascites, contain these spherical or cup-shaped structures [11]. Exosomes have been studied in great detail due to their varied and evolving functions in the pathogenesis of cancer. These widely dispersed micro-vesicles have been shown to prime potential metastatic sites for the attachment and growth of tumor material, as well as to regulate immune system activity, particularly in tumorigenesis. Into the extracellular milieu, they are released. In order to create a pro-tumor environment necessary for carcinogenesis, exosomes can interact with a variety of host tissues [12]. Exosomes contain a variety of physiologically active molecules that provide a real-time image of the tumor's heterogeneity. Furthermore, exosomes are abundant and stable and can be found in nearly all body fluid types. As such, it could potentially serve as a biomarker for early detection of bladder cancer. [13]. This study aims to evaluate the validity of serum and urinary exosomes as tumor biomarkers for bladder cancer.

METHODS

Study design and setting

This case-control study was conducted at the Biochemistry Department, College of Medicine at the University of Baghdad, Iraq. Every patient at Bagdad's Ghazi Hariri Hospital for surgical specialties was selected between January and June of 2023.

Patients' selection

A total of 90 individuals were enrolled in this study and divided into two groups. The first group included 45 patients (37 males and 8 females) with an age range of 47–82 years. Nearly thirty-five patients have been smoking for at least five years. The second group consisted of 45 individuals in good health (control) (8 females and 37 males) with normal bladder tissue and no history of renal systemic diseases.

Inclusion and exclusion criteria

The study included all hospital patients with hematuria and pelvic pain, excluding kidney stones. The patients were referred for ultrasound imaging and a CT scan. Under general or spinal anesthesia, patients with bladder tumors had the bladder tumor transurethral removed. A resectoscope will also be used to completely remove the tumor before it is sent for a histopathological analysis, which will provide important information about the tumor, such as diagnosis, staging, and grading. Based on the results of the histopathological analysis, we will select the superficial tumor (T0 and T1) and exclude the muscle-invasive tumor (T2, T3, and T4).

Sample collection

A venous blood sample of about 10 milliliters was taken from each participant between 9:00 and 1:00 in the morning. After that, the blood was transferred into gel tubes, given time to clot, and centrifuged for ten minutes at 3000 rpm in order to extract the serum. Following that, the serum was placed in Eppendorf tubes with labels and frozen at -80 ºC until the time of measurement of urine and serum exosome amounts. The Exosomes Kit is a patented double-sealed

enzyme-linked immunoassay for quantitative and qualitative analysis of exosomes. It is a successful platform for exosome quantification and characterization from small amounts of human biological fluids (plasma, serum, urine, saliva) or cell media.

Ethical consideration

The study proposal was approved by the local Scientific Committee of the College of Medicine, University of Baghdad, and also by the Center of Training and Research in the Medical City Directorate, Baghdad, Iraq.

Estimation of serum and urinary exosomes

The Exosomes Kit is a patented double-sealed enzyme-linked immunoassay for quantitative and qualitative exosome analysis. Exosomes extracted from minute amounts of human biological fluids, such as cell media, urine, serum, or saliva, are well-measured and described using this platform. This platform enables the detection of exosomes released by cancer cells in the urine and plasma of tumor patients, thereby providing benefits for various medical conditions [14]. It is possible to create a standard curve by graphing the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, then creating a best-fit curve through the points on the graph. Computer-based curve-fitting software is the most efficient tool for performing these calculations, using regression analysis to determine the best fit line.

RESULTS

The study involved the equal division of ninety participants into two groups. Table 1 shows that among the 74 male participants in the study group (37 patients and 37 healthy controls), there was no significant difference in gender between the 16 female participants (8 patients and 8 healthy controls) and the male participants (p>1.00).

Table 1: Demographic characteristics in patients and controls

Variables		Groups		Total	*p*-value
		Patients	Controls		
Gender	Female	8(17.8)	8(17.8)	16(17.8)	1.00
	Male	37(82.2)	37(82.2)	74(82.2)	
Age group (year)	< 40	0(0.0)	5(11.1)	5(5.6)	0.008
	41-50	10(22.2)	8(17.8)	18(20)	
	51-60	12(26.7)	19(42.2)	31(34.4)	
	61-70	13(28.9)	12(26.7)	25(27.8)	
	> 70	10(22.2)	1(2.2)	11(12.2)	
Age (year)		61.47±11.28	58.87±9.41		0.03
Smoking	Non-smoker	12(26.7)	40(88.9)	52(57.8)	<0.001
	Smoker	33(73.3)	5(11.1)	38(42.2)	
Total		45(100)	45(100)	90(90)	

Values were expressed as frequency, percentage, and mean±SD.

Regarding age group, 5.6% of respondents were under 40, 20% were between 41 and 50, 34.4% were between 51 and 60, 27.8% were between 61 and 70, and 12.2% were over 70. A significant correlation (p-value of 0.008) was observed between being a patient and belonging to the older age group (> 70 years), as well as between the younger age group (< 40 years) and the healthy control. The patients' group's mean age (61.47 ± 11.28 years) was considerably greater than the mean of the control group (58.87±9.41) as demonstrated in Figure 1 and Table 1 (p<0.03).

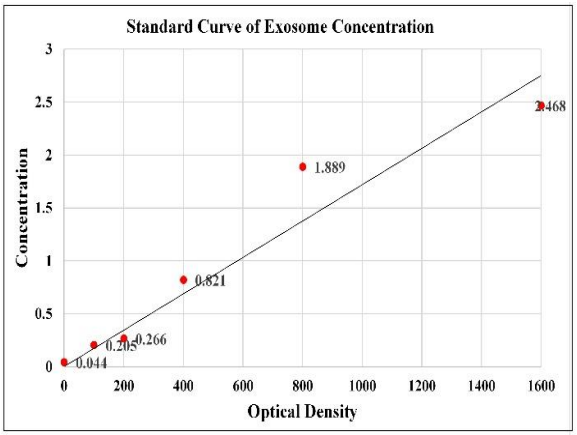

Figure 1: Stander curve of Exosome

Table 1 shows that smoking and the patient's group have a significant correlation (73.3% *vs*. 26.7%) with a p<0.001. Table 2 shows that the average amount of exosomes in the patients' serum and urine was 14.18±2.62 and 20.04±4.67 µg/ml, respectively.

Table 2: Serum and urinary levels of exosomes

Exosomes level	Group		*p*-value
Serum exosomes (µg/mL)	Patients	14.18±2.62	<0.001
	Control	8.21±1.35	
Urine exosomes (µg/mL)	Patients	20.04±4.67	<0.001
	Control	8.94±1.74	

Values were expressed as mean±SD.

These amounts were significantly higher than the average amounts in the control group (8.21±1.35 µg/ml for serum and 8.94±1.74 µg/ml for urine), with p<0.001 for both. As shown in Figure 2, the serum exosome had a significant positive moderate correlation with the urine exosome (r=0.45, p<0.001). Table 3 shows that the urine exosome had an AUC of 0.965, sensitivity of 95.6%, and specificity of 98.44%, with a cut-off value >10.65, whereas the serum exosome had an AUC of 0.95, sensitivity of 88.9%, and specificity of 78%, with a cut-off value >11.3 according to the ROC test.

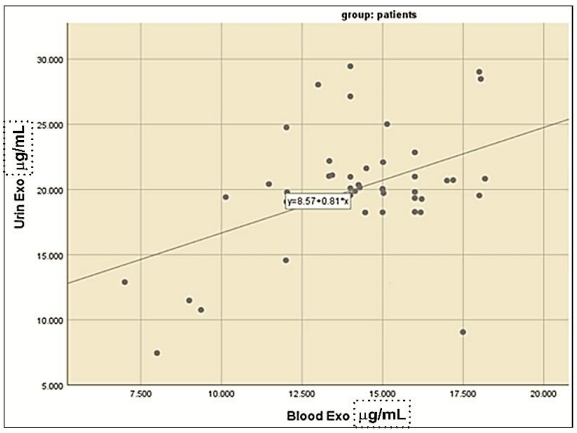

Figure 2: Correlation between serum and urinary exosomes levels in patients with bladder cancer.

DISCUSSION

Several studies, such as those performed by Ibraheem *et al*. at Bagdad University. The mean age of the studied population was 63.5±11.4 years, and the most prevalent age was more than 60 years [15]. In adult males aged ≥ 60 years, accounting bladder cancer was the most common type, representing 11.94% of cancers in other studies at Basra University [16].

Table 3: Sensitivity and Specificity of serum and urine Exosomes levels (ROC analysis)

Variable	Area	Cut off	Sensitivity	Specificity	*p*-value
Serum exosomes	0.957	>11.3	88.9%	78%	<0.001
Urine exosomes	0.965	>10.65	95.6%	98.44%	<0.001

According to numerous studies conducted in Iran, people over the age of sixty are most susceptible to bladder cancer [17]. Table 1 shows that this is consistent with all global and local studies, including those reported by Baqer and Stage (2019). The study's results, which included 66.7% of the patients, agreed with the NCI's conclusions about the relationship between smoking and bladder cancer risk [18]. This suggests that smoking is the primary risk factor for bladder cancer. Abdolahinia *et al*. link high rates of cigarette smoking in Iran to a higher risk of bladder cancer compared to non-smokers [19]. Exosomes, a subtype of extracellular vehicles (EVs), are tiny particles that play a role in several stages of the cancer development process. Almost all human bodily fluids, including urine, contain extracellular vehicles (EVs), especially in tumor microenvironments, making them attractive candidates as potential biomarkers for bladder cancer [20]. Elsharkawi *et al*. found a correlation between the elevated exosome levels and the invasiveness of bladder cancer in Egypt when they compared the exosome levels in urine and serum samples with a control group. According to this study, urine exhibited a greater sensitivity to tumor-derived exosomes than serum did [21]. According to Table 3, urine-based biomarkers may be helpful in a number of important areas of the current clinical landscape for bladder cancer, including diagnosis, frequency of surveillance, type and degree of intravenous treatment, choice of radical treatment in high-risk non-mutually inhibitory bladder cancer, choice of adjuvant chemotherapy and/or immunotherapy, and tracking disease progression [22].

Study limitations

The current study's major limitations are the small sample size and the single-center setting approach used during the study.

Conclusion

Patients with bladder cancer reported larger quantities of exosomes in both serum and urine than healthy patients, with urine exosomes outnumbering serum exosomes. Cut-off >10.65 urine exosomes have an AUC of 0.965, a sensitivity of 95.6%, and a specificity of 98.44%, making them an effective tool for early diagnosis and screening bladder cancer.

Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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