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Original Research Article

A Hospital-Based Assessment of the Diagnostic Accuracy of Urinary Aquaporin-1 (Uaqp-1), for Renal Cell Carcinoma

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Abstract

Aim: The aim of the present study was to assess the diagnostic accuracy of urinary aquaporin-1 (uAQP-1), an upcoming urinary biomarker, for renal cell carcinoma.

Methods: The present study was conducted in Department of General surgery, Patna Medical College and Hospital, Patna, Bihar, India. In the final analysis, 50 patients and 50 healthy controls were included.

Results: Among cases, 38 were men and 12 were women, whereas among controls, 26 were men and 24 were women. The mean (standard deviation [SD]) age of the cases and controls was 46.7 (9.6) years and 47.6 (11.8) years, respectively. The BMI of cases and controls was 24.20 (3.6) and 22.48 (4.1) respectively. Majority of the tumors (47/50) were reported as renal cell carcinoma with a clear-cell histology. Sixty percent of the tumors were pT1 and the mean size of the tumor was 7 cm (2.4–14 cm, SD 3.62 cm). Two-thirds of these patients had a radical nephrectomy, the rest underwent partial nephrectomy.

Conclusion: The present study concluded that uAQP-1 may not be a useful diagnostic urinary biomarker for renal cell carcinoma. This test had a poor sensitivity and specificity in diagnosing renal cell carcinoma in the study population.

Keywords: diagnostic accuracy, urinary aquaporin-1 (uAQP-1), an upcoming urinary biomarker, renal cell carcinoma

Introduction

Cancers of the kidney and renal pelvis are the most lethal urologic malignancies. There has been a steady rise in their incidence, and they now account for almost 4% of adult malignancies. [1-5] The overall age-adjusted renal cancer incidence is 51.7/100,000 for individuals over 50 years of age. [5] Due to increased general diagnostic use of abdominal imaging, there has been a consequent increase in incidental discovery of occult small renal masses. Additionally, incidental rather than symptomatic discovery has resulted in a stage migration towards smaller tumors, and, consequently a higher potential for cure.[1,3,4] Importantly, early detection of smaller, intrarenal RCC presages better patient outcome. Patients with pre-symptomatic, incidentally detected tumors have a 5-year disease-free survival of 85%, while patients with cancers detected symptomatically have a 5-year disease-free survival of only 62%. [6,7]

The prognosis for metastatic RCC is even worse; the 5-year RCC-specific survival ranges from about 40% with nodal metastases to about 20% with distant metastases. [8] There are other substantial benefits to early detection. If the tumor is confined to the renal capsule at diagnosis and nephrectomy, survival can exceed 70%. Additional benefits include the opportunities for laparoscopic vs open nephrectomy and partial versus total nephrectomy. Minimally invasive laparoscopic surgery, as well as percutaneous radiofrequency and cryoablation techniques offer shorter hospitalization, faster recovery, less pain and disability, fewer complications and lower costs compared to open nephrectomy. [9,10] Nephron-sparing partial nephrectomy rather than total nephrectomy preserves renal mass and long-term renal function, and minimizes future chronic kidney disease. [9-13] Thus, early diagnosis of asymptomatic RCC portends identification of smaller, earlier-stage tumors, with targeted and less morbid intervention, and better prognosis.

Biomarkers are broadly defined as objective, quantifiable characteristics of biological processes that measure a physiological state and may be used as surrogate endpoints to predict outcomes. [14] Biomarkers may be classified based on their parameters, including diagnostic biomarkers (i.e., detection of a disease state), disease prognosis biomarkers, and predictive biomarkers (i.e., prediction of clinical response to a therapy). Currently, a wide array of biomarkers exist that may help guide individualized care of kidney cancer patients. [15]

The aim of the present study was to assess the diagnostic accuracy of urinary aquaporin-1 (uAQP-1), an upcoming urinary biomarker, for renal cell carcinoma.

Materials and Methods

The present study was conducted in the Department of General surgery, Patna Medical College and Hospital, Patna, Bihar, India from January 2020 to December 2020. In the final analysis, 50 patients and 50 healthy controls were included.

Study participants Consecutive patients, above the age of 16 years, who were scheduled for a radical or partial nephrectomy for a renal mass suggestive of RCC were eligible to be recruited as cases. The investigators reviewed the laboratory investigations and imaging to exclude those in whom alternative diagnoses other than RCC were suspected. Healthy controls were recruited from among those who had undergone a contrast-enhanced CT abdomen during evaluation for voluntary kidney donation from the urology transplant outpatient department. The authors reviewed the CT scans of eligible controls to rule out any lesions in the kidney. Cases were matched for age up to two years.

Urinary aquaporin-1 measurement

In the initial studies by Morrissey et al., uAQP-1 levels were measured by the Western blot technique. However, in this study, the authors used a sensitive and specific enzyme-linked immunosorbent assay (ELISA) technique similar to the recent study. [16] ELISA techniques are less cumbersome and hence could potentially be used in testing larger numbers of patients. First-morning mid-stream spot urine samples were collected in sterile-labeled containers and transported to the laboratory by one of the investigators. The time of collection and receipt of the sample were noted on the label. The urine sample was discarded if there was a delay of more than 1 h between sample collection and processing. A protease inhibitor cocktail tablet (Roche Diagnostics, Indianapolis) was added to the sample to stabilize the proteins. Urine was centrifuged at 2000 rpm and stored at -80°C in the laboratory. Urine was recentrifuged before ELISA estimation. uAQP-1 concentrations were estimated by an ELISA kit (Universal Biotechnology, New Delhi) based on the biotin double antibody sandwich technology to assay human AQP-1 protein. Absorbance (O. D) was measured with a microplate reader at 450 nm wavelength, at 10 min. A standard curve was plotted between the O. D units and the known standard protein concentrations. This was used for the calculation of uAQP-1 concentrations in the sample. The assay sensitivity was 0.042 ng/ml, and intra-assay and inter-assay precision measured by coefficient of variation (CV) was <8 CV% and <10 CV%, respectively. All values were normalized based on urinary creatinine levels estimated by the Jaffe's method. uAQP-1 ELISA kits had a limited shelf life of 1 month and was procured in batches. For financial and logistical reasons, the study was limited to duration of 6 months.

Statistical Methods

The data were analyzed with SPSS v23.0 (IBM Corporation, Armonk, NY, USA).

Results

Table 1: Baseline characteristics of cases with suspected renal cell carcinoma and healthy volunteers enrolled in the study

Baseline characteristic	With renal masssuspicious of RCC (*n*=50)	Healthycontrols(*n*=50)
Sex (%)		
Male	38 (76)	26 (52)
Female	12 (24)	24 (48)
Age (years), mean (SD)	46.7 (9.6)	47.6 (11.8)
BMI (kg/m^2), mean (SD)	24.20 (3.6)	22.48 (4.1)
Hypertension (%)	24 (48)	0
Diabetes mellitus (%)	16 (32)	0
Smoking (%)	13 (26)	9 (18)
Serum creatinine (mg/dl), mean (SD)	0.88 (0.22)	0.86 (0.22)

Among cases, 38 were men and 12 were women, whereas among controls, 26 were men and 24 were women. The mean (standard deviation [SD]) age of the cases and controls was 46.7 (9.6) years and 47.6 (11.8) years, respectively. The BMI of cases and controls was 24.20 (3.6) and 22.48 (4.1) respectively.

Table 2: Profile of the imaged renal masses

Tumor characteristics	*n*=50
Tumor size (cm)	7 (2.4-14)
Histological subtypes (%)	
Clear cell RCC	47 (94)
Papillary RCC	2 (4)
Chromophobe RCC	1 (2)
T stage (%)	
T1a	15 (30)
T1b	15 (30)
T2a	1 (2)
T2b	6 (12)
T3	12 (24)
T4	1 (2)
N stage (%)	
N0	45 (90)
N1	5 (10)
M stage (%)	
M0	43 (86)
M1	7 (14)

Majority of the tumors (47/50) were reported as renal cell carcinoma with a clear-cell histology. Sixty percent of the tumors were pT1 and the mean size of the tumor was 7 cm (2.4–14 cm, SD 3.62 cm). Two-thirds of these patients had a radical nephrectomy, the rest underwent partial nephrectomy.

Sensitivity & specificity

The sensitivity and specificity of AQP1 (normalized to urine creatinine) to identify patients with clear cell kidney cancer was determined using receiver operating characteristic (ROC) analysis Compared to healthy controls, urine AQP1 had 48.5% sensitivity and 67.3% specificity.

Discussion

Incidentally detected renal tumors have increased from 13% in the 1970s to about 50%–60% in contemporary practice [17,18] due to the widespread use of imaging modalities, such as ultrasound, computed tomography (CT), and magnetic resonance imaging. Although CT has a staging accuracy of 91% for RCC [19], it cannot reliably differentiate between benign and malignant tumors [20] or identify aggressive tumor biology that is present in up to 30% of the small renal tumors. [21] The unpredictable tumor biology and the increased use of nephron-sparing surgery and active surveillance in the management of renal tumors have brought about the need for a biomarker that would aid optimal patient selection and treatment decisions. [22] A sensitive and specific biomarker for RCC that can differentiate between benign and malignant renal tumors as well as identify those with aggressive tumor biology will be a useful adjunct to imaging.

Aquaporin-1 is a water channel protein present throughout the human body with many physiological functions involving transmembrane water and ion transport. It is known that aquaporin-1 is overexpressed in several cancers such as colon, lung, central nervous system, and kidney. [23,24] Although the exact pathways and mechanisms are yet to be discovered, some of the mechanisms that have been suggested are [25] (i) AQP-1-modulated tumor cell migration and invasion, (ii) AQP-1-modulated tumor angiogenesis, (iii) AQP-1-modulated tumor proliferation, (iv) induction of AQP-1 by hypoxia/glycolysis, and (v) tumor progression mediated by downstream effectors and signaling pathways such as beta-catenin, Lin-7, MMP2, MMP9, Rho, and TGF beta. Among cases, 38 were men and 12 were women, whereas among controls, 26 were men and 24 were women. The mean (standard deviation [SD]) age of the cases and controls was 46.7 (9.6) years and 47.6 (11.8) years, respectively. The BMI of cases and controls was 24.20 (3.6) and 22.48 (4.1) respectively. Majority of the tumors (47/50) were reported as renal cell carcinoma with a clear-cell histology. Sixty percent of the tumors were pT1 and the mean size of the tumor was 7 cm (2.4–14 cm, SD 3.62 cm). Two-thirds of these patients had a radical nephrectomy, the rest underwent partial nephrectomy.

Based on published reports, as these tumors originate from the proximal tubule, one would have expected uAQP-1 concentrations to be high in this study arm. Evidence suggests that tumors that do not arise from the proximal nephron do not result in a rise in uAQP-1 levels. [26] Furthermore, common renal diseases such as glomerulonephritis and diabetes mellitus, as well as benign tumors such as oncocytoma and angiomyolipoma, do not seem to

affect the uAQP-1 concentrations. In this study, higher uAQP-1 concentrations were not associated with larger tumors. In contrast, in a prospective observational study, preoperative uAQP-1 levels showed a linear correlation (Spearman's coefficient - 0.78, $P < 0.001$) with the T-stage of the tumor. [27] The investigators examined the potential causes for negative results of the study. uAQP-1 was measured in batches in a nationally accredited laboratory (NABL, India) and standard procedures of collection and storage for biomarker quantification were followed. [28] Strict protocol was followed for sample collection and processing and samples were discarded if there was a deviation from the protocol. Storage at −80°C allowed samples to be completely recovered even after 7 months. [29]

Conclusion

The present study concluded that uAQP-1 may not be a useful diagnostic urinary biomarker for renal cell carcinoma. This test had a poor sensitivity and specificity in diagnosing renal cell carcinoma in the study population.

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