Morphological findings in frozen non-neoplastic kidney tissues of patients with kidney cancer from large-scale multicentric studies on genomics of renal cancer

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Abstract

Background

There are unexplained geographical variations in the incidence of kidney cancer with the high rates reported in Baltic countries, as well as eastern and central Europe. Analysis of non-neoplastic tissues is a way to better understand the carcinogenesis.

Methods

Having access to a rich, well-annotated collection of “tumor/non-tumor” pairs of kidney cancer patients from Czech Republic, Romania, Serbia, United Kingdom, and Russia for studying genomics of kidney cancer, we aimed to analyze morphology of non-neoplastic renal tissue. By applying digital pathology, we performed microscopic examination of 1012 frozen non-neoplastic kidney tissues from patients with renal cell carcinoma. Renal parenchyma was evaluated and scored for the interstitial inflammation and fibrosis, tubular atrophy, glomerulosclerosis and arterial wall thickening, globally called chronic renal parenchymal changes.

Results

Moderate or severe changes was observed in 54 (5.3%) of patients with predominance of occurrence in Romania (OR = 2.67, CI 1.07–6.67) and Serbia (OR = 4.37, CI 1.20-15.96) in reference to those from Russia. Further adjustment for comorbidities, tumor characteristics and stage did not change risk estimates. In multinomial regression model, relative probability of non-glomerular changes were 5.22 times higher for Romania and Serbia compared to Russia.

Conclusion

Our findings show that the frequency of chronic renal parenchymal changes in kidney cancer patients varies by country, significantly more frequent in countries located in central and southeastern Europe where the incidence of kidney cancer has been reported to be high. We suggest that these parenchymal changes, possibly linked to environmental exposures, may be relevant to renal carcinogenesis in these countries.

Background

Worldwide, kidney cancer was reported as the 9th most common malignancy in men and 14th in women in 2018, with an estimated 136,515 and 67,424 new cases in North America and Europe respectively. There is considerable geographic variation in the incidence of kidney cancer, the reasons for which are unknown. The highest incidence rates are reported in Baltic countries, as well as central and southern

Europe, with age-standardized incidence rates of 16.8 in Belarus, followed by Latvia (15.2), Lithuania (14.8), Czech Republic (14.7), and Estonia (14.6) [1]. Renal cell carcinoma (RCC), the most common malignant neoplasm in kidney, is composed of a heterogeneous group of carcinomas derived from renal tubular epithelium. The most common carcinomas in this category are clear cell RCC, papillary RCC, and chromophobe RCC, which globally comprise 85–90% of RCC cases.

Previous data indicate that non-neoplastic kidney tissues are rarely normal and often show variable levels of pathological changes such as glomerular abnormalities, diabetic or hypertensive nephropathies, and chronic pyelonephritis [2–4]. Such changes in nephrectomy specimens may have an impact on optimal treatment planning and on early identification of unrecognized kidney disease and postoperative renal outcomes' prediction [5]. Through examination of non-neoplastic renal parenchymal changes in 110 cancer-related nephrectomy specimens, Bijol *et. al.* showed that only 10% of the cases have unremarkable parenchyma and those with considerable pathological changes such as severe parenchymal scarring and more than 20% glomerulosclerosis had progressive worsening of renal function in 6 months follow-up after radical nephrectomy [6]. Chronic kidney disease and kidney cancer are connected in both directions: controversial results have been reported the carcinogenic role of chronic kidney diseases, and conversely, nephrectomy, as the standard therapeutic approach to renal cancer, can lead to chronic kidney disease [7, 8].

During 2007 to 2016, the International Agency for Research on Cancer (IARC) conducted hospital and population-based case-control studies in Czech Republic, Romania, Serbia, and Russia (K2 study) to recruit incident cases with kidney cancer diagnosis. In parallel, incident kidney cancer cases were recruited in the United Kingdom (UK) by the Leeds Multidisciplinary Research Tissue Bank. Both collections were used in the Cancer Genomic of Kidney (CAGEKID) project (<http://www.cng.fr/cagekid/>). This rich collection of samples through utilising harmonized and standard operation procedures comprises more than 2000 frozen “tumor/non-tumor” pairs with annotated clinico-epidemiological data of kidney cancer patients. Understanding of molecular events such as somatic mutations in non-neoplastic cells is central to understand cancer development and is taking a considerable place in research on cancer [9, 10]. Morphological features in non-neoplastic tissue of cancer patients could also provide key information towards better understanding of cancer development. Having access to a rich collection of paired tumor/non-tumor kidney, we designed this study to evaluate the post-nephrectomy non-neoplastic tissues of kidney cancer patients in a subset of our collection.

Methods

Aim and design

We aimed to evaluate the morphological features in non-neoplastic tissues of patients from countries with variable incidence of RCC to understand if there is any pathological morphology that could be linked to carcinogenesis. 1045 non-neoplastic frozen kidney tissues of patients with RCC from the IARC biorepository were selected for this study. The selection was based on the availability of at least one

hematoxylin and eosin (H&E) stained slide, irrespective to the tumor type or the country of recruitment. High-resolution whole slide images were used for digital microscopic examination by one pathologist. Through applying a harmonized protocol for multicentric biological sample collection in both K2 and CAGEKID studies, immediately after nephrectomy, one piece of non-neoplastic kidney tissue was collected from renal cortex, distant from tumor, and at least 5 mm in the largest diameter, in addition to tumor tissue. All collected tissues were snap frozen and stored in liquid nitrogen or directly immersed in RNA/later followed by freezing in -80°C freezers, and were then shipped to IARC for storage and processing. Patients were interviewed and detailed information including tobacco use, alcohol consumption, body mass, medical history, and family history of cancer, as well as clinical data from medical records were collected. For the purpose of this study, microscopic examination was performed blind to the type and status of tumor, demographic and clinical data, country of recruitment, and laboratory results. We first evaluated the composition of renal parenchyma and the percentage of medulla, if present, was recorded. Four structures of renal cortex were examined: renal glomeruli and the presence of various degrees of sclerosis (GS), renal tubules and the presence and severity of atrophy, presence and severity of interstitial fibrosis (the latter two together called IFTA), presence, severity, and type of inflammation; and also blood vessels and the presence or absence of atherosclerosis. These changes, that we call them chronic renal parenchymal changes (CRPC), were scored from none to severe as follows: none, if no significant pathological changes were observed, and mild, moderate and severe if any or a complex of parenchymal changes were seen in 1–10%, 11–20% and more than 21%, respectively, of the examined sections. We re-examined and re-scored all available images that we scored as mild, moderate, or severe parenchymal changes by applying the scoring method that is proposed by the International Society of Nephrology (ISN) to report the microscopic findings of the non-neoplastic renal tissues in native kidney biopsies [11].

Statistical analysis

Dependent on our findings, we categorized outcome into two main groups: those with none-mild CRPC versus those with moderate-severe CRPC. Demographic variables were then categorized and compared between two outcomes including country of recruitment, age at diagnosis, body mass index (BMI) at diagnosis, tobacco smoking status and alcohol consumption habit. Further, we added variables that could have a negative impact on our defined protocol to collect the non-neoplastic tissue from renal cortex such as tumor size and stage, surgical approach (radical or partial), and percentage of medulla. Kidney-related diseases or medical conditions that could potentially confound the relation between CRPC and demographic variables were analyzed, including self-reported history of hypertension, diabetes mellitus, nephrolithiasis, renal cyst, kidney diseases, and ever use of non-steroidal anti-inflammatory drugs (NSAIDs). Logistic regression models were used to calculate unadjusted and adjusted odds ratios (ORs) and 95% confidence intervals (CIs). In all analyses, unexposed groups were the reference. For numerical variables, lowest category was considered as a reference. Our scoring method in this study was defined to evaluate the non-neoplastic morphological features in patients that “cancer” has been their main pathology and were not reported as having clinical symptoms of renal disease. To evaluate the performance of our scoring, we used the scoring method of ISN as gold standard and compared the two.

Also, we used multinominal regression analysis to compare geographical residence (as predictor) association with the presence of individual components of CRPC without applying any score which included: any degree of GS, non-glomerular pathologies (IFTA), presence of both GS and IFTA, and absence of any pathological change. Age, gender, and presence of inflammation were included in the model.

Results

Of the 1045 initially selected tissues, 33 were excluded due to the exclusive presence of renal medulla in which evaluation of renal cortical structures was not possible. Table 1 summarizes the demographic characteristics as well as potential confounders of the examined patients by grouping them into two categories of CRPC as the outcome of interest. In 1012 examined ones, 836 (82.6%) did not show significant parenchymal changes, 122 (12.0%) were scored as mild CRPC (Fig. 1A), 28 (2.8%) as moderate, and 26 (2.6%) as severe CRPC (Figs. 1B to 1D). 958 (94.7%) were grouped into none-mild CRPC category, and 54 (5.3%) subjects met our criteria for being considered as having moderate-severe CRPC. Except for age and country of recruitment, no significant difference was observed between proportion of moderate-severe CRPC relative to tumor type, sex, BMI, smoking habit, and alcohol consumption. Mean age of patients recruited from Russia and UK was 57 and 62 years old. Patients from Serbia, Romania, and Czech Republic were recruited at the mean age of 60, 63, and 64 year-old. In univariate analysis, odds of having moderate-severe CRPC showed a significant trend with older age ($P = 0.001$). Proportion of both glomerular and tubular components of CRPC increased by age (Fig. 2).

There was a variation in number of recruited cases in this study. Czech Republic with 426 patients provided the highest number of cases from four different recruiting cities, followed by Romania (Bucharest), UK (Leeds), Russia (Moscow), and Serbia (Belgrade). Clear cell RCC was the most common histological type of RCC in the entire examined patients (84.0%), followed by papillary RCC (7.3%). Other tumor types included chromophobe RCC, collecting duct carcinoma, unclassified RCC, and oncocytoma, altogether comprised 8.7% of the total examined cases. Proportion of major RCC histological types did not vary across CRPC scores (Table 2). The relative probability of detecting GS rather than no changes for patients from Czech Republic is more than double the corresponding probability for patients from Russia or UK with the same level of inflammation. Variable degrees of GS were not significantly higher among Romanians and Serbians compared to Russians. In contrast, IFTA was observed four and five times higher in patients from Czech Republic and Romania-Serbia, respectively, than Russians. The relative probability remained unchanged when those with both GS and IFTA were tested (Table 3).

In order to evaluate whether the observed morphological changes were secondary to the effect of tumor on adjacent tissue, we used variables such as percentage of medulla in non-neoplastic tissues, size of original tumor, stage at which tumor was operated upon, and type of surgical approach to study their impact on outcome. Similar proportion of CRPC across tumor size and other variables was observed (Table 4). Since other medical conditions such as hypertension and diabetes mellitus could cause pathological changes in renal parenchyma, we evaluated the association of the observed CRPC with

medical conditions and risk factors for RCC (Table 5). Almost half of RCC patients reported history of hypertension in this study. In spite of variation in prevalence of hypertension across countries, hypertensive patients were equally distributed among CRPC categories ($P = 0.4$). Self-reporting of diabetes mellitus was recorded in 12.0% of patients. Similar proportion of our two CRPC groups did not report history of diabetes (73.0% vs. 70.0%). Although percentage of missing data for diabetes was significantly higher in none-mild category, there was a trend towards less cases with diabetes among moderate-severe CRPC ($P = 0.03$). NSAIDs users were defined as those who take NSAIDs at least once a week for a year. Both groups of CRPC showed similar proportion of non-users (68.5% vs. 70.6%), while missing data amongst advanced CRPCs was less than half of none-mild group (7.0% vs. 17.0%). After excluding missing data, NSAIDs use was less common among moderate-severe CRPC group than none-mild one ($P = 0.04$).

Table 6 shows ORs for association between country of recruitment and the CRPCs as outcome. Due to limited number of events in Serbia (with modest sample size), we pooled Serbia and Romania to achieve more stable estimate. Minimally adjusted model (including age, gender, and the percentage of medulla) showed that those living in Romania and Serbia experienced more than three-fold increased risk of moderate-severe CRPC comparing to those living in Russia or UK. Neighbouring geographic sites (Czech Republic) shows a 1.7-fold increase in risk, although this point estimate does not remain significant. Adjustment for tumor size, stage, history of hypertension, history of diabetes, and regular NSAIDs use did not materially change the estimate.

We compared our scoring with the standard approach from ISN. Two methods were highly correlated. Estimated diagnostic accuracy of grading used in this study with the gold standard was calculated based on 82% sensitivity (95% CI: 59.7%-94.8%), 100% specificity (95% CI: 87.2%-100%), and 0.91 area under ROC (95% CI: 0.83–0.99) (Table 7).

Discussion

Here we report the CRPC in the non-neoplastic kidney tissues of kidney cancer patients in different geographical sites across central and eastern Europe, Russia, and the UK, showing a three-fold increase in odds associated with living in Romania and Serbia.

Previous reports on the microscopic examination of non-neoplastic kidney tissue in cancer-related nephrectomies have shown that the pressure effect from the adjacent tumor causes pathologic morphological and clinical features while this effect reduces with distance. Azhar *et. al.* showed that the severity of pathological findings such as inflammation, nephrosclerosis, and glomerulosclerosis decrease from severe to mild at 1 mm to 5 mm distance from tumor [12]. Studies focused on functionality of adjacent-tumor non-neoplastic renal parenchyma shows glomerular viability reaching to 92% in 1.0 cm from tumor [13]. Additionally, renal tumors might produce a fibrotic pseudocapsule around themselves and pathological changes in the parenchyma have been reported in the outer aspect of pseudocapsules starting from tubules in the form of tubular atrophy, then progressively to interstitial fibrosis [14].

To address the possibility of tumor pressure effect being the origin of our observations, we first relied on our harmonized sample collection procedure in which the collection of non-neoplastic tissue distant from the tumor and from renal cortex was emphasized. Although the exact distance has not been recorded, the collection of non-neoplastic tissues from such a close distance to tumor seems unlikely. In addition, all non-neoplastic tissues were free of tumor in microscopic examination. On the other hand, adjusted models for confounders such as tumor size and type of nephrectomy did not change the initial risk estimates. These findings support the idea that the observed CRPC are not caused by the pressure effect from tumor and are most probably independent events.

Hypertension and diabetes mellitus are among the main clinical conditions that can lead to chronic parenchymal changes. We did not find any convincing morphological evidences to support the presence of hypertensive or diabetic nephropathies. Lack of hypertension and diabetes mellitus history were similarly reported in the both groups of patients with none-mild and moderate-severe CRPC. When adjusted for these factors, the odd estimate did not change.

The significant presence of CRPC in Romania and Serbia is of particular interest and might be relevant to renal carcinogenesis due to unknown exposures. In the 1950s, a progressive renal disease in the form of chronic interstitial nephritis was reported affecting the people living in the Balkan Peninsula along the tributaries of the Danube River in Bosnia and Herzegovina, Bulgaria, Croatia, Romania, and Serbia, so called Balkan endemic nephropathy (BEN). The similarity of morphological and clinical features of BEN and Chinese herbs nephropathy support the etiologic role of dietary exposure to aristolochic acid (AA), emphasizing that AA may not be the only factor causing this kind of nephropathy. AA nephropathy is associated with a high incidence of urothelial carcinoma of the upper urinary tract but its association with RCC is not reported [15–17]. We have previously published the results of whole genome sequencing of 94 RCC patients from the same collection that revealed a striking elevation of A:T:T:A transversions in 12 out of 14 RCC patients from Romania, a mutational pattern that is consistent with the exposure to AA [18]. Although the microscopic examination on non-neoplastic tissues of these patients did not show typical characteristics of BEN, exposure to AA was further studied by mass spectrometric measurements of the aristolactam DNA adduct 7-(deoxyadenosine- N^6 -yl) aristolactam I (dA-AL-I) in the 14 Romania patients as well as 15 patients from other countries. Vraibly high levels of AA DNA adducts, ranging from 0.7 to 27.0 adducts per 10^8 , were detected only in the 14 Romanian cases [19]. The non-neoplastic kidney tissues of eight out of the 14 above-mentioned Romanian patients were included in this study. Only two of them had focal and mild level of interstitial fibrosis and inflammation with AA DNA adduct level reported as 26.84 and 6.08 per 10^8 DNA bases, but no typical morphological evidence to support diagnosis of BEN. We observed the highest proporation (13.3%) of moderate-severe changes in Serbians, a finding that urges further exploration of the etiopathogenesis of kidney cancer in Serbia, currently unknown. Large collection of kidney cancer patients from Romania and Serbia will be analysed for discovery of mutational signatures caused by environmental exposures through our ongoing collaborative project, Mutographs of cancers (<https://www.mutographs.org>).

With our best knowledge, this study is unique to report pathological changes in non-neoplastic kidney tissues regarding to both sample size and geographical diversity of the patients from countries with variable incidence of RCC and with detailed clinico-epidemiological annotations. In addition, this is the first report relying on fresh frozen tissues for detailed microscopic examination. We believe that in the current context of a growing number of molecular studies based on the use of frozen tissues to optimize the quality of the high throughput technologies, it is critical to assess the reliability of morphological examination of frozen tissues. Our study demonstrates that at least some features, mainly tubulo-interstitial structures, can be adequately assessed on frozen tissues. The scoring method that we applied in this study is highly correlated to the proposed one by the ISN. Lower sensitivity in this method ensures us that our estimated risk might be even underestimated due to misclassification of true positive among CRPC negative category.

On the other hand, we are aware of the limitations of this study: 1) We used retrospectively collected material from multiple centres and it was impossible to get access to the diagnostic formalin-fixed paraffin embedded material. This limits our findings because some morphological and diagnostic indices are difficult to evaluate in frozen tissues due to freezing-induced artifacts and similarly, the application of ancillary diagnostic techniques and stainings as part of routine diagnostic practice of nephropathology, particularly to evaluate glomerular diseases, is not possible on frozen tissues, although the quality of our samples and images has been good enough to evaluate essential morphological features. Our data shows that IFTA are the main drive for the observed difference between the occurrence and severity of CRPC across study regions. Notably, presence of GS, that might be more difficult to precisely categorize in frozen tissues, did not add to the estimate of difference which indicates the probability of GS being secondary event following IFTA. 2) We acknowledge that the findings from the examination of one single piece of tissue might not be indicative of the status of the entire organ. This indicates that we might have missed some diagnostic morphological features. To overcome this possibility we categorized all of the mild CRPC with those with no significant pathological changes in one group to avoid any overestimation of the observations and provided our analyzes based on significant (moderate and severe) changes.

Conclusion

Our data shows that the frequency of CRPC in RCC cases varies across countries with significant occurrence in southeastern Europe. This is unlikely to be due to sampling method or pressure effect of the tumor alone. Instead, our findings suggest that the observed CRPC might be linked to some unknown or plausible environmental exposures leading to significant predominance of IFTA than glomerulopathies, and might be relevant to renal tumorigenesis in Romania and Serbia. This hypothesis deserves further investigations and dedicated study designs to answer the remaining questions about the source of these environmental exposures and their possible role in renal carcinogenesis.

Abbreviations

RCC: Renal cell carcinoma; ; IARC: International Agency for Research on Cancer; CAGEKID : Cancer Genomics of Kidney; H&E: Hematoxylin and eosin; GS: Glomerulosclerosis; IFTA: Interstitial fibrosis and tubular atrophy; CRPC: Chronic renal parenchymal changes; BMI: Body mass index; NSAIDs: Non-steroidal anti-inflammatory drugs; ISN: International society of nephrology; BEN: Balkan endemic nephropathy; AA: Aristolochic acid.

Declarations

Ethical approvals and consent to participate

Local and institutional review board approvals were obtained for individual case-control studies (Ref. IEC 09-24 & IEC 06-11). All recruited patients consented to participate in those studies with approval of using their data for future research studies.

Consent for publication

Not applicable

Availability of data and materials

The data of this study cannot be shared publicly due to presence of confidential participants' information. The data are available from the corresponding author upon reasonable request.

Competing interest

The authors declare no competing interests. Dr. Anne Y Warren reports personal fees from ROCHE, outside the submitted work.

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Authors' contributions

BAA designed the study, performed the microscopic examination, and wrote the main manuscript. LE, CP, AP, AYW, and DN are pathologists in the CAGEKID study and performed diagnosis of RCC cases. REB, NV, IH, LF, VJ, DM, ViJ, SM, MO, and SO provided the biological material and data from each contributing centers. JV created the CAGEKID pathology web-base facility. PB, GS, and REB conducted, managed, and harmonized the CAGRkid and K2 studies among centers. ML and YR provided and supervised the sequencing facilities . CC provided all pathology technical assistance for the entire CAGEKID and K2 studies. EC, JM, and all the authors reviewed the manuscript.

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Tables

Table 1. Demographic characteristics of patients with moderate-severe chronic renal parenchymal changes and otherwise in non-neoplastic kidney tissues

Chronic Renal Parenchymal Changes			
	None-mild	Moderate-severe	P value
	N=958	N=54	
Recruiting country			
Czech Republic	402 (42.0)	24 (44.4)	0.007
Romania	271 (28.3)	9 (16.7)	
UK	144 (15.0)	4 (7.4)	
Russia	115 (12.0)	13 (24.1)	
Serbia	26 (2.7)	4 (7.4)	
Sex			
Male	601 (62.7)	32 (59.3)	0.6
Female	357 (37.3)	22 (40.7)	
Age at diagnosis			
<55	238 (24.8)	6 (11.1)	0.02
55-59	162 (16.9)	5 (9.3)	
60-64	190 (19.8)	11 (20.4)	
65-69	156 (16.3)	13 (24.1)	
>70	212 (22.1)	19 (35.2)	
BMI at diagnosis			
<24.9	221 (23.1)	14 (25.9)	0.9
25.0-29.9	364 (38.0)	19 (35.2)	
30.0-34.9	224 (23.4)	14 (25.9)	
>35.0	88 (9.2)	6 (11.1)	
Missing	61 (6.4)	1 (1.8)	
Tobacco smoking status			
Never smoker	484 (50.5)	24 (44.4)	0.4
Ex-smoker	232 (24.2)	18 (33.3)	
Current smoker	233 (24.3)	12 (22.2)	
Missing	9 (0.9)	0 (0.0)	
Alcohol drinking status			
Never drinker	481 (50.2)	29 (53.7)	0.8
Ex- drinker	86 (9.0)	7 (13.0)	
Current drinker	241 (25.2)	14 (25.9)	
Missing	150 (15.6)	4 (7.4)	

Table 2 .Comparison of severity of chronic renal parenchymal changes in non-neoplastic kidney tissues with tumor type and recruitment country

None -mild						Moderate -severe					
Czech	Romania	Russia	Serbia	UK	Total	Czech	Romania	Russia	Serbia	UK	Total
321	231	93	18	144	807	19	8	10	2	4	43
42	16	4	6	0	68	3	1	1	1	0	6
39	24	18	2	0	83	2	0	2	1	0	5

Table 3. Odds ratio of observing four groups of parenchymal changes relevance to geographic residence of cancer patients (adjusted for age, gender and presence of inflammation)

Study region	None (reference)	Only glomerulosclerosis		Interstitial fibrosis or tubular atrophy (IFTA)		Both	
	N (%)	N (%)	OR (95%CI)	N (%)	OR (95%CI)	N (%)	OR (95%CI)
Russia	255 (31.7)	15 (15.8)	Reference	3 (8.6)	Reference	6 (7.7)	Reference
Czech Republic	303 (37.7)	56 (58.9)	2.41* (1.22-4.74)	22 (62.9)	4.38* (1.20-15.93)	45 (57.7)	4.29** (1.53-12.04)
Romania & Serbia	110 (13.7)	18 (18.9)	2.16 (0.95-4.88)	10 (28.6)	5.22* (1.30-20.98)	21 (26.9)	5.51** (1.77-17.10)
UK	136 (16.9)	6 (6.3)	0.50(0.17-1.48)	0 (0)	NA	6 (7.7)	1.03 (0.25-4.191)

* p<0.05, ** p<0.01

Table 4. Tumor and tissue variables among two categories of patients subdivided by presence/severity of chronic renal parenchymal changes in non-neoplastic kidney tissues

Chronic Renal Parenchymal Changes			
	None-mild	Moderate-severe	<i>P</i> value
	N=958	N=54	
Presence of medulla (%)			
0-9	687 (71.7)	41 (71.9)	0.9
10-30	154 (16.1)	8 (14.8)	
31-50	51 (5.3)	3 (5.6)	
>50	66 (6.9)	2 (3.7)	
Type of nephrectomy			
Radical	834 (87.1)	48 (88.9)	0.6
Partial	109 (11.4)	6 (11.1)	
Unknown/missing	15 (1.6)	0 (0.0)	
Stage at diagnosis			
I	430 (44.9)	18 (33.3)	0.6
II	99 (10.3)	7 (13.0)	
III	190 (19.8)	12 (22.2)	
IV	127 (13.3)	9 (16.7)	
Missing	112 (11.7)	8 (14.8)	
Tumor size (cm)			
T≤4	189 (19.7)	7 (13.0)	0.4
4<T≤7	373 (38.9)	26 (48.0)	
7<T≤10	221 (23.1)	11 (20.4)	
T>10	164 (17.1)	8(14.8)	
Missing	11 (1.0)	2 (3.7)	

Table 5. Past medical histories and NSAIDs use among two categories of chronic renal parenchymal changes in non-neoplastic kidney tissues

Chronic Renal Parenchymal Changes			
	None-mild	Moderate-severe	<i>P</i> value
	N=958	N=54	
History of hypertension			
No	466 (48.6)	23 (42.6)	0.4
Yes	480 (50.1)	31 (57.4)	
Missing	12 (1.2)	0 (0.0)	
History of diabetes			
No	700 (73.1)	38 (70.4)	0.04
Yes	109 (11.4)	12 (22.2)	
Missing	149 (15. 5)	4 (7.4)	
History of chronic renal disease			
No	728 (76.0)	44 (81.5)	0.2
Yes	26 (2.7)	3 (5.6)	
Missing	204 (21.3)	7 (13.0)	
History of renal cyst			
No	710 (74.1)	44 (81.5)	0.5
Yes	37 (3.9)	2 (3.7)	
Missing	211 (22.0)	8 (14.8)	
Regular NSAIDs user			
No	676 (70.6)	37 (68.5)	0.02
Yes	123 (12.8)	13 (24..1)	
Missing	159 (16.6)	4 (7.4)	

Table 6. Odds ratio and 95% confidence interval for association between country and observing moderate-severe chronic renal parenchymal changes in non-neoplastic kidney tissues of RCC patients

Recruiting country	Odds ratio (95% CI)			
	unadjusted	model 1*	model 2**	model 3***
Russia	Reference	Reference	Reference	Reference
UK	0.84 (0.25-2.76)	0.64 (0.19 - 2.16)	0.59 (0.17-2.01)	0.39 (0.10-1.49)
Czech Republic	1.79 (0.82-3.92)	1.34 (0.59-3.00)	1.54 (0.67-3.57)	1.28 (0.56-2.91)
Romania	3.40 (1.41-8.18)	3.12 (1.26-7.74)	3.16 (1.24-8.03)	2.67 (1.07 - 6.67)
Serbia	4.63 (1.33-16.08)	5.06 (1.39-18.44)	6.27 (1.40-28.02)	4.37 (1.20 - 15.96)
Romania and Serbia	3.62 (1.57-8.32)	3.42 (1.44 -8.12)	3.53 (1.45-8.58)	2.96 (1.24 - 7.03)

* adjusted for age, gender, percentage of medulla

**adjusted for age, gender, percentage of medulla, stage, tumor size

***adjusted for age, gender, diabetes, hypertension, NSAIDs use

Table 7. Comparison of scoring method applied in this study with the one proposed by the International Society of Nephrology

		Grading proposed by ISN			
		Mild	Moderate	Severe	Total
Grading used in this study	Mild	27	4	0	31
	Moderate	0	5	1	6
	Severe	0	3	9	12
	Total	27	12	10	49

Figures

Figure 1

Whole slide images of the observed chronic renal parenchymal changes (CRPC) from mild to severe: 1A- Mild CRPC: A few number of sclerotic glomeruli (arrows), mild interstitial fibrosis (arrow head) with focal, mild interstitial inflammation in a patient from Romania (5X digital magnification); 1B- Severe interstitial fibrosis and inflammation (stars), plus extensive glomerulosclerosis (arrows) and thick-wall vessels (arrow head) in a patient from Romania (4X digital magnification); 1C- Severe infiltration of interstitium by mononuclear inflammatory cells, interstitial fibrosis, and graded levels of glomerulosclerosis from periglomerular wall thickening (1) to partial (2) and full sclerosis (3) in a patient from Serbia (12X digital magnification); 1D- Interstitial fibrosis and severe tubular atrophy leading to thyroidization pattern (arrows) in a patient from Serbia (12X digital magnification).

Figure 2

Division of the tubular and glomerular chronic renal parenchymal changes by age categories.