Original Paper

Histopathological Study of Renal Cell Carcinoma

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ABSTRACT **Background.** Prognostic markers for renal cell carcinoma (RCC), such as tumor stage, grade, and necrosis or microvascular invasion are useful for determining appropriate follow-up and selecting patients for adjuvant therapy. Hence, the objective of the current study was to evaluate these pathological variables and also we attempted to establish possible correlations between them. **Patients and Methods.** Seventy-two patients from the Pathology Department of Emergency County Hospital no. 1 from Craiova, Romania, were included in this retrospective study. Age, sex, histologic subtype, pT stage, Fuhrman grade, tumor necrosis and microvascular invasion were determined in all cases. **Results.** Clear cell, papillary, chromophobe carcinomas and renal oncocytoma accounted for 53 (73.61%), 12 (16.66%), 3 (4.16%) and 3 (4.6%) cases, respectively. Fuhrman grade 2 and pT3 stage were predominant features, tumor necrosis was identified in one third of investigated tumors and microvascular invasion in 9 tumors (12.5%).**Conclusions.** Most tumors were clear cell type and had a predilection for G2 and pT3 categories, instead papillary form, the second as frequency, had higher values regarding grading and tumor necrosis, but lower stage. Tumor necrosis correlates with higher grade and tumor stage.

KEY WORDS *renal, carcinoma, clear, papillary, chromophobe, oncocytoma*

Introduction

Renal cell carcinoma (RCC) accounts for over 2.5% of all cancers [1], 2% of cancer mortality [2] and 95,000 deaths per year worldwide [3]. Around 208,500 new cases of kidney cancer are diagnosed in the world each year [4]. The peak incidence is in the sixth and seventh decades of life and the male to female ratio is 1.6:1.0 [5]. The highest rates are recorded in Northern America and the lowest rates in Asian and African regions [6]. Epidemiological studies have indicated that the worldwide and European annual increase in incidence of RCC is approximately 2% [7]. The lack of demonstrable efficacy of chemotherapy and radiation therapy in Stage IV RCC has led to a 5-year survival ranging from 5% to 10% [8, 9].

Moreover, RCC it is a heterogenous disease comprising different types with specific histopathological and genetic characteristics [10]. The four main subtypes of RCC, as defined by the Heidelberg classification system, are clear-cell, papillary, chromophobe and collecting-duct [10]. In addition, the 2004 World Health Organization (WHO) classification of RCC, also include medullary carcinoma, multilocular cystic RCC, mucinous tubular and spindle cell carcinoma, and unclassified categories [11, 12]. The Mainz classification of renal cell tumors also includes renal oncocytoma [13]. The clear cell type is the most common adult RCC, representing 70% of all RCCs. Papillary RCC accounts for 10%– 15%, chromophobe RCC for 4%–6%, renal oncocytoma for 5%, and unclassified lesions for 4%–5% of RCCs.

In our study we aimed to evaluate the pathological variables, such as histologic subtype, tumor grade, pT classification, tumor necrosis, resection margins status and microvascular invasion in a series of consecutive patients with renal cell cancer. We also attempted to establish possible correlations between these variables.

Materials and Methods

This was a retrospective study made on renal cell carcinoma diagnosed in the Pathology Department of Emergency County Hospital no. 1 from Craiova, Romania, between 2004 and 2008. Surgical specimens from patients who underwent radical nephrectomy for renal tumors provided tissue samples. These were processed by classical technique of formalin fixation and paraffin embedding, followed by serial sections Haematoxylin-eosin standard staining.

The clinicopathologic features including sex, age, tumor size, histologic subtype, growth pattern, nuclear grade, tumor stages, resection margins status, tumor necrosis, and microvascular invasion were evaluated by reviewing the medical records, pathologic slides and reports. Histologic subtypes were classified based upon the 2004 WHO classification [12], tumor staging was based

on the 2002 AJCC staging system [14], and nuclear grading was based on the Fuhrman system [15]. The image acquisition was performed with a Nikon Eclipse 600 microscope and a Nikon DS200 camera, using Lucia 5 acquisition software. Resulting data registering and analysis were performed with Microsoft Excel software (Microsoft Office package, version 2007).

Results

Our study consisted out of 72 consecutive RCCs, accounting for 2% of urologic pathology examined and diagnosed in the Pathology Department between 2004 and 2008.

Forty-three (59.72%) patients were male, and 29 (40.28%) were female (ratio 1.48:1). Regarding patients age distribution, the peak incidence of renal carcinomas was seen in 60-69 age group consisting out of 41 (56.94%) patients, followed by 16 (22.22%) patients in 50-59 age group. Patients were between 38 and 76 years of age, with a mean age of 60.79.

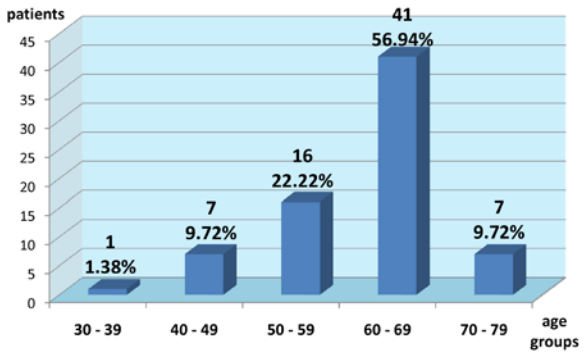

Figure 1. Age distribution of patients with RCC showing a peak incidence in 60-69 years age group.

Table 1. summarizes the tumor histology. Of the patients studied, the clear cell RCCs variant was seen in 53 (73.61%). Many tumors demonstrated a predominant compact alveolar architecture (22 of 53 clear cell type RCCs, 41.5%). Other growth patterns one could observe, were solid in 17 cases, acinary in 10, and mixed patterns for the rest. We also observed tubular and cystic areas, or focal papillary architecture associated with the alveolar pattern. Most clear cell RCC contained numerous capillaries and thin-walled blood vessels in the supporting stroma - a helpful diagnostic feature. Due to its rich content in lipids and glycogen, which dissolved during processing, the cytoplasm of many tumor cells exhibited characteristic clear appearance. Scattered tumor cells with eosinophilic granular cytoplasm, especially near necrosis, it was not an uncommon observation. Tumor cells exhibited round and centrally placed nuclei. Nuclear pleomorphism was variable depending on tumor grade. One clear cell RCC underwent sarcomatoid differentiation, defined as areas of spindle cell proliferations that had the histologic appearance of a sarcoma.

Table 1: Tumor histology on studied renal cell carcinomas

Tumor type	Clear cell RCC	Papillary RCC	Chromophobe RCC	Renal oncocytoma	Mixed – oncocytic and chromophobe – RCC
Number of patients	53	12	3	3	1
Percents %	73,61%	16,66%	4,16%	4,16%	1,38%

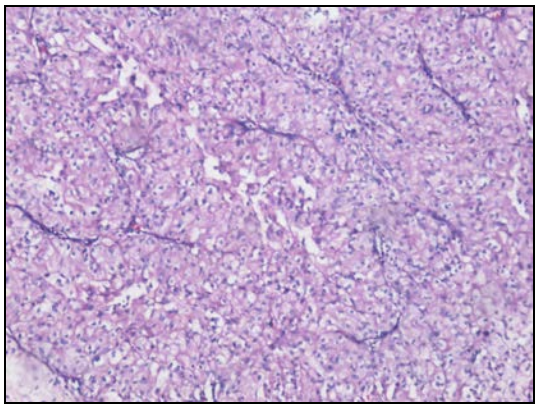

Figure 2. Clear cell RCC. Well demarqued tumoral cells exhibited characteristic clear cytoplasm and centrally placed nuclei. Thin-walled blood vessels are apparent in the stroma. Hematoxillin and eosin stain, X 100.

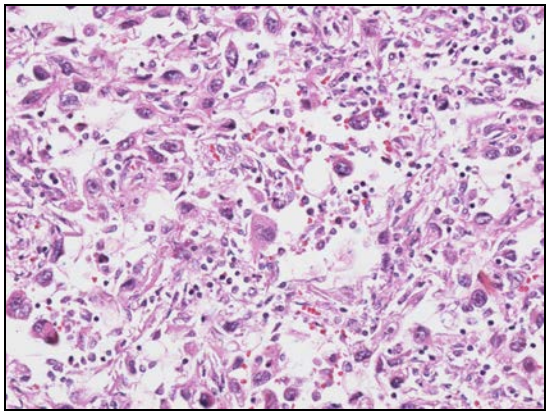

Figure 3. Sarcomatoid dedifferentiation area in a clear cell RCC. Note pleomorphism and nuclear atypia. Hematoxillin and eosin stain, X 200.

Papillary RCC was seen in 12 (16.66%) patients. The papillae were made of thin fibrovascular cores covered by cuboidal tumor cells, which might demonstrate complex branching and contain aggregates of foamy macrophages. Tumor cells vary from small size with scanty cytoplasm and large nuclei resulting in high N:C ratio (the *basophilic variant*) to large tumor cells with abundant eosinophilic and granular cytoplasm (the *eosinophilic variant*). The nuclei of tumor cells are usually round and uniform and without nucleoli. Occasionally, tumor cell nuclei may be pleomorphic and with prominent nucleoli, depending on the grade of the tumor. The same grading and staging systems proposed for clear cell RCC are recommended for chromophil RCC.

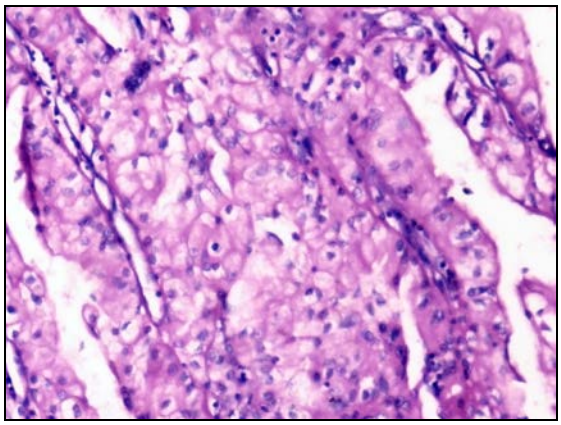

Figure 4. Papillary renal cell RCC. Thin fibrovascular cores covered by tumor cells. Hematoxillin and eosin stain, X 400.

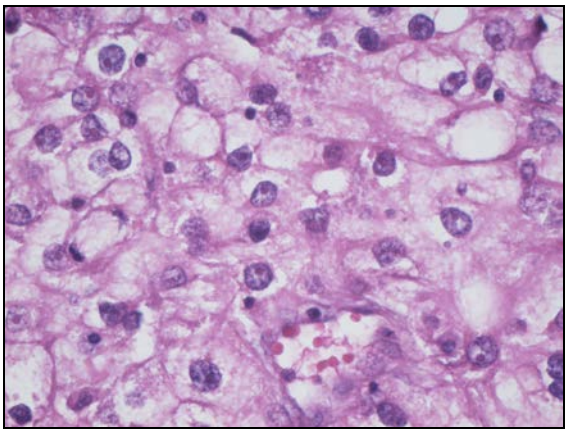

Figure 5. Cromophobe renal cell RCC. Hematoxillin and eosin stain, X 400.

The chromophobe RCCs variant was seen in 3 (4.16%) of the patients studied. Those usually had a solid growth pattern, but could show some foci of tubular, and alveolar areas. Neoplastic cells showed an abundant and pale or eosinophilic cytoplasm. A narrow zone of cytoplasmic condensation was frequently found along the cell periphery. The nuclei were centrally placed, and some were binucleated. Occasionally, one could see mitotic activity (Fig. 4). Areas of necrosis, hemorrhage, and fibrosis could be identified.

Renal oncocytoma was encountered in 3 patients (4.16%). The histology exhibited very characteristic features. Strongly eosinophilic tumor cells forming islands and tubules dominated throughout the tumor. Tumor cells exhibited large and finely granular cytoplasm, uniform round nuclei, clumped chromatin and small nucleoli (Fig. 5). Bizarre, enlarged nuclei may be scattered throughout the tumor, but mitoses are rare.

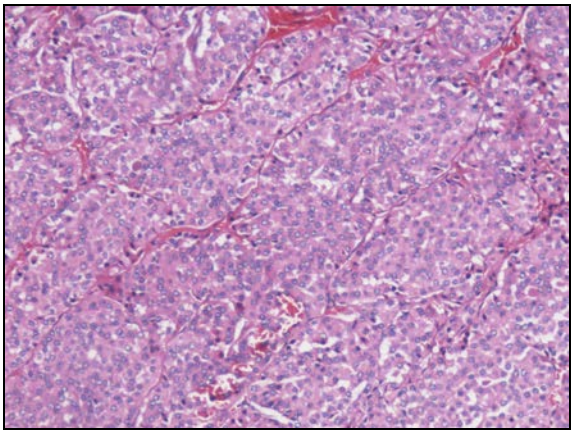

Figure 6. Renal oncocytoma. Hematoxillin and eosin stain, X 200.

We also encountered one case of mixed (hybrid) renal carcinoma – oncocytic and chromophobe type.

As regards tumor grade, we observed 4 tumors (5.55%) with nuclear grade one, according to Fuhrman grading system, 43 tumors (59.72%) corresponding to G2 criteria, 23 (31.95%) tumors with G3 and 2 (2.77%) in G4. Among the 12 patients with papillary variant, Fuhrmann grade 3 was seen in 7 tumors (58.3% of papillary renal cell carcinomas), while 37 of 53 clear cell type (69.81% of clear cell variant) were G2 tumors.

Table 2: Tumor grade, according to Fuhrman grading system (see ref. abi)

Tumor grade	Grade I	Grade II	Grade III	Grade IV
Number of patients	4	43	23	2
Percents %	5.55%	59.72%	31.95%	2.77%

Owing to inconstant disposable clinical data regarding metastases (M) status, a rigorous classification on **pTNM** stage could not be achieved for all the patients included in our study. However, we attempted to characterize the tumors distribution according to **pT** stage. We noted 30 (41.66%) records corresponding to pT3 stage, 26 (36.11%) to pT1, and 16 (22.22%) to pT2. No

records on pT4 stage were noted. The pT distribution in patients with clear cell carcinoma was as follows: 18 pT1 tumors (33.96%), 12 pT2 tumors (22.64%) and 23 pT3 (43.39%). As regards papillary variant, 5 tumors were pT1 (41.66% of papillary renal cell carcinoma in our study), 3 were pT2 (25%), and 4 tumors were pT3 (33.33%).

Resection margins status, tumor necrosis and microvascular invasion were also evaluated. There were 22 (30.55%) cases in which tumor necrosis was present, from which 13 in clear cell variant. Tumor necrosis was more present in stage pT3 (n=15, 50%) than pT1 and 2, and also in G3 and 4 (n=10, 40%). 9 tumors (12.5%) had microvascular invasion evidence in our study.

Discussion

RCC is the most common malignancy of the human kidney, with a variable outcome. Currently, tumor stage, size, RCC subtype, and nuclear grade are widely accepted as important pathologic prognostic indicators for RCC [16].

The incidence of RCC in our series was higher in the seventh decade of life, with a mean age of 60.79. This value was slightly higher than those of previous reports and reviews [17]. The male to female ratio of 1.48:1 seen in our casuistry is close to that of referenced studies [7].

RCC histologic subtype is the first parameter taken into account for pathologic evaluation. The main histologic subtype encountered in our casuistry was clear cell carcinoma. We also found similar results as seen in referenced reports and reviews regarding papillary and cromophobe forms and renal oncocitoma. Clear cell RCC is the most common adult RCC, representing 70% of all RCCs. Papillary RCC accounts for 10%– 15%, chromophobe RCC for 4%–6%, renal oncocytoma for 5%, and unclassified lesions for 4%–5% of RCCs. Histologic subtyping have a clinical relevance for patients outcome. Clear cell RCC have a less favorable prognosis (stage for stage) than do papillary RCC and chromophobe RCC. Sarcomatoid dedifferentiation that is thought to represent the high-grade end of all subtypes, noted in one case in our casuistry, occurs in 5% of clear cell RCC cases and implies a poor outcome, associated with reduced response to immunotherapy treatments [11, 17, 18]. Although each of the histological variants displays a spectrum of clinical behavior, conventional carcinomas tend to behave aggressively, while chromophobe carcinomas and papillary carcinomas follow a more indolent clinical course [19]. Each histological variant of renal cell carcinoma shows distinct karyotypic abnormalities including loss of chromosome 3p in clear cell carcinomas [20] and trisomy of chromosomes 7, 12, 16, 17, and 20 in papillary carcinomas [21].

More than half of all investigated cases were G2 tumors in our series. Regarding the distribution of this parameter we found inconclusive results in several reports [22, 23]. There was a significant difference in nuclear grade between clear cell type, with predominant G2 tumors, and papillary tumors, predominantly G3, although its statistical significance is difficult to assess owing to few number of patients in papillary carcinoma group. The Fuhrman nuclear grading system is based on tumor-cell nuclear characteristics – size, contour and nucleoli. The tumor grading of clear-cell carcinomas is a well documented and well recognized independent prognostic tool.

The evaluation of pT distribution showed most cases in pT3 stage in our casuistry. We found a similar distribution pattern in clear cell carcinoma group. However, papillary variant revealed predominance of pT1 tumors in these patients. A large, multicenter study [24] showed similar results for pT distribution according to histologic subtypes. Instead, the general distribution of pT stage in our study did not matched with the results on overall distribution of pT stage in the same multicenter study, revealing slightly higher values in pT2 and pT3 stages in our casuistry. Currently, the most extensively used prognostic tool for RCC is the Tumor, Nodes and Metastasis (TNM) staging system, and a number of studies have shown the system to be accurate in reflecting RCC prognosis. However, a number of recent studies have debated the prognostic accuracy of using the TNM system for RCC staging, in particular in relation to prognoses of locally advanced renal cell carcinoma (pT3–T4 RCC) [25]. Recent studies reported a relative risk of death over 10 times higher in pT3-T4 stages than pT1 [26].

One third of investigated tumors showed the presence of necrosis in our study. Necrosis had higher values in papillary form when compared with its presence in clear cell tumors, more specific almost half of papillary tumors, respectively ¼ in clear cell tumors. Also, we demonstrate that RCC tumors with coagulative necrosis tend to exhibit other adverse pathologic features, including high nuclear grade and advanced tumor stage compared with tumors without necrosis. These results are comparable with those of other studies [22]. Our study also revealed that tumor necrosis correlates with higher grade and tumor stage. The presence of

histological necrosis has been shown to be an adverse prognostic feature of clear-cell carcinomas. It has been shown in a recent study to confer a two- to three-fold higher risk of death from RCC than in patients with no tumour necrosis [25, 26]. Histologic coagulative tumor necrosis is an independent predictor for clear cell and chromophobe RCC outcome, and it should be routinely reported and used in clinical assessment [22].

Microvascular invasion is a reliable prognostic factor with a high risk for the development of metastatic disease when demonstrated [27]. We noted a prevalence of 12.5% in our study, lower than the results of other groups. However, the difference may be explained by the detection method used for microvascular invasion from our study (classical hematoxilin and eosine routine examination) to others (using Factor VIII and / or CD34 staining for this purpose).

One limitation of the current study is that the few number of patients in cromophobe carcinoma and renal oncocitoma impeded us to establish correlations for these groups of patients, either for grading, pT stage and necrosis or microvascular invasion status.

Conclusions

RCC consists of a heterogeneous group of malignant neoplasms that have characteristic histopathologic features and biologic behavior. By far, the main histologic subtype encountered in our casuistry was clear cell carcinoma. We also found more than half G2 tumors, most cases in pT3 stage and necrosis in one third of evaluated tumors. Different histologic subtypes have different predilections for specific pathologic features, such as pT stage, Fuhrman grade or tumor necrosis. Clear cell tumors were established more often in G2 and pT3 categories, instead papillary form had higher values regarding grading and tumor necrosis, but lower stage. Our study also revealed that tumor necrosis correlates with higher grade and tumor stage.

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