Diagnostic accuracy of urinary aquaporin-1 as a biomarker for renal cell carcinoma

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INTRODUCTION

Incidentally detected renal tumors have increased from 13% in the 1970s to about 50%–60% in contemporary practice1,2 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,3 it cannot reliably differentiate between benign and malignant tumors4 or identify aggressive tumor biology that is present in up to 30% of the small renal tumors.5 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.6 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.

ABSTRACT

Introduction: Optimal patient selection plays a vital role in management of renal tumors with the introduction of nephron-sparing approaches and active surveillance. A reliable and accurate diagnostic biomarker will be a useful adjunct to decision-making. We studied the diagnostic accuracy of urinary aquaporin-1 (uAQP-1), an upcoming urinary biomarker, for renal cell carcinoma.

Materials and Methods: In this prospective biomarker study, urine samples were obtained preoperatively from 36 patients with an imaged renal mass suggestive of RCC and 24 healthy age-matched controls, chosen from among voluntary kidney donors. uAQP-1 concentrations were estimated with a sensitive and specific enzyme-linked immunosorbent assay (ELISA) and normalized by estimation of urinary creatinine. The Mann–Whitney U-test was used to compare differences between any two groups. A receiver operator characteristic (ROC) curve was plotted to analyze the diagnostic accuracy of uAQP-1 for RCC.

Results: The median uAQP-1 concentration among the cases and controls was 8.78 ng/mg creatinine (interquartile range [IQR]: 5.56–12.67) and 9.52 ng/mg creatinine (IQR: 5.55–12.45), respectively. There was no significant difference in uAQP-1 concentrations between the two groups. ROC analysis showed that, for a cutoff value of 8 ng/mg creatinine, the sensitivity and specificity of uAQP-1 as a diagnostic test were 47.2% and 66.7%, respectively, and area under the curve was 0.52 (95% confidence interval: 0.42–0.62).

Conclusions: uAQP-1 concentrations did not discriminate between healthy individuals and patients with RCC. The results of this study suggest that uAQP-1 may not be a suitable diagnostic biomarker for RCC in the study population.

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the proximal nephron, the expression of the aquaporin-1 protein is increased. In a prospective cohort study of patients undergoing radical nephrectomy for RCC, Morrissey et al. reported an 88% decrease in concentrations of urinary aquaporin-1 (uAQP-1) between the pre- and postoperative urine samples. [7] In another prospective observational study that compared uAQP-1 levels in RCC, non-RCC tumors, prostate cancer, and bladder cancer, uAQP-1 had a sensitivity and specificity of 99% to 100% for clear-cell and papillary RCC. [8] Another study investigated the role of uAQP-1 as a screening tool with promising results. [9] While evidence suggests that uAQP-1 may be a potential biomarker, a study from Serbia reported contrasting findings. [10] This emphasizes the need for validation of this marker in other populations. Accordingly, we conducted a prospective study to determine the diagnostic accuracy of uAQP-1 as a diagnostic biomarker in our population.

MATERIALS AND METHODS

Study design
This was a prospective phase II biomarker study to estimate the accuracy of uAQP-1 as a diagnostic biomarker, by a two-gated design with healthy controls, and was conducted at a tertiary care center in India over a duration of six months. [11] The aim of a phase II study in biomarker development is to estimate the receiver operator characteristic (ROC) curve of a test and therefore its ability to distinguish subjects with cancer from those without cancer. [12] The study was approved by the Institutional Review Board and Ethics Committee of the Christian Medical College and Hospital, Vellore, India (IRB No. 10735, dated March 7, 2017). Participants were recruited with written informed consent. The hypothesis of the study was that uAQP-1 levels are significantly higher in those with RCC, when compared to a healthy control population. Based on the study by Morrissey et al. [9] 90% sensitivity and specificity were assumed for the index test. The sample size calculated with the above assumption with a precision of 10% with a Z-score of 1.96 was 72, with 36 cases and 36 controls. Histopathological examination of the tumor was considered the gold standard for diagnosis of RCC and its subtypes. The authors confirm the availability of, and access to, all original data reported in this study.

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. [9] ELISA techniques are less cumbersome and hence could potentially be used in testing larger numbers of patients. All laboratory methods were performed by an experienced laboratory scientist (Dr. AJN) who was blinded to the study arm. 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 a duration of 6 months.

Statistical methods
Descriptive statistics were used to illustrate the baseline characteristics of the participants Supplementary Figure 1 (a,b and c). Shapiro–Wilk test was used to test normality of data [Supplementary Table 1(a,b)]. The sensitivity and specificity of uAQP-1, normalized with urine creatinine measurements, for diagnosing renal cell carcinoma were calculated by plotting a ROC curve. The data were analyzed with SPSS v23.0 (IBM Corporation, Armonk, NY, USA). The median uAQP-1 levels were calculated for each category with the interquartile ranges (IQRs). Nonparametric Mann–Whitney U-test was performed to compare two categories and Kruskal–Wallis test was used if there were more than two categories. The ROC curve was drawn and the sensitivity and specificity were calculated. Spearman’s correlation rank test was used to test the correlation between tumour size
and uAQP-1. The area under the curve (AUC) of uAQP-1 was estimated with a 95% confidence interval. Detailed statistical data are available in the Supplementary Material.

RESULTS

From March to June 2018, 52 cases and 35 prospective kidney donors were screened for eligibility. Urine samples were collected from 43 patients and 28 healthy controls. In the final analysis, 36 patients and 24 healthy controls were included. The mean (standard deviation [SD]) age of the cases and controls was 45.9 (9.7) years and 46.3 (10.9) years, respectively. Among cases, 27 were men and 9 were women, whereas among controls, 12 were men and 12 were women. The baseline characteristics of the study arms are summarized in Table 1. Majority of the tumors (34/36) were reported as renal cell carcinoma with a clear-cell histology. Sixty-one 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 (24/36) of these patients had a radical nephrectomy, the rest underwent partial nephrectomy. The details of the tumors among patients who underwent radical or partial nephrectomy are summarized in Table 2.

The median uAQP-1 concentration (Figure 2) among the patients with renal mass was 8.78 ng/mg creatinine (IQR: 5.56–12.67). Among controls, the median uAQP-1 concentration was 9.52 ng/mg creatinine (IQR: 5.55–12.45). There was no significant difference in uAQP-1 levels between cases and controls (P = 0.74). The median (IQR) values of uAQP-1 levels (in ng/mg creatinine) for men and women among cases were 6.92 (IQR: 4.42–11.69) and 9.80 (IQR: 6.31–14.66) and among controls were 8.81 (IQR: 5.56–10.71) and 10.67 (IQR: 5.81–13.49), respectively. The uAQP-1 concentrations were not statistically different between genders (P = 0.201). This was tested by the Mann–Whitney U-test for both the cases (P = 0.34) and controls (P = 0.36). There was no difference between uAQP-1 concentrations with respect to the size of the tumor (Spearman’s coefficient - 0.4) or T stage of the tumors (P = 0.93) [Supplementary Figure 2] and the nuclear grade of the tumors (P = 0.173) [Supplementary Table 2]. uAQP-1

There was no difference between uAQP-1 concentrations with respect to the size of the tumor (Spearman’s coefficient - 0.4) or T stage of the tumors (P = 0.93) [Supplementary Figure 2] and the nuclear grade of the tumors (P = 0.173) [Supplementary Table 2]. uAQP-1

| Baseline characteristic | With renal mass suspicious of RCC (n=36) | Healthy controls (n=24) |
|-------------------------|------------------------------------------|-------------------------|
| Sex (%)                 | Male 27 (75) | 12 (50) |
| Age (years)             | 45.8 (9.7) | 46.3 (10.9) |
| BMI (kg/m²)             | 25.23 (3.6) | 23.54 (4.1) |
| Hypertension (%)        | 17 (47.2) | 0 |
| Diabetes mellitus (%)   | 11 (30.6) | 0 |
| Smoking (%)             | 9 (25) | 4 (16.7) |
| Serum creatinine (mg/dl)| 0.88 (0.21) | 0.85 (0.22) |

SD = Standard deviation, BMI = Body mass index, RCC = Renal cell carcinoma

| Tumor characteristics | n=36 |
|-----------------------|------|
| Tumor size (cm)       | 7 (2.4-14) |
| Histological subtypes |  |
| Clear cell RCC        | 34 (94.4) |
| Papillary RCC         | 1 (2.8) |
| Chromophobe RCC       | 1 (2.8) |
| T stage (%)           |  |
| T1a                   | 11 (30.6) |
| T1b                   | 11 (30.6) |
| T2a                   | 1 (2.8) |
| T2b                   | 3 (11.1) |
| T3                    | 8 (22.2) |
| T4                    | 1 (2.8) |
| N stage (%)           |  |
| N0                    | 32 (88.9) |
| N1                    | 4 (11.1) |
| M stage (%)           |  |
| M0                    | 31 (86.1) |
| M1                    | 5 (13.9) |

RCC = Renal cell carcinoma

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**Figure 1:** Study flow diagram showing eligible participants and the participants included in the final analysis.
levels based on histology could not be analyzed as 34 out of 36 cases had a clear-cell histology. There was no difference in uAQP-1 with respect to N stage, M stage, tumor necrosis, or renal vein thrombosis. ROC curve analysis showed that the sensitivity and specificity were 47.2% and 66.7%, respectively, for a cutoff value of 8 ng/mg creatinine. Likelihood ratio was 1.26 for the cutoff value. The AUC or the c-index was 0.52 (95% CI: 0.42–0.62) [Figure 3]. The Youden’s index of the diagnostic test was 0.139.

DISCUSSION

Most of the experience with AQP-1 as a urinary biomarker for RCC has been reported by Morrissey et al.[9,13] However, AQP-1 as a urinary biomarker has not been studied previously in the Asian-Indian population. Furthermore, in a report from Serbia, uAQP-1 was not found to be a useful test.[10] 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.[14-17] Although the exact pathways and mechanisms are yet to be discovered, some of the mechanisms that have been suggested are[18] (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.

In order to establish the clinical validity as a diagnostic biomarker in our population, this phase 2 study to assess the specificity, sensitivity, and accuracy of uAQP-1 to diagnose RCC was undertaken.[12] Establishing the baseline concentrations of uAQP-1 among healthy individuals is an important initial step. The study design was tailored to achieve this, by recruiting healthy individuals as the control population. Voluntary kidney donors, in the authors’ opinion, are the ideal control population. They undergo comprehensive medical evaluation, including contrast-enhanced CT of the abdomen before donation as a part of donor workup. This ensures no added radiation exposure to a healthy control. Although the sample size calculated at the beginning of the study was a total of 72 patients with an equal number of patients and healthy controls (36 each), only 24 participants in the control arm were analyzed. This was largely because ELISA kits had limited shelf life and a strict timeline had to be followed. Moreover, the cases and the controls had to be age matched. The higher proportion of women in the control group compared to the patients with renal cell carcinoma is representative of the voluntary kidney donor population in most countries, especially in Asia and also the higher incidence of RCC in males. The authors did not find any significant difference in uAQP-1 levels between genders. Moreover, there is no evidence to suggest that uAQP-1 levels are affected by gender. Other baseline characteristics including BMI and serum creatinine were comparable between the two groups.

In this study, contrary to previous reports, uAQP-1 was not elevated consistently in patients with RCC. The median uAQP-1 concentration was 8.78 ng/mg creatinine (IQR: 5.56–12.67) among patients with RCC and 9.52 ng/mg creatinine (IQR: 5.55–12.45) in healthy individuals. Morrissey et al. reported the median uAQP-1 concentration of 255 ng/mg creatinine for those with RCC and 1.1 ng/mg creatinine in those without RCC[9] and therefore reported a sensitivity and specificity of over 95% for this biomarker. The majority of the patients had a clear-cell histology (34/36).
and one patient had a papillary cell histology. Due to insufficient numbers, no statistical comparisons between various subtypes of RCC were made. 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.\(^{[13]}\) 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.\(^{[13]}\) We did not find any difference in uAQP-1 concentrations with respect to the nuclear grade of RCC, which is consistent with prior reports. Although it was expected in the context of the main results of the study, it further emphasizes the point discussed by Morrissey et al. that these markers should be only used as an adjunct to imaging as small aggressive tumors may be missed.\(^{[13]}\)

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.\(^{[13]}\) 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.\(^{[20]}\) The authors recognize that despite all these precautions, protein degradation can still ensue, which can lead to a falsely low value of uAQP-1 in patients with renal carcinoma. Second, this study included only participants from the Indian subcontinent and all patients were of Asian-Indian ethnic background. It is not known if uAQP-1 levels are affected by the racial/ethnic profile of the population. In this paper, controls had a higher median uAQP-1 concentration in comparison with patients with RCC, although this finding was not statistically significant. These results mirror the results of Mijugković et al.\(^{[10]}\)

The investigators reported a sensitivity and specificity of 47.2% and 66.7%, respectively, for uAQP-1 as a diagnostic biomarker. This was far from the assumed 90% sensitivity and specificity at the onset of the study based on available literature. Current imaging modalities provide more than 80% sensitivity and specificity in diagnosing and characterizing renal masses. Unless a biomarker for RCC has a higher sensitivity and specificity than the standard imaging modalities, it may not have any practical relevance in terms of identifying malignancy in those with atypical imaging features or high-grade tumors where one would prefer radical treatment. One of the important limitations of this study was inability to recruit the required number of controls. To address this, we performed a post hoc analysis of the power as precision. The precision of the estimates of sensitivity and specificity of the test were \(\pm 15.3\%\) and \(\pm 20\%\), respectively. The precision of the estimate of specificity, if all controls were recruited, would have improved by \(\pm 4\%\) to \(\pm 16.3\%\) whereas the precision for sensitivity will remain the same [Supplementary Table 3]. The authors reason that although the study failed to recruit the required number of healthy controls as per the sample size calculated, the observed results of this study should caution future researchers investigating this marker. Current evidence for the use of uAQP-1 is conflicting; however, novel and refined methods of uAQP-1 estimation may change the scenario.\(^{[21]}\)

An ideal biomarker for renal cell carcinoma is yet to be identified. uAQP-1 , although deemed a promising biomarker, is still in its early phase of development and validation. The clinical validity of uAQP-1 as a diagnostic marker could not be reproduced in this study.

**CONCLUSIONS**

The results of this paper suggest 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.

**REFERENCES**

1. Luciani LG, Cestari R, Tallardo C. Incidental renal cell carcinoma-age and stage characterization and clinical implications: Study of 1092 patients (1982-1997). Uroloko 2000;56:58-62.
2. Jayson M, Sanders H. Increased incidence of serendipitously discovered renal cell carcinoma. Urology 1998;51:203-5.
3. Catalano C, Fraioli F, Laghi A, Napoli A, Pediconi F, Danti M, et al. High-resolution multidetector CT in the preoperative evaluation of patients with renal cell carcinoma. AJR Am J Roentgenol 2003;180:1271-7.
4. Millet I, Doyon FC, Hoa D, Thuret R, Mereeaud S, Serre I, et al. Characterization of small solid renal lesions: Can benign and malignant tumors be differentiated with CT? Am J Roentgenol 2011;197::887-96.
5. Pierorazio PM, Hyams ES, Mullins JK, Allaf ME. Active surveillance for small renal masses. Rev Urol 2012;14:13-9.
6. Sun M, Abdollah F, Bianchi M, Trinh QD, Jeldres C, Thuret R, et al. Treatment management of small renal masses in the 21st century: A paradigm shift. Ann Surg Oncol 2012;19:2380-7.
7. Morrissey JJ, London AN, Luo J, Kharasch ED. Urinary biomarkers for the early diagnosis of kidney cancer. Mayo Clin Proc 2010;85:413-21.
8. Morrissey JJ, Mobley J, Figenshu RS, Vetter J, Bhayani S, Kharasch ED. Urine aquaporin 1 and perilipin 2 differentiate renal carcinomas from other imaged renal masses and bladder and prostate cancer. Mayo Clin Proc 2015;90:35-42.
9. Morrissey JJ, Molinick VM, Luo J, Siegel MJ, Figenshu RS, Bhayani S, et al. Evaluation of urine aquaporin-1 and perilipin-2 concentrations as biomarkers to screen for renal cell carcinoma: A prospective cohort study. JAMA Oncol 2015;1:204-12.
10. Mijugkovic M, Stanojevic I, Milovic N, Cernoic S, Petrovic D, Jovanovic D, et al. Urinary KIM-1 and AQP-1 in patients with clear renal cell carcinoma: Potential noninvasive biomarkers. Vojnosanit Pregl 2016;73:266-72.
11. Rutjes AW, Reitsma JB, Vandenbroucke JP, Glas AS, Bossuyt PM. Case-control and two-gate designs in diagnostic accuracy studies. Clin Chem 2005;51:1335-41.

12. Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, et al. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst 2001;93:1054-61.

13. Morrissey JJ, Mobley J, Song J, Vetter J, Luo J, Bhayani S, et al. Urinary concentrations of aquaporin-1 and perilipin-2 in patients with renal cell carcinoma correlate with tumor size and stage but not grade. Urology 2014;83:256.e9-14.

14. Yoshida T, Hojo S, Sekine S, Sawada S, Okumura T, Nagata T, et al. Expression of aquaporin-1 is a poor prognostic factor for stage II and III colon cancer. Mol Clin Oncol 2013;1:953-8.

15. Yun S, Sun PL, Jin Y, Kim H, Park E, Park SY, et al. Aquaporin 1 is an independent marker of poor prognosis in lung adenocarcinoma. J Pathol Transl Med 2016;50:251-7.

16. Deb P, Pal S, Dutta V, Boruah D, Chandran VM, Bhatoe HS. Correlation of expression pattern of aquaporin-1 in primary central nervous system tumors with tumor type, grade, proliferation, microvessel density, contrast-enhancement and perilesional edema. J Cancer Res Ther 2012;8:571-7.

17. Huang Y, Murakami T, Sano F, Kondo K, Nakaigawa N, Kishida T, et al. Expression of aquaporin 1 in primary renal tumors: A prognostic indicator for clear-cell renal cell carcinoma. Eur Urol 2009;56:690-8.

18. Tomita Y, Dorward H, Yool AJ, Smith E, Townsend AR, Price T, et al. Role of aquaporin 1 signalling in cancer development and progression. Int J Mol Sci 2017;18. https://www.mdpi.com/1422-0067/18/2/299#cite.

19. Thomas CE, Sexton W, Benson K, Sutphen R, Koomen J. Urine collection and processing for protein biomarker discovery and quantification. Cancer Epidemiol Biomarkers Prev 2010;19:953-9.

20. Zhou H, Yuen PS, Pisitkun T, Gonzales PA, Yasuda H, Dear JW, et al. Collection, storage, preservation, and normalization of human urinary exosomes for biomarker discovery. Kidney Int 2006;69:1471-6.

21. Jakowiecki J, Sztyler A, Filip S, Li P, Raman K, Barathiraja N, et al. Aquaporin-graphene interface: Relevance to point-of-care device for renal cell carcinoma and desalination. Interface Focus 2018;8. https://doi.org/10.1098/rsfs.2017.0066.

Supplementary tables available online at www.indianjurol.com

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Supplementary Figure 1a: Histogram showing urinary aquaporin-1 distribution for both cases and controls

Supplementary Table 1a: Shapiro-Wilk test for both cases and controls

| Variable              | Shapiro-Wilk |
|-----------------------|--------------|
| AQP/mg creatinine     | 0.827        |
| df                   | 60           |
| Significant           | 0.000        |

AQP = Aquaporin

Above Shapiro–Wilk test shows a statistically significant result, which means the data are not normal. By looking the distribution of data also we can conclude that the data are skewed.

To check normality assumption by group

For control

Supplementary Figure 1b: Histogram showing urinary aquaporin-1 distribution in the control group

For cases
STATISTICAL TESTS FOR TESTING NORMALITY OF DATA

For the entire study

Supplementary Figure 1c: Histogram showing urinary aquaporin-1 distribution in the case group

Supplementary Table 1b: Shapiro-Wilk test for cases and controls

| Variable        | Arm   | Shapiro-Wilk |
|-----------------|-------|--------------|
|                 |       | Statistic    | df  | Significant |
| AQP/mg creatinine | Control | 0.962        | 24  | 0.484       |
|                 | Case   | 0.793        | 36  | 0.000       |

AQP = Aquaporin

Here, in control group uAQP is following normal distribution. However, in cases group AQP is not met normality assumption. Therefore, we have used non parametric methods for the comparison of AQP across the group.

Supplementary Table 2: Comparison of uAQP-1 in various groups

| Variables       | AQP creatinine Median (IQR) | P     |
|-----------------|------------------------------|-------|
| Gender          |                              |       |
| Male            | 5.6 (3.8-10.0)               | 0.201 |
| Female          | 5.0 (4.3-10.5)               |       |
| Nuclear grade   |                              |       |
| I               | 4.2 (3.6-5.3)                | 0.930 |
| II              | 7.2 (4.0-11.5)               |       |
| III             | 9.5 (6.5-11.8)               |       |
| IV              | 7.5 (2.9-7.5)                |       |
| T stage         |                              |       |
| T1              | 4.1 (3.6-5.1)                | 0.173 |
| T2              | 11.5 (10.0-13.5)             |       |
| T3              | 10.0 (7.2-11.9)              |       |
| T4              | 13.7 (13.7-13.7)             |       |
| Tumor size      | Correlation coefficient 0.407| 0.014 |

uAQP-1 = Urinary aquaporin-1, AQP = Aquaporin, IQR = Interquartile range
**Supplementary Figure 2:** Box plot showing Urinary AQP-1 levels in various T stages of tumor. Box plot depicts the median with the 1<sup>st</sup> and 3<sup>rd</sup> quartiles.

**Power analysis – based on observed values**

| Parameter                  | Calculated sample size (n=36) | Collected sample size (n=24) |
|----------------------------|-------------------------------|------------------------------|
| Sensitivity (precision)    | 68.0 (15.3)                   |                              |
| Specificity (precision)    | 46.0 (16.3)                   | 46.0 (20.0)                  |

**Power analyses:**

**As precision:** In the sensitivity as the numbers have been kept as planned, that is 36. Therefore the precision of the estimates is 15.3% for sensitivity. However, in the Control we were able to study only 24 subjects that increased the precision by 4% more from the planned number of subjects 36. That is had we studied 36 subjects as control then the precision would have been 16.3%.

The sensitivity and specificity are estimates and based on the proposed values the sample size was calculated. But they are not tested against any other test values or studies, while doing the sample size calculation and therefore the typical power analyses may not be possible.