# RENAL CELL CARCINOMA DIAGNOSED BY THE DEPARTMENT OF ANATOMICAL PATHOLOGY AT THE UNIVERSITY OF THE FREE STATE, SOUTH AFRICA: A 10 YEAR HISTOPATHOLOGICAL REVIEW

by

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in the

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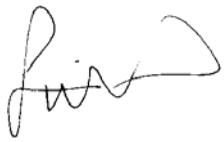
**DECLARATION**

I, Louis Johannes Muller, declare that the coursework Master's Degree mini-dissertation that I herewith submit in a publishable manuscript format for the Master's Degree qualification in Anatomical Pathology at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.



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**Louis Johannes Muller** **Date**

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**ABSTRACT**

**Background:** Globally, the incidence of renal cell carcinoma varies widely between populations and geographic areas, with the lowest incidence reported in Africa. Very little information is available on the epidemiology or histopathological profile of renal cell carcinoma (RCC) in the South African population, and most studies on RCC in Africa are from Nigeria. The determination of the incidence, demographic and histopathological features of RCC would provide the latest and most up to date information on RCC epidemiology in the state sector in central South Africa and would serve as a foundation for future research.

**Aim:** The purpose of this study was determine the number of cases of RCC seen over a ten year period by the Department of Anatomical Pathology, University of the Free State and National Health Laboratory Service and to describe the demographic profile of the patients identified as well as the histological spectrum. We further aimed to determine the age-standardised incidence rate (ASR) of RCC for state sector patients from the Free State Province.

**Methods:** A retrospective descriptive review with an analytical component was performed. All histologically confirmed cases of RCC identified between 1 January 2005 and 31 December 2014 were included in the study. The pathology reports were reviewed to collect the demographic information for each case and the H&E slides were reviewed to confirm the diagnosis and the specific histological subtype. ASRs were calculated using the population data from the South African census as performed in 2011. ASRs were only calculated for the Free State Province and patients from the North West and Northern Cape Provinces were excluded for these calculations as the department does not receive all the specimens from these two provinces.

**Results:** A total of 105 patients with RCC were diagnosed with a male:female ratio of 1.2:1 and a mean age of 55.1 years. The age standardized incidence rate for the Free State province was 0.4 per 100 000 population. Ten patients (9.5%) were younger than 40 years. The majority of cases were identified in the age group 50 to 59 years (33.3%). The majority of patients (58.1%) were black and they were found to present on average a decade earlier than white patients. The most common histological subtype was clear cell RCC (58.1%). Patients diagnosed with papillary RCC were found to be significantly

more likely to be male than female (72.7% vs 27.3%; p=0.03) and were also more likely to be black than white (81.8% vs 13.6%; p<0.01). Patients with chromophobe RCC were more likely to be female (80%) and black (60%). Two cases of a new entity, clear cell RCC with giant cells and emperipolesis were identified. All five patients with MiT family translocation RCC were diagnosed in black females.

**Conclusion:** To our knowledge this is the most comprehensive study on RCC performed in our region. Our study demonstrated that the incidence of RCC in our population is lower than reported in the rest of the world. The age distribution correlates closely with other African studies, with black patients presenting a decade earlier than white patients. In addition, our findings identified distinct age, sex and racial differences for the various RCC subtypes, which warrants further research.

**Keywords:**

Renal cell carcinoma, Epidemiology, Histological type, Incidence, Central South Africa

## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| ALK | - | Anaplastic lymphoma kinase |
| AMPK | - | Adenosine monophosphate-activated protein kinase |
| ASR | - | Age-standardized incidence rate |
| BHD | - | Birt-Hogg-Dubé |
| ccRCC | - | Clear cell renal cell carcinoma |
| DNA | - | Deoxyribonucleic acid |
| FH | - | Fumarate hydratase |
| H&E | - | Hematoxylin and eosin |
| HIF-α | - | Hypoxia-inducible factor-α |
| HIV | - | Human immunodeficiency virus |
| HPRC | - | Hereditary papillary renal cell carcinoma |
| HPT-JT | - | Hereditary hyperparathyroidism-jaw tumour syndrome |
| ISUP | - | International Society of Urological Pathology |
| JARID1C | - | Jumonji AT-rich interactive domain 1C |
| MET | - | Mesenchymal-epithelial transition |
| MiT | - | Microphthalmia transcription |
| mTOR | - | Mammalian target of rapamycin |
| NCR | - | National Cancer Registry |
| NHLS | - | National Health Laboratory Service |
| NSAIDs | - | Nonsteroidal anti-inflammatory drugs |
| pRCC | - | Papillary renal cell carcinoma |
| RCC | - | Renal cell carcinoma/s |
| SDH | - | Succinate dehydrogenase complex |
| SDHA | - | Succinate dehydrogenase complex Subunit A |
| SDHB | - | Succinate dehydrogenase complex Subunit B |
| SDHC | - | Succinate dehydrogenase complex Subunit C |
| SDHD | - | Succinate dehydrogenase complex Subunit D |
| SWI/SNF | - | Switch/Sucrose Nonfermentable |
| TSC | - | Tuberous sclerosis complex |
| UFS | - | University of the Free State |
| US | - | United States of America |
| VHL | - | Von Hippel-Lindau |
| WHO | - | World Health Organization |

| | | |
|---|---|---|
| 2SC | - | S-(2-succino) cysteine |

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# CHAPTER 1

# LITERATURE REVIEW

## INTRODUCTION

Renal cell carcinoma (RCC) represents a group of neoplasms having a common origin from the epithelium of the renal tubules. The different subtypes comprise a variety of distinct clinicopathological entities, each of which display different morphological, immunohistochemical and molecular features. In the past decade, advances in the understanding of these features led to an expansion in the number of distinct tumour entities that are currently recognized.

### Epidemiology

Approximately 2% to 3% of all new cancer cases worldwide originate in the kidney [1]. The incidence has been increasing in many countries with approximately 70% of new cases occurring in countries with high and very high levels of socioeconomic development [2]. Globally, the incidence of renal cell carcinoma varies widely. The highest incidence rates are found in Europe, North America and Australia and the lowest rates are found in East Asian and African countries [2]. These differences could be explained by possible under-diagnosis and/or under-reporting, which again could be explained by poor socioeconomic development in African and Asian countries. However, differences in environmental and genetic risk factors most likely also contribute to the variation in incidence [3–5].

**Gender** - RCC is more common in men with a male:female ratio of approximately 1.5:1 [1]. The worldwide age-standardized rate (ASR) for men is 6.0/100 000 and 3.1/100 000 for women. Globocan 2012, however, has shown that the incidence has been increasing more rapidly in women than in men [6, 7]. Data from studies done in Nigeria have even shown a reversal in incidence with a female predominance with a male: female ratio varying between 1:1.2 to as much as 1:2.8 [8].

**Age** – The incidence of RCC is the highest in the sixth to eighth decades of life, with only 5% of cases being reported in patients under 40 years of age [9]. Multiple studies performed in Africa and the Middle East consistently reported a younger peak age incidence in the fifth

decade [5, 8, 10]. Claassen *et al.* from South Africa, reported a mean age of 54.2 years in black patients compared to a mean age of 70 years in white patients [11].

**Race** – When looking at race, one has to keep in mind the country or region's population breakdown with regards to race. Globally RCC incidence has previously been reported to be very similar when comparing black and white patients [12]. The reported incidence of RCC in Africa is relatively low, which can be due to many variables, such as lack of healthcare as well as underreporting. Studies done in the United States such as those by Lipworth *et al.* and Chow *et al.* found that RCC incidence was significantly higher in the black population compared to the white population[12, 13]. They also found that over the past 3 decades, the incidence rates for black Americans have been rising more rapidly than for white Americans. Furthermore, it has been reported that RCC incidence in Asian patients is much lower compared to white patients, as found in studies done in Asian countries and the United States [6, 14]

**Mortality** – Mortality patterns follow the incidence of RCC. However, RCC incidence has risen three times higher than the mortality rate, with stabilized mortality trends achieved in some developed countries, but not in low-income countries. This is probably due to improved screening and therefore earlier detection of the tumours and more effective treatment strategies. Incidence:mortality ratios were the lowest in African countries and the highest in North America, suggesting a higher survival rate in the latter region [15].

## Risk factors

Currently, the only well-established risk factors are male sex, black race, cigarette smoking and obesity [16–20]. Other factors associated with an increased risk are: end-stage renal disease/acquired cystic kidney disease, exposure to asbestos, petroleum products and heavy metals, hypertension and/or its treatment, cytotoxic chemotherapy, chronic hepatitis C infection, sickle cell disease and kidney stones [21–28]. Factors that may increase the risk for RCC are nitrite intake from processed meat, reproductive factors (e.g. multiple pregnancies) and prior radiation therapy [23, 29, 30].

No evidence has been found thus far to establish a significant relationship between the occurrence of RCC and the consumption of alcohol and coffee. Studies by Gupta *et al*, and Belloco *et al*, have actually found alcohol to be associated with a protective effect on the risk

of RCC [31, 32]. The evidence linking analgesic use with RCC has thus far been contradictory in many studies. Cho *et al.* reported an increased risk for RCC with use of nonsteroidal anti-inflammatory drugs (NSAIDs), but aspirin and acetaminophen (paracetamol) use was not associated with the development of RCC [33]. In contrast, Karami *et al.* found that acetaminophen use increased the risk of developing RCC, but that aspirin and NSAIDs do not [34].

## Genetics

Most RCCs are sporadic, however, 2-4% are associated with multiple specific inherited cancer syndromes [2]. The chance of developing RCC is two to three times higher in someone who has a first-degree relative with RCC [35]. Features that favour a hereditary contribution in patients without a clear familial syndrome include first degree relatives with a tumour, onset before the age of 40 years, and bilateral or multifocal disease [36]. Inherited polycystic kidney disease may be a high-risk factor for RCC [25].

## Pathogenesis

Various molecular abnormalities are involved in the pathogenesis of RCC.

**1. The hypoxia-inducible factors pathway and the von Hippel-Lindau (VHL) syndrome** – VHL disease is caused by a germline mutation in the *VHL* tumour-suppressor gene, which is situated on chromosome 3p25-26. The VHL protein's role is to assist in the degradation of hypoxia-inducible factor-α (HIF-α). With loss-of-function variants, the HIF-α accumulates and activates expression of many HIF's, each of which plays an important role in tumour genesis [37]. Variants of the *VHL* gene were also detected in 70% of sporadic RCCs [38].

**2. The mesenchymal-epithelial transition (MET) pathway and hereditary papillary renal cell carcinoma (HPRC)** - The *MET* gene is located on chromosome 7q31 and corresponds to the *HPRC* gene. *MET* is a proto-oncogene, with gain-off-function variants resulting in successive activation of the receptor [37].

**3. The fumarate hydratase gene (FH) and the hereditary leiomyomatosis renal cell carcinoma** – Germline variants in the *FH gene*, situated on chromosome 1q42 are found in

patients with hereditary leiomyomatosis RCC [39]. The *FH* gene is a tumour suppressor gene, thus loss-function mutations lead to decreased levels of FH and an increase in fumarate, which causes HIF-α level stabilization [39].

**4. Birt-Hogg-Dubé (BHD) syndrome** - is caused by a germ-line loss-of-function variant in the *folliculin gene* (FLCN) on chromosome 17p11.2[37]. The product of the *FLCN* gene is folliculin, a tumour-suppressor protein that is involved in the control of adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) signalling pathways [37]. mTOR is a multifunctional serine-threonine kinase that plays an important role in the regulation of cellular activities.

**5. The phosphatidylinositol-3-kinase/Akt pathway –** Alterations in the phosphatidylinositol-3-kinase/Akt pathway are known to be associated in the process of oncogenesis of numerous carcinomas [40].

**6. *SDHB*-associated hereditary paraganglioma/phaeochromocytoma** – is characterised by the germ-line variants in one of the succinate dehydrogenase (SDH) genes, *SDHB*, *SDHA* or *SDHC* [41]. Deactivation of the SDH-enzyme complex leads to the disablement of oxidative phosphorylation and to a dependence on glycolysis for energy production. Renal cell carcinomas were discovered as another extraparaganglial tumour associated with *SDHB*-associated hereditary paraganglioma [42].

**7. Hereditary hyperparathyroidism-jaw tumour syndrome (HPT-JT)** – RCC is only one of several renal manifestations of this syndrome. The *HPT-JT* gene functions as a tumour suppressor gene [37]. Thus a mutation of this gene leads to a decrease in parafibromin, which plays a role in inhibiting the *c-MYC* proto-oncogene [37].

**8. Translocation renal cell carcinomas** – are associated with gene fusions in microphthalmia transcription (MiT) family transcription factors, *TFE3* and *TFEB*. The *TFE3* gene on locus Xp11.2 is the most common gene involved, while RCCs with the t(6;11) translocation are less common and have the *MALAT1-TFEB* gene fusion [43].

**9. Other genetic aberrations**

- The tuberous sclerosis complex (TSC) is associated with inactivating variants of *TSC1 (hamartin)* or *TSC2* (tuberin) that causes inhibition of mTOR, that ultimately causes an increased risk for RCCs in these patients. [44, 45].
- Fifteen percent of clear cell RCCs show evidence of a mutated *BRCA1* associated protein-1 (*BAP1*) gene, located at 3p [46].
- *PBRM1* (polybromo1), is another tumour suppressor gene that was discovered as another major gene involved in the pathogenesis of RCC [47].

**10. Epigenetic alterations in renal cell carcinoma –** These include DNA hypermethylation and histone modifications. Two genes have been identified in RCC that are involved in histone modification namely the SET domain-containing protein 2 (*SETD2*) and the Jumonji AT-rich interactive domain 1C (*JARID1C*) [48].

## Pathology of renal cell carcinomas

## Classification of renal cell carcinomas

As the histological classification of RCC has developed, the terminology used for designating its subtypes has referred to various descriptive or characteristic features like cell type, growth pattern, predominant cytoplasmic features, staining characteristics, architectural features and/or a combination of these features. More recently the molecular basis of the different types of RCCs has been included to more accurately classify the different subtypes. The classification and evolution of RCC subtypes are summarized in Table 1 [2, 49–51].

The classification of renal cell carcinomas is based on correlative cytogenetic, genetic and histological studies of both familial and sporadic tumours. Clear cell RCC (ccRCC) is the most common histological subtype (65-70%), followed by papillary RCC (pRCC) (10-15%) and chromophobe RCC (5-7%) [2, 15].

**Table 1: Classification of renal cell carcinomas - timeline**

| **1997 – Heidelberg classification** [50] | **2004 – WHO classification**[51] | **2016 – WHO classification** [2] | **New and emerging RCCs – post-WHO 2016 classification:** [49] |
|---|---|---|---|
| • Conventional RCC<br>• Papillary RCC<br>• Chromophobe RCC<br>• Collecting duct carcinoma<br>• Medullary carcinoma<br>• Unclassifiable | • Clear cell RCC*<br>• Multilocular clear cell RCC#<br>• Papillary RCC *<br>• Chromophobe RCC*<br>• Carcinoma of the collecting ducts of Bellini*<br>• Renal medullary carcinoma*<br>• Renal carcinoma associated with Xp11.2 translocations (MiT family translocation)*<br>• Carcinoma associated with neuroblastoma<br>• Mucinous tubular and spindle cell carcinoma*<br>• RCC unclassified* | • *As classified in 2004<br># Multilocular clear cell RCC is now classified as multilocular cystic renal neoplasm of low malignant potential<br><br>**Newly added entities**<br>• Tubulocystic RCC<br>• Acquired cystic disease-associated RCC<br>• Clear cell papillary RCC<br>• Hereditary leiomyomatosis and RCC-associated renal cell carcinoma<br>• Succinate dehydrogenase-deficient RCC | • Thyroid-like follicular carcinoma of the kidney<br>• Anaplastic lymphoma kinase rearrangement-associated RCC<br>• RCC with prominent smooth muscle stroma<br>• Fumarate hydratase deficient RCC<br>• Biphasic squamoid papillary RCC<br>• Eosinophilic solid and cystic RCC<br>• Atrophic kidney-like RCC<br>• Clear cell RCC with giant cells and emperipolesis<br>• Warthin-like papillary RCC<br>• Low-grade oncocytic tumour – CD117 negative and CK7 positive |

## Morphological features

On macroscopic examination, most RCCs are well-circumscribed and centred in the cortex and can involve the venous structures and renal sinus. Satellite nodules and areas of cystic change are also common, while calcification, necrosis and haemorrhage may be present. On cut section of a typical case of RCC, most tumours have a fibrous pseudocapsule separating the tumour from the surrounding normal tissue.

**Clear cell RCC** (Figure 1) — is mostly solid with a golden yellow colour but can also be cystic. They are usually unilateral and unicentric with an expansile appearance and clear demarcation from the normal kidney. Microscopically, ccRCC displays a variety of growth patterns. The most common pattern being solid with nests, alveolar and acinar structures, separated by a network of blood vessels. The classic clear cells of ccRCC are large with prominent cell membranes and clear cytoplasm. The cytoplasm may, however, range from clear to eosinophilic and even granular appearance. The nuclei can show variable pleomorphism with prominent nucleoli, but are mostly centrally located [52].

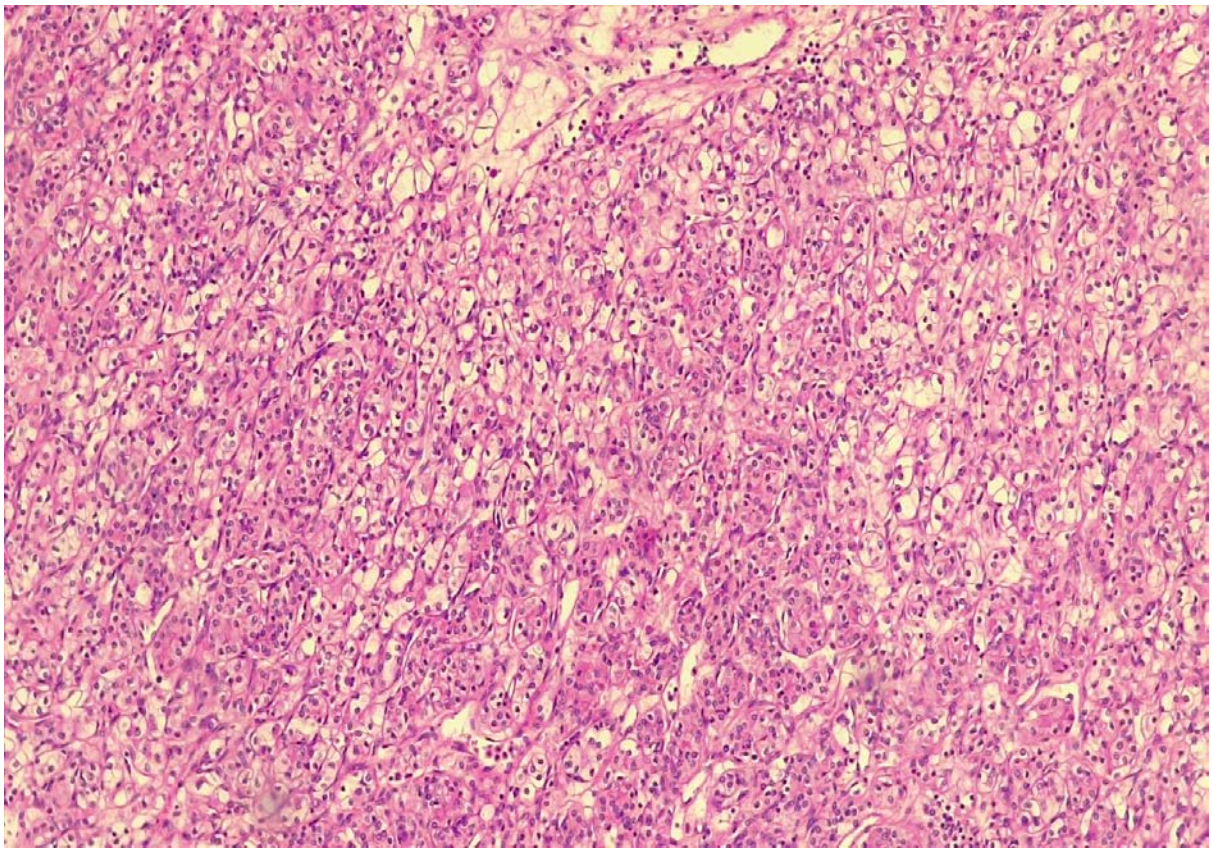


**Figure 1.** H&E image of a clear cell RCC (10x magnification)

**Papillary RCC** — Macroscopically, they are typically well-circumscribed and mostly confined to the renal parenchyma [53]. They may be multifocal, particularly in cases of hereditary pRCC [54]. In most cases, they also have a thick pseudocapsule [55]. Microscopically they have mixture of papillary and tubular structures [55]. Papillary RCC can be sub-classified into two morphologic variants [56]:

- Type 1 (Figure 2) - is composed of complex to solid papillae lined by a single layer of small cuboidal epithelial cells with scant pale cytoplasm. The nuclear grade varies but is often low grade. The papillary structures often has an infiltrate of foamy macrophages or neutrophils [57].

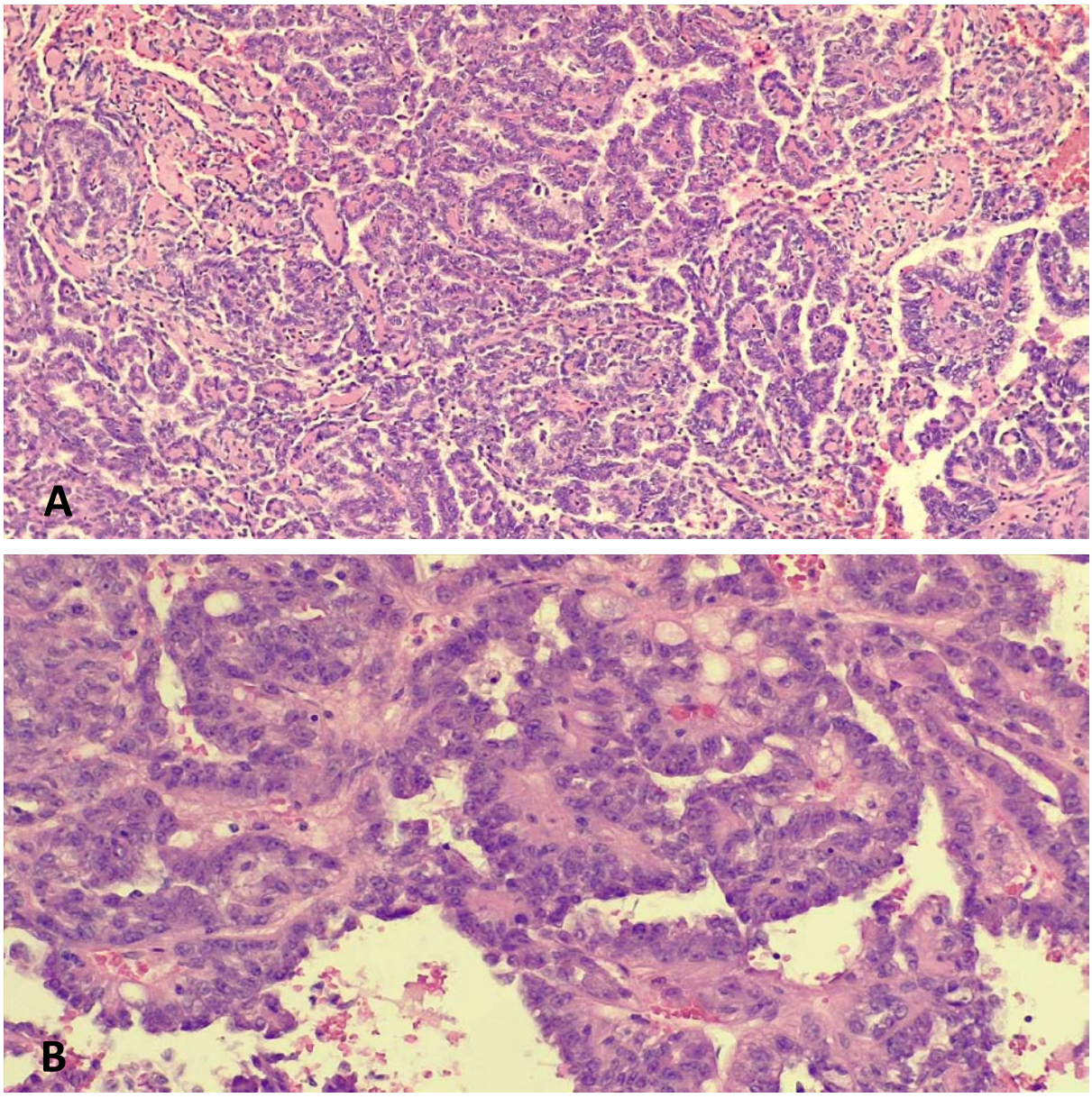



**Figure 2.** H&E images of a type 1 papillary RCC (A) 10x, (B) 20x magnification

- Type 2 (Figure 3) – the tumour cells lining the papillae are more pseudostratified and larger with abundant eosinophilic cytoplasm. The nuclei are of higher grade, at least International Society of Urological Pathology (ISUP) grade 3 [57].

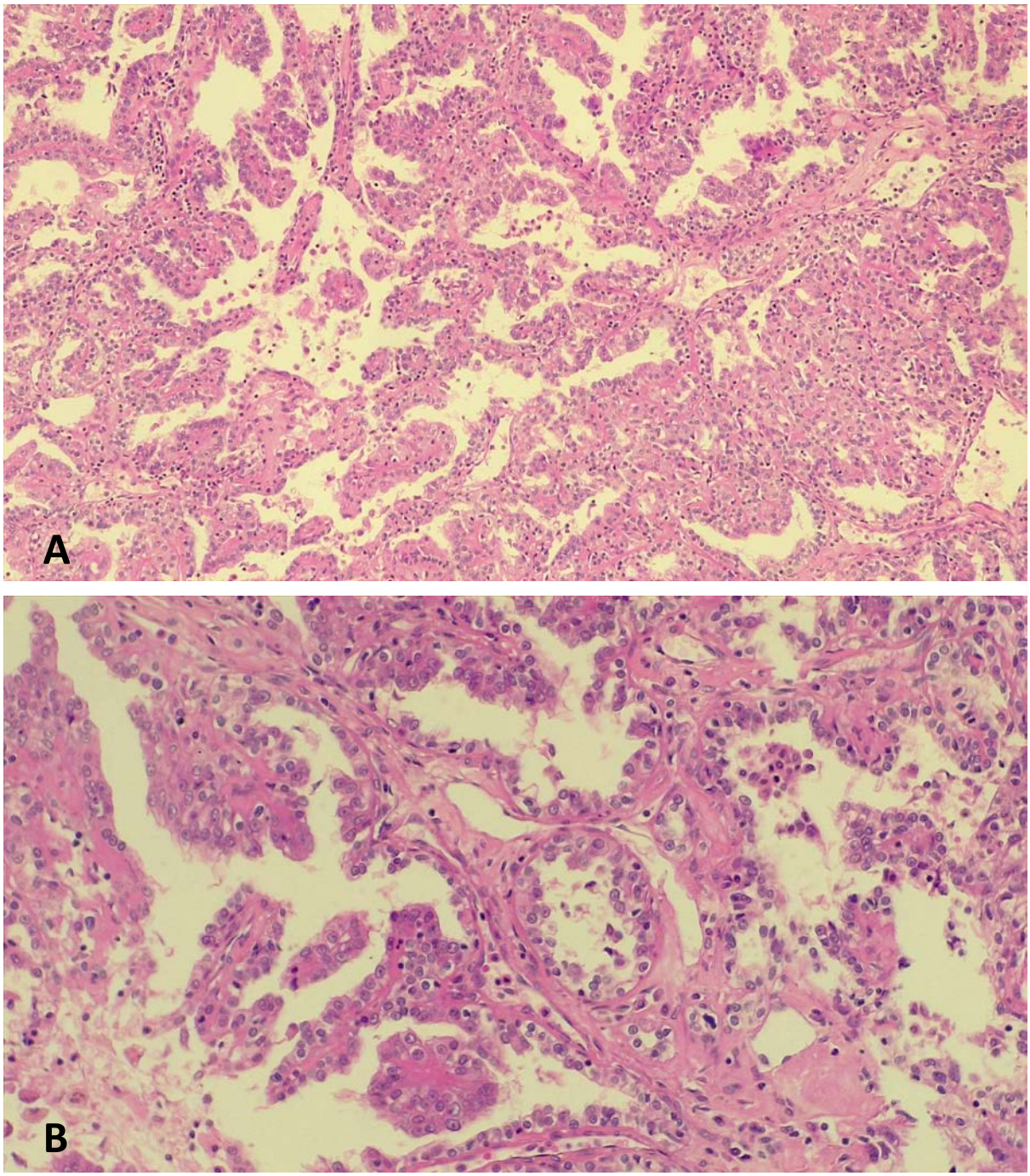



**Figure 3.** H&E images of a type 2 papillary RCC (A) 10x, (B) 20x magnification

- Some cases have a mixed histological appearance, making it difficult to classify as either type 1 or type 2 [58].

**Clear cell-papillary RCC** (Figure 4) **–** Grossly these tumours are usually small, well circumscribed and encapsulated. Histologically they can vary from tubular to solid and sometimes even have a cystic appearance. The tumour cells lining the papillae are mostly columnar with prominent clear cytoplasm and has characteristic small nuclei arranged in a straight line away from the basement membrane. The stromal component are mostly hyalinised but can consist of a prominent smooth muscle component [59].

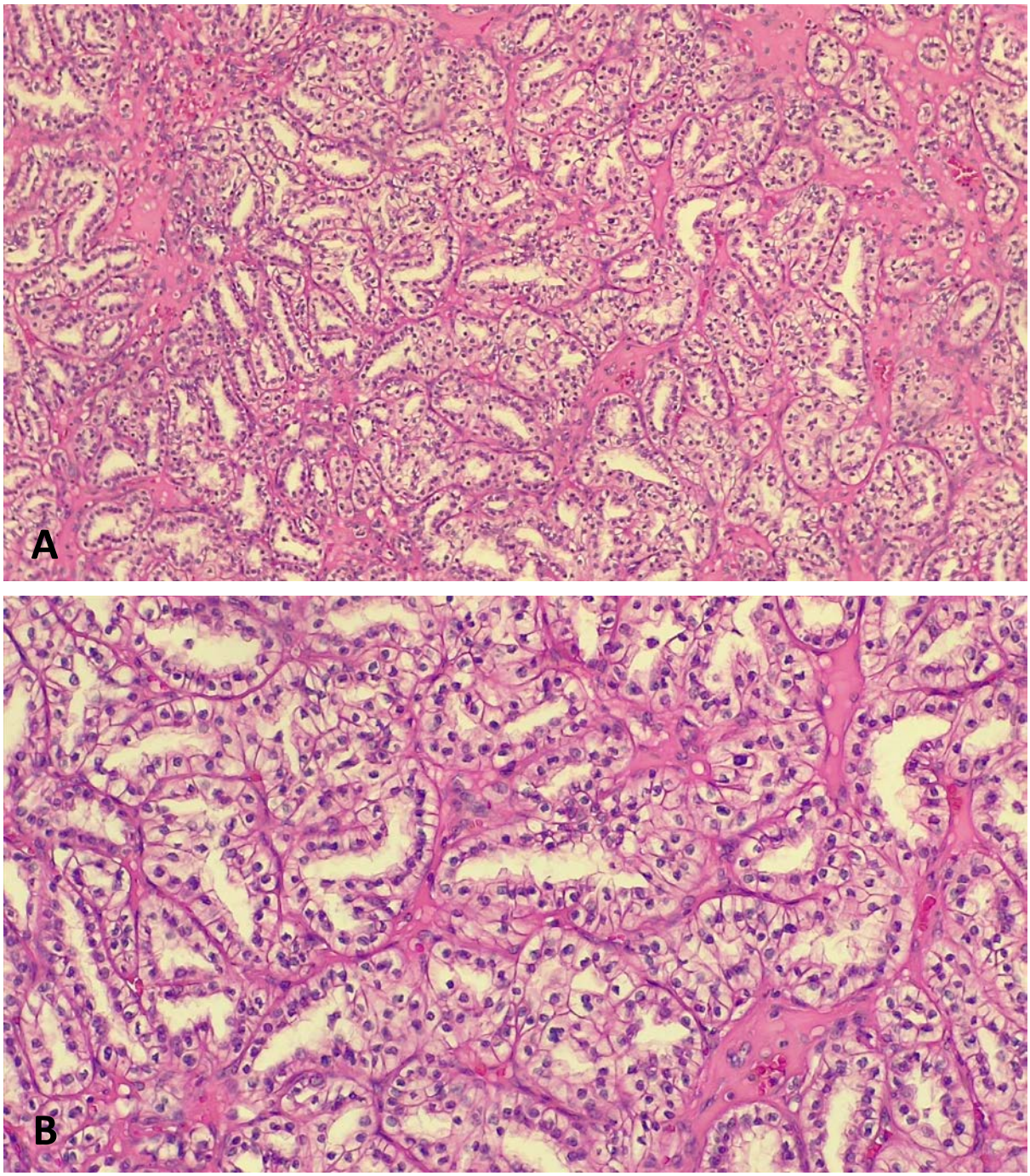
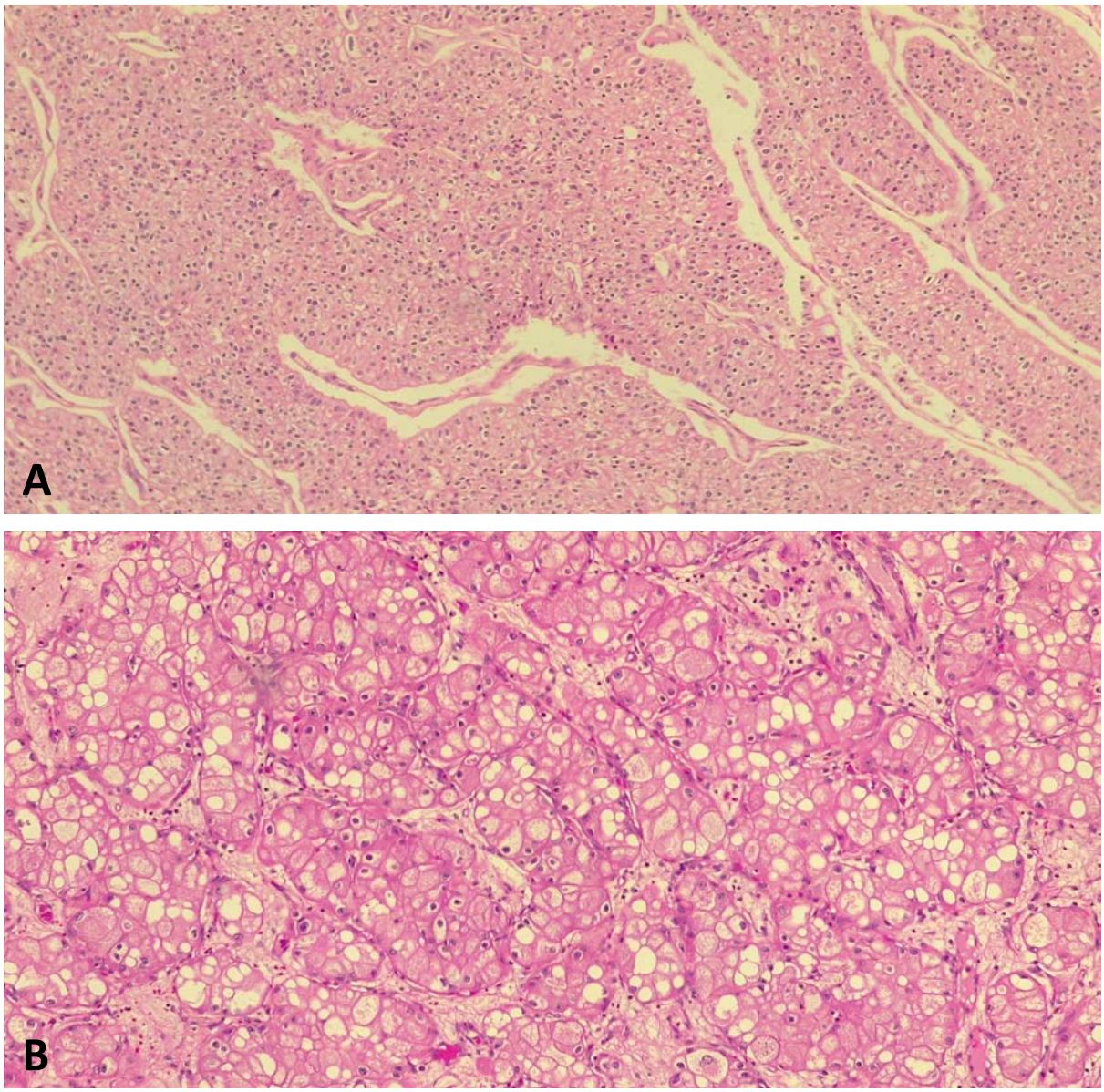



**Figure 4.** H&E images of a clear cell papillary RCC (A) 10x, (B) 20x magnification

**Chromophobe RCC** (Figure 5) — Grossly these tumours are mostly well delineated, single and brown-grey in colour. A central scar can be present in approximately 20% of cases. Histologically, they can have varied growth patterns, with a characteristic broad alveolar appearance being the most common pattern. The two main histological variants are the classic type and the eosinophilic variant. The classic type consists of cells with thick, plant-like cell membranes and abundant cytoplasm, with vague perinuclear clearing. The eosinophilic variant, consists of tumour cells with more prominent perinuclear halos and less prominent cell membranes. Some tumours may have a mixture of these features. These tumours have characteristic nuclear features which are essential for the diagnosis and consist of irregular nuclear membranes and hyperchromasia. The nuclei are often referred to as having a raisinoid appearance. [60].



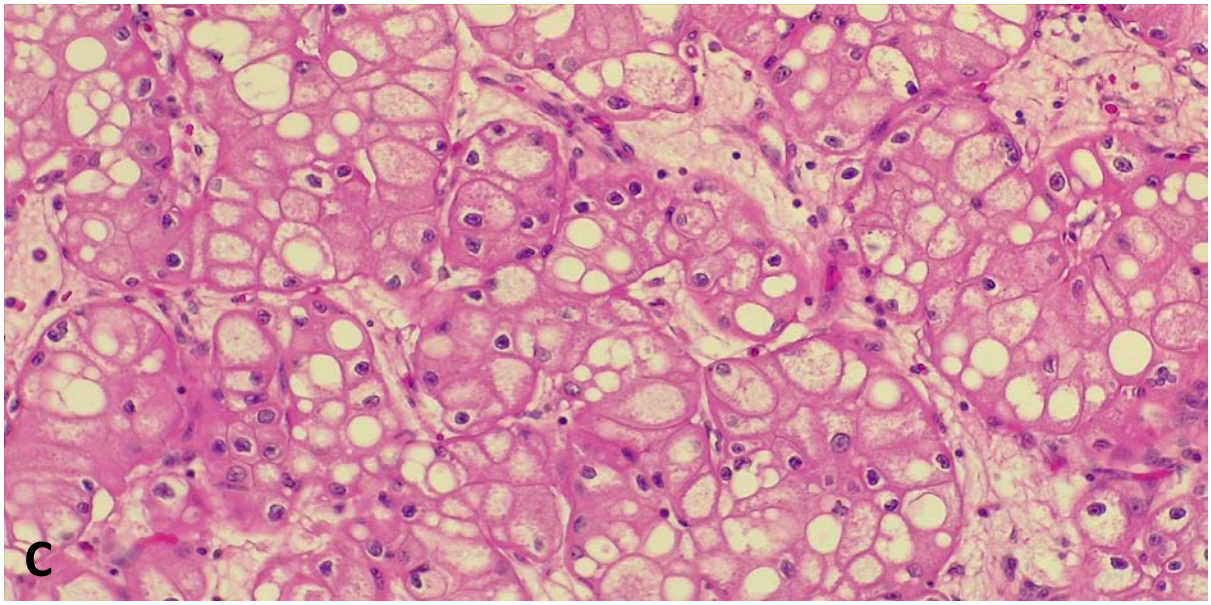


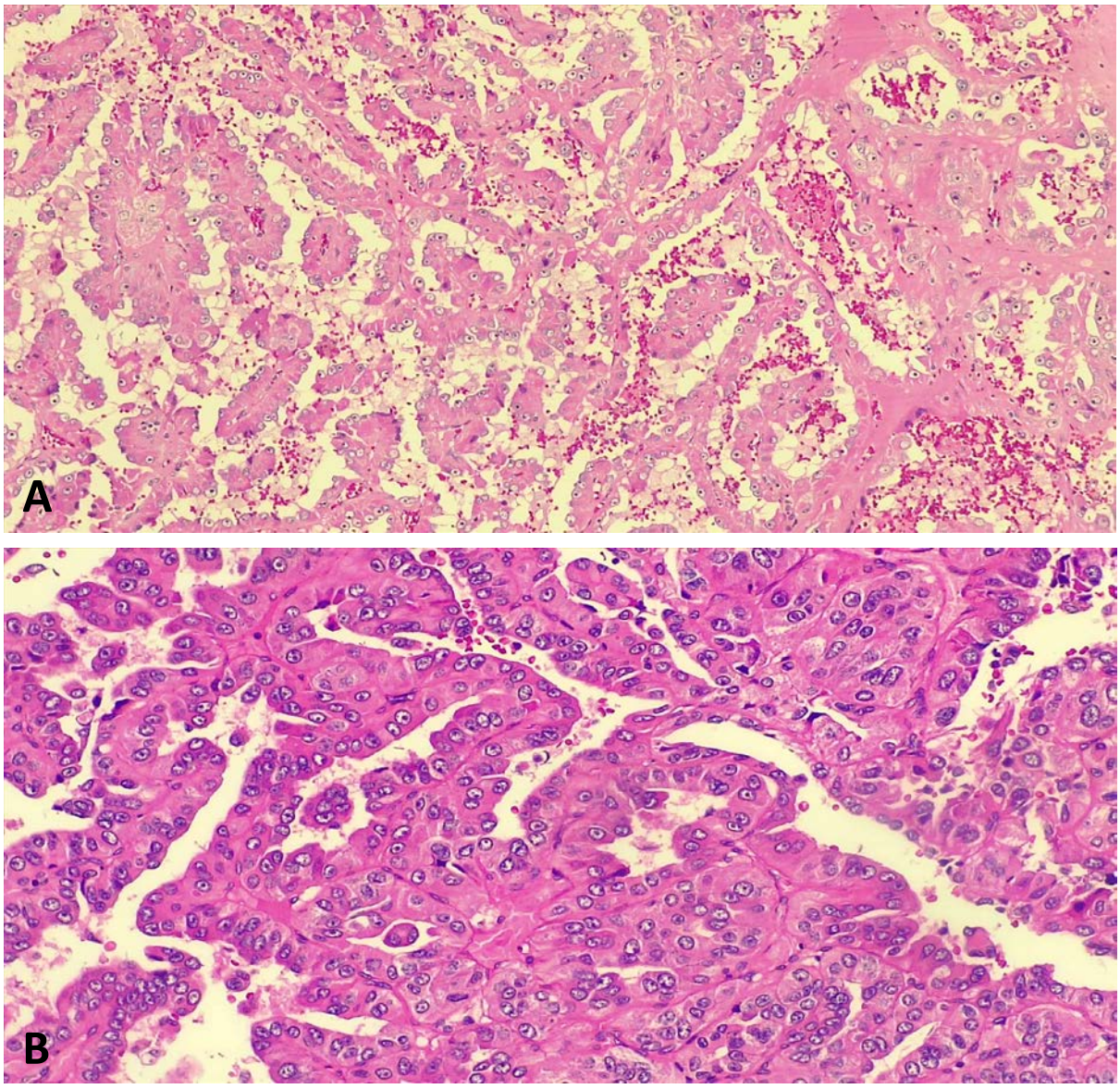

**Figure 5.** H&E images of a chromophobe RCC (A) 10x, (B) 20x, (C) 40x magnification

**Collecting duct RCC** – Macroscopically, these tumours are centrally localized within the medullary pyramid. They are grey-white and firm on cut section with an indistinct tumour border. They often involve adjacent structures, like the adrenal gland, peri-renal fat and regional lymph nodes. The hilar area with lymph nodes may be involved. Microscopically, they show a complex tubulo-papillary architecture with poorly defined borders and high grade nuclei. The adjacent parenchyma may show evidence of a prominent inflammatory cell infiltrate. Prominent stromal desmoplasia is a constant feature [61].

**Renal medullary carcinoma** – is common in young patients of African ancestry with sickle cell trait [62]. Macroscopically, they are typically located in the renal medulla. The often have a lobulated appearance and are firm and rubbery in consistence. Microscopically, they are heterogeneous in appearance, but the most characteristic growth pattern is a reticular or microcystic appearance. The tumour cells are typically large with prominent nuclei and nucleoli and they may have a rhabdoid appearance. The stromal background is often desmoplastic with abundant neutrophils present. The majority show mucin production. Sickled erythrocytes are present on most sections [52].

**MiT family translocation RCC** (Figure 6) — A distinct variant of RCC that is found to be more common in younger patients [63]. Two different tumours have been identified in this group. The first involves region Xp11.2, resulting in a *TFE3* gene fusion. Histologically these tumours are diverse and can vary from papillary to more solid in appearance with the

cytoplasm varying between clear to granular and eosinophilic. Psammoma bodies can be abundant. The t(6;11) RCCs are less common and they involve fusion of the *TFEB* gene. These tumours show a more nested and acinar growth pattern with round nuclei and clear to granular cytoplasm. They sometimes show a biphasic appearance consisting of small cells clustered around basement membrane material and larger epithelioid cells [63].



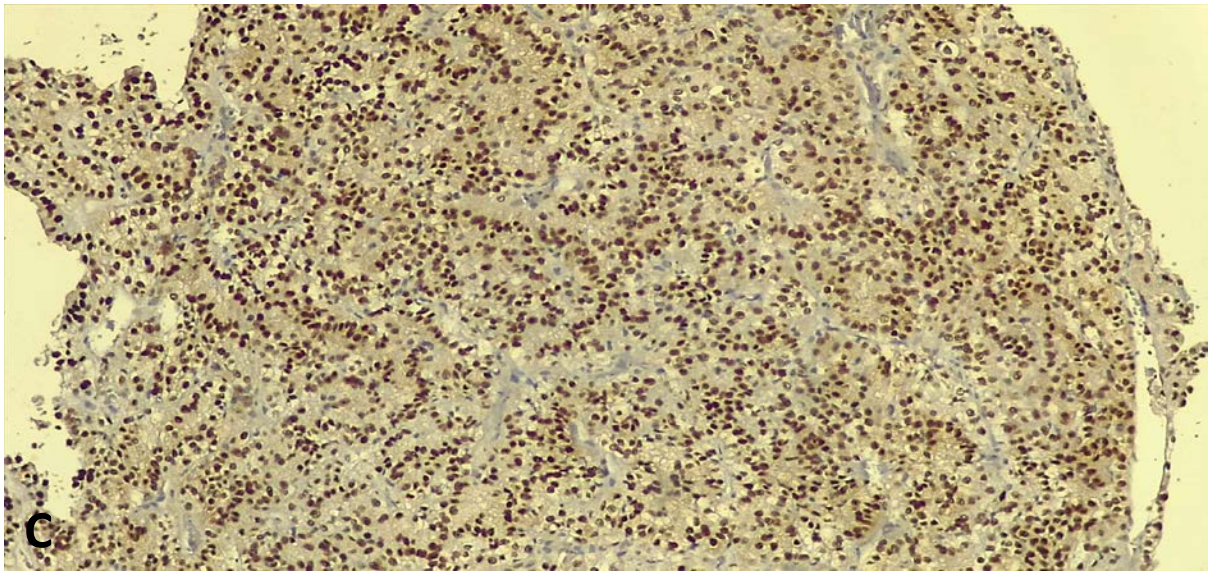



**Figure 6.** H&E images of a translocation RCC
(A) 10x magnification (B) 20x magnification, (C) TFE3 positivity, 10x magnification

**SDH-deficient RCC –** These are usually solitary, solid tumours, but multifocal and bilateral tumours have been documented. They are mostly well-circumscribed tumours with cystic areas being common. Microscopically, they can have different growth patterns, varying from solid to nested and even tubular in appearance. The tumour cells are typically monomorphic with bland round nuclei. The presence of intracytoplasmic vacuoles in the eosinophilic to flocculent cytoplasm is a characteristic feature of this lesion [49].

**Thyroid-like follicular carcinoma of the kidney –** Macroscopically, these tumours are mostly solitary and encapsulated. Histologically they consist predominantly of macro- and microfollicles that are lined by cuboidal epithelial cells. The follicles contain an amorphous colloid-like material. The tumour cells appears monomorphic with round nuclei and evenly distributed chromatin [64].

**ALK rearrangement-associated RCC** – Grossly these tumours vary from solid to cystic and can be well-circumscribed. Microscopically, the tumour cells are arranged in various growth patterns, which can vary from solid areas to tubular structures. Some tumours resemble papillary RCC histologically. The tumour cells vary from polygonal to spindle to cuboidal in shape with eosinophilic cytoplasm [65].

**RCC with smooth muscle stroma (also referred to as RCC with leiomyomatous stroma)** – Microscopically these tumours consists of two distinct components: The first

component represents the epithelium and has the appearance of conventional ccRCC while the second is represented by a smooth muscle stromal component [66].

**Fumarate hydratase (FH) - deficient RCC** – typically shows multiple architectural patterns varying from tubulocystic, papillary, solid to cribriform. Most commonly they have a papillary growth pattern consisting of tumour cells with focal macronucleoli and peri-nucleolar halos [67].

**Biphasic squamoid papillary RCC** – Macroscopically, these tumours are mostly solid and papillary with occasional small areas of haemorrhage and necrosis. Microscopically, they are biphasic consisting of small monomorphic cells with scant cytoplasm. These cells are mixed with larger cells with a squamoid appearance. The presence of emperipolesis in the squamoid cells is a hallmark of this entity [68].

**Eosinophilic solid and cystic RCC –** Macroscopically these tumours are classically solid and cystic. They are usually solitary lesions. Microscopically the tumour cells have abundant eosinophilic cytoplasm. The solid areas consist mostly of nests and acinar structures, often mixed with lymphocytes and histiocytes. The cytoplasm appears granular with a stippled appearance. The nuclei are irregular with prominent nucleoli. The solid areas are separated by septae of variable appearance, forming cysts that are lined by prominent eosinophilic neoplastic cells [69].

**Atrophic kidney-like RCC –** Macroscopically, they are typically solitary, well-circumscribed and encapsulated tumours with a fine spongy appearance on cut surface. Microscopically the tumour consists mainly of follicles that gives the lesion an appearance similar to that of atrophic kidney tubules. The tumour cells that line the follicles can often become detached in a colloid-like material. Calcifications are common in these lesions, some of which has a typical psammomatous appearance [70].

**Clear cell RCC with giant cells and emperipolesis** (Figure 7) **–** These tumours commonly show areas of necrosis and haemorrhage. Microscopically, they have a biphasic appearance consisting of a classic clear cell RCC component with areas showing histiocyte-like giant cells with abundant, granular cytoplasm. These giant cells resemble syncytiotrophoblasts and are often mixed with cells with a rhabdoid appearance. Emperipolesis is only found in the giant cells [71].

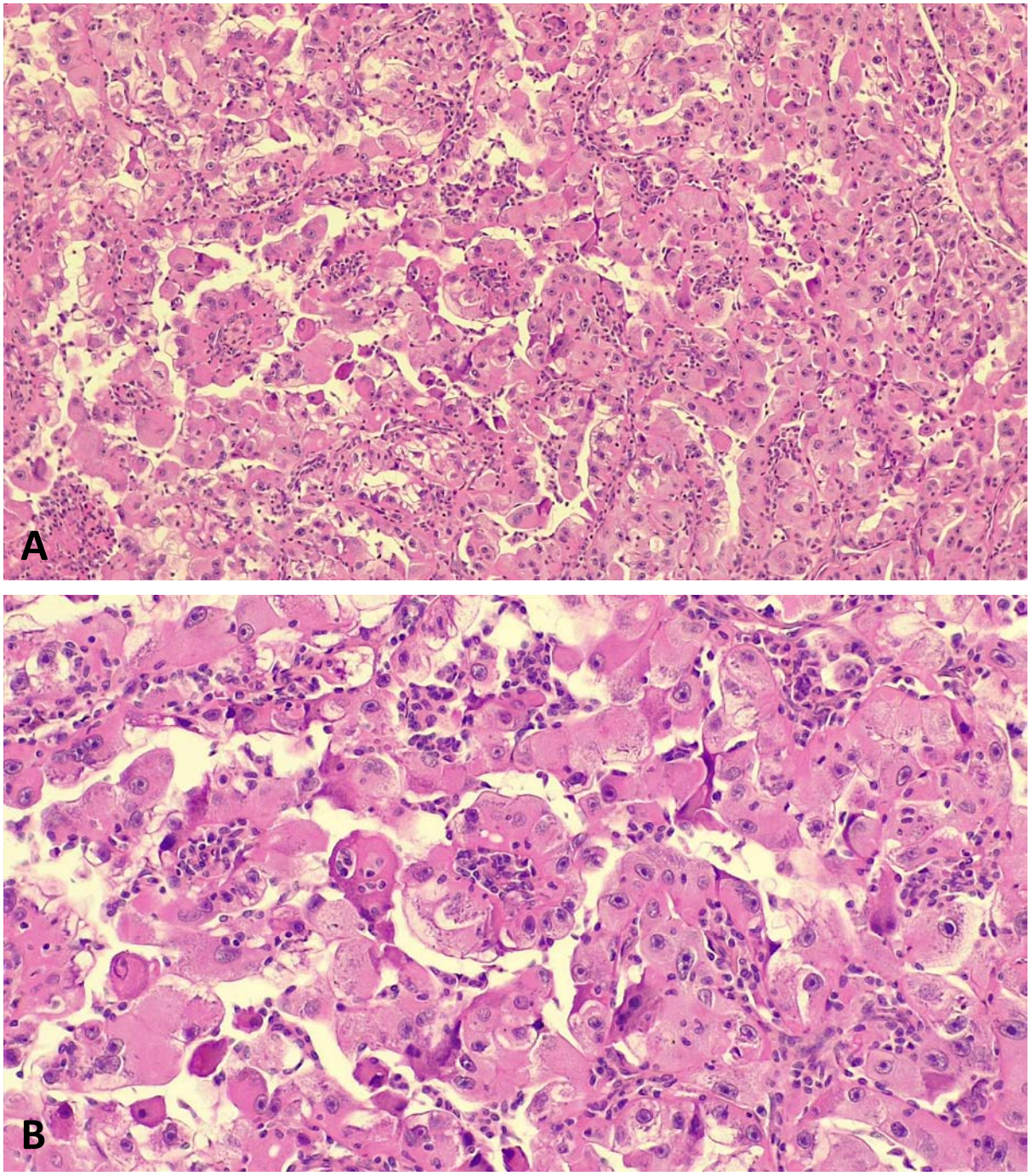



**Figure 7.** H&E images of a clear cell RCC with giant cells and emperipolesis
(A) 10x magnification, (B) 20x magnification

**Warthin-like papillary RCC –** On histological examination these tumours predominantly have a papillary growth pattern and consist of large eosinophilic tumour cells and a prominent stromal lymphocytic inflammatory cell infiltrate [72].

## Immunohistochemical stains for RCC

RCC cases that are difficult to diagnose or subtype may need the use of immunohistochemical stains for confirmation. These stains are also used to distinguish RCCs from metastatic tumours to the kidney or to confirm the renal origin of an unknown tumour outside the kidney.

The following immunohistochemical panel is recommended in the workup of challenging renal epithelial cell tumours: PAX8/PAX2, CD10, CK7, CAIX, AMACR, CD117, Vimentin, TFE3, HMB45, Melan A, 34BE12, INI1, SDHB [73]. Table 2 provides a summary for the use of the above markers.

Immunohistochemical stains can be used to identify certain molecular subtypes of RCC. SDHB, FH, S-(2-succino) cysteine (2SC) and TFE3/TFEB are current immunohistochemical markers that can be used for this purpose. SDH-deficient RCC can be identified by loss of immunohistochemical expression of SDHB [41]. Hereditary leiomyomatosis and RCC syndrome (HLRCC)-associated RCC caused by a germline mutation of the Fumarate Hydratase (*FH*) gene, can be identified by loss of staining for the FH immunohistochemical marker [67]. HLRCC-associated RCC can also be diagnosed by strong and diffuse cytoplasmic and nuclear staining of 2SC [67]. This staining pattern is indicative of biallelic inactivation of FH. TFE3 and TFEB immunohistochemical stains can respectively be used to identify Xp11 and t(6;11) translocation RCCs. Positive nuclear staining in translocation RCC is indicative of the specific molecular abnormality [74].

**Table 2: Immunohistochemical markers in the differential diagnosis of RCC** [75, 76]

| Antibody | Clear cell RCC | Papillary RCC | Clear cell papillary RCC | Chromophobe RCC | MTSCC | Collecting duct carcinoma | Renal medullary carcinoma | MiT family translocation RCC | SDH deficient RCC |
|---|---|---|---|---|---|---|---|---|---|
| PAX8/PAX2 | + | + | + | +/- | + | +/- | +/- | + | + |
| CA-IX | + | - | + | - | - | - | - | -/+ (focal) | - |
| CD10 | + | + | | +/- | +/- | - | - | + | -/+ (diffuse) |
| CK7 | - | + (Type 1) -/+ (Type 2) | + | +/- | + | -/+ | -/+ | - | - |
| AMACR | - | + | - | - | + | -/+ | -/+ | - | + (focal) |
| CD117 | - | - | - | + | - | - | - | - | - |
| Vimentin | +/- | +/- | - | - | - | - | - | - | - |
| TFE3/TFEB | - | - | - | - | - | - | - | + | - |
| 34BE12 | - | - | - | - | - | +/- | -/+ | - | -/+ (focal) |
| INI1 | + | + | + | + | + | + | - | + | + |
| SDHB | + | + | + | + | + | + | + | + | - |
| HMB45 | - | - | - | - | - | - | - | -/+ | - |
| Melan-A | - | - | - | - | - | - | - | -/+ | - |

AMACR = alpha-methylacyl-CoA racemase; CA-IX = carbonic anhydrase IX; MiTF = microphthalmia-associated transcription factor; MTSCC = mucinous tubular and spindle cell carcinoma; RCC = renal cell carcinoma; SDH = Succinate dehydrogenase

**South African and African data:**

Little information is available on the epidemiology or histopathological profile of RCC in the South African population. After an advanced Pubmed search of the English literature, only three articles related to the epidemiology or histopathological profile of RCC in the South African population could be found. The only other related information found was from the National Cancer Registry (NCR) from 2014. The NCR is the principal cancer surveillance system in South Africa. The registry falls within the structures of the National Health Laboratory Service (NHLS) which is the service provider for the diagnostic histopathology service for the public health system in South Africa. They manage cancer surveillance for both the public healthcare system, serving approximately 84% of the population, as well as the smaller private healthcare system that serves the rest of the population [77]. Also of importance is to note that the NCR does not report on RCC separately. The report is on kidney cancer in general, which includes all types of cancer diagnosed histologically in the kidney, including RCC. Renal cell carcinoma does, however, represent 90% of all renal malignancies [21].

The NCR reported that kidney cancer in males and females, made up 1.11% and 0.64% respectively of all cancers in South Africa and they calculated the age-standardized rates (ASR) for kidney cancer in South Africa at 2.0/100 000 for men and 1.0/100 000 for women [78]. They also reported a male to female ratio of 1.7:1 which is slightly higher than the reported ratio in the literature [78].

The registry further showed the highest incidence of kidney cancer in the sixth and seventh decades for both men and women [78]. However, when corrected for race, black patients had a slightly younger incidence, with a peak incidence in the fifth and sixth decades for both men and women [78].

Claassen *et al.* performed a study on the demographics of the patients diagnosed with RCC in Bloemfontein in central South Africa. Their study stretched over 15 years from 1996 to 2010. They found that black patients presented with renal cell carcinoma at a younger age (mean age of 54.2 years) compared to white patients (mean age of 70 years) [11]. They also found that patients with HIV presented at a younger age (mean age 32 years) compared to patients that tested negative for HIV (mean age 62 years) [11].

Du Plessis *et al.* did a prospective observational study of the epidemiology and pathological profile of RCC in a South African population. They reported a peak incidence in the sixth decade, which is earlier than the reported incidence in the United States and Europe [79]. They found that their gender and pathological profile was very similar to international literature with a male:female ratio of 1.4:1 and ccRCC being the most common subtype, followed by pRCC and Chromophobe RCC [51]. In contrast to international literature, they also reported Type 2 pRCC to be more common compared to Type 1 pRCC [58].

Sing *et al.* did a retrospective chart review of patients having undergone nephrectomy for RCC in a regional hospital in Kwazulu-Natal, South Africa between 2010 and 2015. They also reported a peak incidence in the sixth decade, with HIV positive patients presenting a decade earlier, agreeing with the results reported by Claassen *et al*. Their male:female ratio was 1.7:1. The pathological profile of their cases was very similar to international literature with ccRCC the most common (54%) followed by pRCC (19%) and chromophobe RCC (4%) [80].

When looking at the rest of Africa, most studies on RCC were done in Nigeria. When comparing the data from Claassen *et al.* and Du Plessis *et al.* with studies done in Nigeria the following differences were noted: The peak incidence was earlier, with a mean age of diagnosis in the fifth decade [10, 79, 81, 82]. A trend that was also seen in studies done in the US, where black American patients also showed a peak incidence at an earlier age [20, 83]. A systematic review of RCC in Nigeria in 2018 found that men are only marginally more affected than women with a male:female ratio of 1.1:1 compared to ratios of 2.1:1 in the US, 2.3:1 in Europe and 1.4:1 in Northern Africa and 1.9:1 in Southern Africa [6, 8]. When comparing the pathological profile, ccRCC was still the most common subtype making up 60-85% of cases, which is very similar to international literature [8]. Papillary RCC although still the second most common subtype, occurred more often with an average frequency of 34% compared to the 10-15% reported in western literature [8].

The determination of the incidence, demographic and histopathological features of RCC would provide the latest and most up to date information on RCC epidemiology in the state sector in central South Africa. This data would also serve as a foundation for future research on RCC in central South Africa.

## AIM AND OBJECTIVES

The aim of the study is to determine the profile of RCCs diagnosed by the Department of Anatomical Pathology, University of the Free State (UFS) and National Health Laboratory Service (NHLS) over a 10 year period.

Objectives:

1. To determine the number of cases of RCC seen over a ten year period from January 2005 to December 2014 by the Department of Anatomical Pathology, UFS and NHLS.
2. To determine the age-standardized incidence rate for state sector patients from the Free State Province.
3. To evaluate the demographic features of patients diagnosed with RCC.
4. To determine the histological spectrum (subtypes) of the RCCs diagnosed.

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CHAPTER 2

ARTICLE: **RENAL CELL CARCINOMA DIAGNOSED BY THE DEPARTMENT OF ANATOMICAL PATHOLOGY AT THE UNIVERSITY OF THE FREE STATE, SOUTH AFRICA: A 10 YEAR HISTOPATHOLOGICAL REVIEW**

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# RENAL CELL CARCINOMA DIAGNOSED BY THE DEPARTMENT OF ANATOMICAL PATHOLOGY AT THE UNIVERSITY OF THE FREE STATE, SOUTH AFRICA: A 10 YEAR HISTOPATHOLOGICAL REVIEW

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**ABSTRACT**

**Background:** Globally, the incidence of renal cell carcinoma varies widely between populations and geographic areas, with the lowest incidence reported in Africa. Little information is available on the epidemiology or histopathological profile of renal cell carcinoma (RCC) in the South African population. The purpose of this study was to determine the age-standardised incidence rate (ASR) of RCC for state sector patients from the Free State Province over a ten year period and to describe the demographic profile and the histological spectrum of the cases identified.

**Methods:** A retrospective descriptive review with an analytical component was performed. All histologically confirmed cases of RCC identified between 1 January 2005 and 31 December 2014 were included. The pathology reports and H&E slides were reviewed to collect the demographic information and confirm the diagnosis and the specific histological subtype. ASRs were calculated using the population data from the South African census as performed in 2011. The ASRs were only calculated for the Free State Province.

**Results:** A total of 105 patients with RCC were diagnosed with a male:female ratio of 1.2:1 and a mean age of 55.1 years. The ASR for the Free State province was 0.4 /100 000 population. The majority of cases were identified in the age group 50 to 59 years (33.3%). The majority of patients (58.1%) were black and they were found to present on average a decade earlier than white patients. The most common histological subtype was clear cell RCC (58.1%). Patients diagnosed with papillary RCC were found to be significantly more likely to be male than female (72.7% vs 27.3%; P=0.03) and were also more likely to be black than white (81.8% vs 13.6%; P<0.01). Patients with chromophobe RCC were more likely to be female (80%) and black (60%). Two cases of a new entity, clear cell RCC with giant cells and emperipolesis were identified.

**Conclusion:** Our study demonstrated that the incidence of RCC in our population is lower than reported in the rest of the world. The age distribution correlates closely with other African studies, with black patients presenting a decade earlier than white patients. Our findings also identified distinct age, sex and racial differences for the various RCC subtypes, which warrants further research.

**Keywords:**

Renal cell carcinoma, Epidemiology, Histological type, Incidence, Central South Africa

## ARTICLE

## INTRODUCTION

In 2018, renal cell carcinoma (RCC) was the sixth most common malignancy in men and the tenth most common malignancy in women globally [1]. Kidney cancer caused approximately 155 000 deaths in 2018, with RCC representing 90% of these cases [2]. RCC further represents 2-3% of all adult cancers worldwide, however, only 3.1% of these cases are diagnosed in Africa [3]. Despite RCC being responsible for only a fraction of all cancers, it is an important urological disease because of its high mortality [4, 5].

Globally, the incidence of RCC varies widely between populations and geographic areas. According to Globocan 2012, the global age-standardized incidence rate (ASR) is 4.4 per 100 000 population [6]. The highest rates of kidney cancer are reported in Northern America, Australasia and Europe, while the lowest rates are reported in the Middle East, Asia and Africa, with the ASR in Africa at 1.2 per 100 000 for the total population [6]. The National Cancer Registry (NCR) in South Africa reported that kidney cancer was the fourteenth most common cancer in males with 1.1% of all male cancers and the eighteenth most common cancer in females with 0.64% of all female cancers in South Africa for the period 2010 – 2014 [7].

Males are more commonly affected than females, with a male predominance of 1.5:1, with the global ASR for men at 6.0 per 100 000 males and women at 3.1 per 100 000 females [6]. The South African NCR reported the ASR for kidney cancer in males and females respectively at 2.0 per 100 000 for males and 1.0 per 100 000 females [7]. Globocan 2012, however, has shown the incidence of RCC in females has been increasing more rapidly compared to males [6]. Data from studies done in Nigeria have even shown a reversal in incidence with a female predominance. They reported a male: female ratio varying from 1:1.2 to as much as 1:2.8 [8].

The incidence of RCC is the highest in the sixth to eighth decade of life, with only 5% of cases being reported in patients under 40 years of age [2, 9]. Globocan 2012 elaborated on this trend, reporting that RCC incidence rates in developed countries appear to be higher in older patients where more than 50% of cases are diagnosed incidentally [10]. Studies performed in Africa and the Middle East consistently reported a younger peak age incidence in the fifth decade [8, 11].

In the majority of the international literature, RCC incidence has been reported to be comparable between black and white patients [12]. Studies by Lipworth et al. and Chow et al. in 2011 and 2013, however, reported that both incidence and mortality rates were much higher in black patients compared to white patients [13, 14]. They also found that over the past three decades, the rise in incidence rates for black American patients has been more pronounced compared to white patients [15]. It is suggested that a higher prevalence of RCC risk factors like hypertension, chronic kidney disease and obesity among black Americans, could be responsible for the difference in RCC incidence between black and white patients [16]. The NCR of South Africa reported a much higher ASR in the white population compared to the black population. The ASR for both sexes was 3.8 per 100 000 for white South Africans compared to an ASR for both sexes of 0.7 per 100 000 black South Africans [7]. The reported incidence of RCC in Africa is the lowest in the world according to Globocan 2012. This can be attributed to many variables, such as the lack of healthcare and underreporting. This racial disparity might be explained by differences in access to health care, lifestyle factors and possible genetic features [2, 11]. Male sex, black race, obesity, cigarette smoking and hypertension are the current well-established risk factors for developing sporadic RCC [13, 17, 18].

RCCs are a group of neoplasms originating from the epithelium of the renal tubules, with different subtypes demonstrating different morphological and genetic characteristics. RCC is classified based on histological subtypes. Clear cell RCC (ccRCC) is the most common histological subtype (65-70%) followed by papillary RCC (pRCC) (10-15%) and chromophobe RCC (5-7%) [3, 10]. Subtyping of RCC is of critical importance as they exhibit different outcomes, with ccRCC being more aggressive compared to the more indolent papillary and chromophobe RCCs [19]. This is predominantly because clear cell tumours more often present at a more advanced stage with evidence of metastatic spread. The histological classification of RCC has developed extensively over the past decade with the emergence of newly recognised entities and the advancement of molecular pathology [20].

Multiple international studies describing the demographic differences by histological subtype have been performed. Papillary tumours are substantially more common in black patients, while clear cell tumours are more common in white patients [12, 21, 22]. Chromophobe RCC is slightly more common in black patients [21]. With regards to gender, the data shows that patients with clear cell and papillary RCC are substantially more likely to be male, while those with chromophobe RCC are most likely to be female [21, 22]. Younger patients are more likely

to have chromophobe RCC, while papillary histology is more common in older age groups [9, 22, 23]. Little information is available on the epidemiology or histopathological profile of RCC in the South African population. After an advanced Pubmed search of the English literature, only three studies related to the epidemiology or histopathological profile of RCC in the South African population could be found.

The aim of this project was therefore to determine the number of cases of RCC seen over a 10 year period by the Department of Anatomical Pathology, University of the Free State (UFS) and National Health Laboratory Service (NHLS) and to describe the demographic and histopathological features of the patients identified.

## METHODOLOGY

A retrospective descriptive study with analytical component was conducted. All histologically confirmed cases of RCC diagnosed by the Department of Anatomical Pathology, UFS and NHLS over a 10 year period from 1 January 2005 to 31 December 2014 were evaluated for possible inclusion in the study. The cases were identified by performing a SNOMED search of the NHLS' electronic databases. The Department of Anatomical Pathology provides histology services to all state hospitals and clinics in the Free State Province and occasionally to the North West and Northern Cape provinces.

Once the cases were identified, the corresponding slides for each case were retrieved from the Department of Anatomical Pathology archives. All repeat specimens, where both biopsy and excision specimens were available for the same patient, were included as one case. Cases without available slides were excluded from the study. The slides and pathology reports were evaluated by a registrar and a pathologist. The slides were reviewed to confirm the diagnosis and to provide a specific histological subtype based on characteristic histological features and where available, immunohistochemical stains. Cases where there was any uncertainty regarding the original diagnosis or where a new diagnosis was proposed, were reviewed by a second pathologist. Demographic data including age and gender were obtained from the pathology reports, while the race was obtained from the Meditech hospital information system.

Approval to perform the study was obtained from the Health Sciences Research Ethics Committee, UFS (UFS-HSD2017/1432). Statistical analysis was performed by the researchers and the Department of Biostatistics, UFS. Categorical variables were summarised by frequencies and percentages. Numerical variables were summarised by means and medians. ASRs were calculated using the population data from the South African census as performed in 2011 [24]. ASRs were only calculated for the Free State Province and patients from the North West and Northern Cape Provinces were excluded for these calculations as the department does not receive all the specimens from these two provinces. Subgroup comparisons of categorical variables were done using chi-squared or Fisher's exact tests.

## RESULTS

A total of 105 cases of RCC were diagnosed in the 10 year study period, of which 58 (55.2%) were male and 47 (44.8%) were female, with a ratio of 1.2:1. Our study showed the overall ASR for RCC for the Free State province to be 0.4 per 100 000 population, with an ASR of 0.4 per 100 000 for males and 0.3 per 100 000 for females.

The highest number of cases were recorded in the years 2006 and 2007, with fifteen cases in each year, while only five cases were recorded in 2008. There was a noticeable change in the male:female ratio over the last three years, with a female predominance of 1:1.5 compared to a ratio of 1.5:1 in the previous 7 years (Table 1).

**Table 1:** Yearly distribution of RCC cases according to gender

| | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 |
|---|---|---|---|---|---|---|---|---|---|---|
| Male | 6 | 8 | 10 | 3 | 8 | 7 | 6 | 3 | 4 | 3 |
| Female | 4 | 7 | 5 | 2 | 3 | 6 | 5 | 5 | 4 | 6 |
| Total | 10 | 15 | 15 | 5 | 11 | 13 | 11 | 8 | 8 | 9 |

The patients' ages ranged from 7 to 81 with a mean age of 55.1 years and a median age of 57 years. There were 10 patients (9.5%) younger than 40 years. The majority of cases were identified in the age group 50 to 59 years (33.3%). Of the 105 cases identified, 61 (58.1%) patients were black, 39 (37.1%) patients were white and five (4.8%) patients were coloured. In 52 (49.5%) patients the tumour was located in the left kidney and in 53 (50.5%) patients the tumour was in the right kidney. No bilateral tumours were identified (Table 2).

**Table 2:** Demographic distribution of RCC by age, gender, race and location (n = 105).

| | n (%) |
|---|---|
| Age range | |
| <40 | 10 (9.5) |
| 40 – 49 | 19 (18.1) |
| 50 – 59 | 35 (33.3) |
| 60 – 69 | 25 (23.8) |
| 70 – 79 | 15 (14.3) |
| >80 | 1 (1) |
| Gender | |
| Male | 58 (55.2) |
| Female | 47 (44.8) |
| Race | |
| Black | 61 (58.1) |
| White | 39 (37.1) |
| Coloured | 5 (4.8) |
| Tumour location (laterality) | |
| Left | 52 (49.5) |
| Right | 53 (50.5) |

In total, 61 (58.1%) cases were subtyped as ccRCC, 22 (20.9%) as pRCC and 5 (4.8%) cases each were diagnosed as chromophobe and MiT family translocation RCC. A total of 9 (8.6%) cases could not be classified and subtyped as unclassified. When the results were separated by race, the distribution of the histological subtypes showed variable differences when comparing the black patients to the white patients depending on the specific subtype. Coloured patients were excluded due to the small sample size. Although ccRCC was still comparatively the most common subtype in black patients, the proportion of cases with clear cell histology was considerably less than in white patients (47.5% vs 76.9%, respectively; $p$ <0.01). In contrast, the proportion of cases of pRCC was substantially higher in black patients than in white patients (29.5% vs 7.7% respectively; $p$ = 0.01). All 5 cases of MiT family translocation RCC were diagnosed in black patients (8.2% vs 0%; $p$ = 0.08). In addition, 4.9% of the RCC cases in black patients were subtyped as chromophobe, while only 2.6% of white patients were diagnosed with chromophobe RCC. Two cases of ccRCC with giant cells and emperipolesis were identified, both of which were white males (Figure 1).

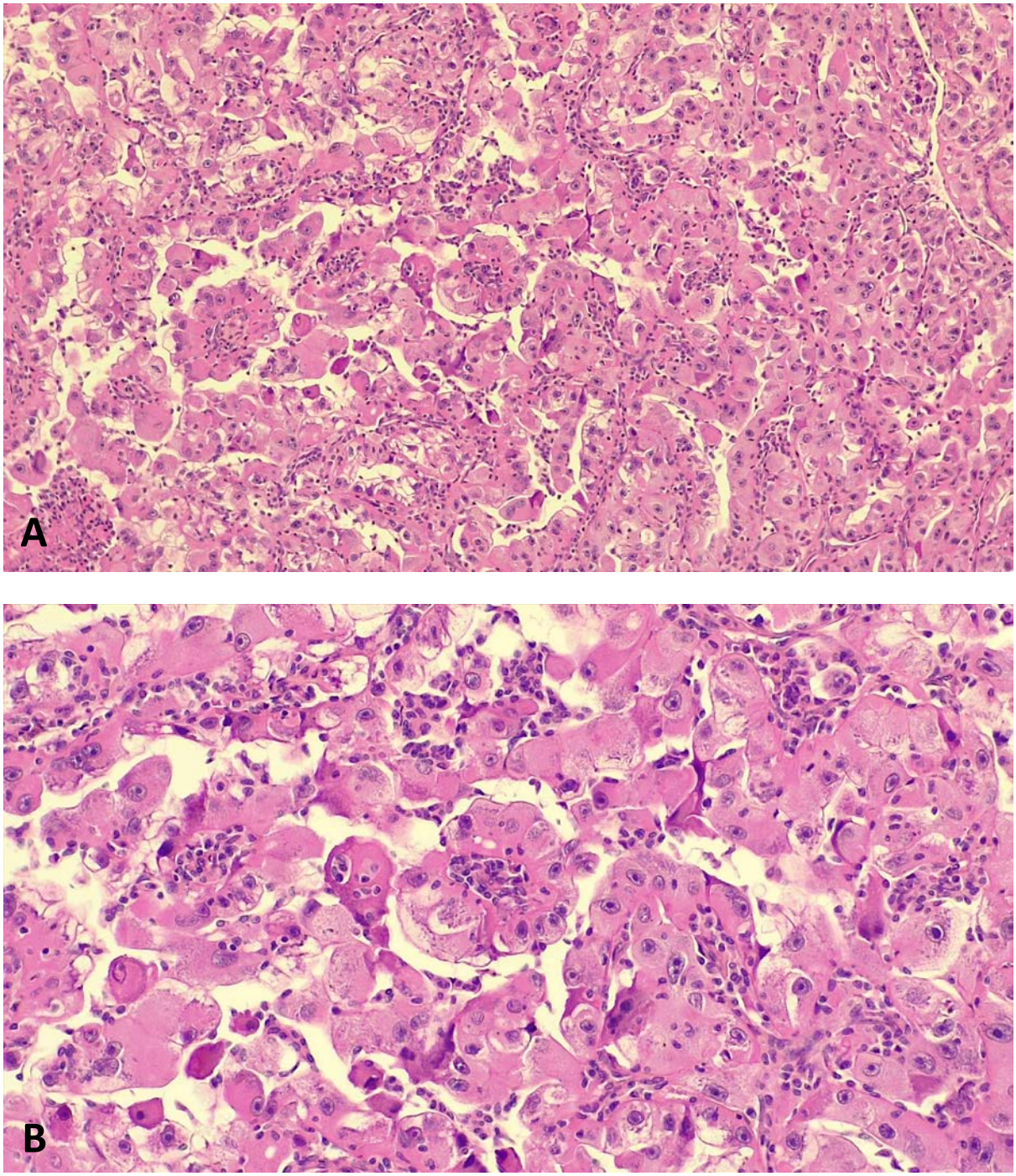


Figure 1: H&E images of a clear cell RCC with giant cells and emperipolesis
(B) 10x magnification, (B) 20x magnification

Black males and females were equally affected by RCC, with a male:female ratio of 1:1.03, while in white patients, RCC was twice as common in males than in females with a male:female ratio of 2:1. With regards to age distribution and race, black patients predominantly presented in the fifth and sixth decades of life (21.3% and 34.4%), with white patients more commonly

presenting in the sixth and seventh decades (35.9% and 33.3%). RCCs were significantly more common in white patients than black patients in the seventh decade of life (33.3% vs 14.8%, respectively; $p$ = 0.03) (Table 3).

**Table 3.** Comparison of the distribution of RCC histological subtypes, gender and age by race

| | Black n (%) | White n (%) | Coloured n (%) | Total n (%) | *p*-value Black versus White |
|---|---|---|---|---|---|
| Number of patients | 61 (58.1) | 39 (37.1) | 5 (4.8) | 105 | |
| Tumour histology | | | | | |
| Clear cell | 29 (47.5) | 30 (76.9) | 2 (40.0) | 61 (58.1) | *<0.01* |
| Papillary | 18 (29.5) | 3 (7.7) | 1 (20.0) | 22 (20.9) | *0.01* |
| Chromophobe | 3 (4.9) | 1 (2.6) | 1 (20.0) | 5 (4.8) | 0.49 |
| Clear cell papillary | 1 (1.6) | 0 (0) | 0 (0) | 1 (0.9) | 0.61 |
| MiT family translocation | 5 (8.2) | 0 (0) | 0 (0) | 5 (4.8) | 0.08 |
| Clear cell RCC with giant cells with emperipolesis | 0 (0) | 2 (5.1) | 0 (0) | 2 (1.9) | 0.15 |
| RCC unclassified | 5 (8.2) | 3 (7.7) | 1 (20.0) | 9 (8.6) | 1.00 |
| Gender | | | | | *p*=0.09 |
| Male | 30 (49.2) | 26 (66.7) | 2 (40.0) | 58 (55.2) | |
| Female | 31 (50.8) | 13 (33.3) | 3 (60.0) | 47 (44.8) | |
| Age | | | | | |
| <40 years | 7 (11.5) | 3 (7.7) | 0 (0) | 10 (9.5) | 0.74 |
| 40-49 years | 13 (21.3) | 4 (10.3) | 2 (40.0) | 19 (18.1) | 0.15 |
| 50-59 years | 21 (34.4) | 14 (35.9) | 0 (0) | 35 (33.3) | 0.88 |
| 60-69 years | 9 (14.8) | 13 (33.3) | 3 (60.0) | 25 (23.8) | *0.03* |
| 70-79 years | 10 (16.4) | 5 (12.8) | 0 (0) | 15 (14.3) | 0.63 |
| >80 years | 1 (1.6) | 0 (0) | 0 (0) | 1 (1.0) | 1.00 |

Table 4 shows the results comparing the histological subtypes of RCC with each other in terms of gender, race and age. Patients with ccRCC were more likely to be male than female with 59% of cases occurring in males and 41% in females ($p$ = 0.15). The percentage of ccRCC occurring in black patients was similar to white patients with 47.5% compared to 49.2%. We further found that patients with pRCC were significantly more likely to be male than female (72.7% vs 27.3%; $p$ = 0.03) and more likely to be black than white (81.8% vs 13.6%; $p$ <0.01). Both ccRCC and pRCC were found to be the most common in the sixth decade (36.1%

and 40.9%), although patients with ccRCC were generally found to be older, with 73.8% of patients being older than 50 years compared to 63.6% of patients with pRCC. Patients with chromophobe RCC were more likely to be female and black with 80% of the cases diagnosed in female patients and 60% of the cases seen in black patients. Our results further showed that all 5 cases of MiT family translocation RCC were diagnosed in black females, of which three were under 40 years of age. One case of clear cell papillary RCC was diagnosed in a 71-year-old black female. Nine cases (8.6%) could not be subtyped and were classified as RCC unclassified.

Table 4. Comparison of the distribution of gender, race and age for the different histologic subtypes of RCC

| | Clear cell RCC<br>n (%) | Papillary RCC<br>n (%) | Chromophobe RCC<br>n (%) | MiT family transloction RCC<br>n (%) | ccRCC with giant cells with emperipolesis<br>n (%) | Clear cell papillary RCC<br>n (%) | Unclassified RCC<br>n (%) | Total |
|---|---|---|---|---|---|---|---|---|
| Total | 61 (58.1) | 22 (20.9) | 5 (4.8) | 5 (4.8) | 2 (1.9) | 1 (0.9) | 9 (8.6) | 105 |
| Gender | | | | | | | | |
| Male | 36 (59.0) | 16 (72.7) | 1 (20.0) | 0 (0) | 2 (100.0) | 0 (0) | 3 (33.3) | 58 |
| Female | 25 (41.0) | 6 (27.3) | 4 (80.0) | 5 (100.0) | 0 (0) | 1 (100.0) | 6 (66.7) | 47 |
| Race | | | | | | | | |
| Black | 29 (47.5) | 18 (81.8) | 3 (60.0) | 5 (100.0) | 0 (0) | 1 (100.0) | 5 (55.6) | 61 |
| White | 30 (49.2) | 3 (13.6) | 1 (20.0) | 0 (0) | 2 (100.0) | 0 (0) | 3 (33.3) | 39 |
| Coloured | 2 (3.3) | 1 (4.5) | 1 (20.0) | 0 (0) | 0 (0) | 0 (0) | 1 (11.1) | 5 |
| Age | | | | | | | | |
| <40 years | 5 (8.2) | 2 (9.1) | 0 (0) | 3 (60.0) | 0 (0) | 0 (0) | 0 (0) | 10 |
| 40-49 years | 11 (18.0) | 6 (27.3) | 1 (20.0) | 0 (0) | 0 (0) | 0 (0) | 1 (11.1) | 19 |
| 50–59 years | 22 (36.1) | 9 (40.9) | 1 (20.0) | 0 (0) | 1 (50.0) | 0 (0) | 2 (22.2) | 35 |
| 60-69 years | 13 (21.3) | 2 (9.1) | 3 (60.0) | 1 (20.0) | 1 (50.0) | 0 (0) | 5 (55.6) | 25 |
| 70-79 years | 9 (14.8) | 3 (13.6) | 0 (0) | 1 (20.0) | 0 (0) | 1 (100.0) | 1 (11.1) | 15 |
| >80 years | 1 (1.6) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 |

Five cases (4.8%) were reclassified after review (table 5). Two cases of ccRCC were reclassified as clear cell RCC with giant cells and emperipolesis. This is a new entity and was not described at the time of the original diagnosis.

**Table 5: Cases where the RCC subtype was reclassified after review**

| Case no | Case demographics | Original diagnosis | New diagnosis | Description | Reason for change of the diagnosis |
|---|---|---|---|---|---|
| 5/2005 | 26 years<br>Black female | RCC unclassified | MiT family translocation RCC – Xp11 translocation RCC | Histology shows a tumour with a papillary and solid architecture, with individual tumour cells having a predominantly clear cell appearance.<br>Immunohistochemical stain: TFE3 - Positive | TFE3 stain shows strong nuclear staining pattern in tumour cells. TFE3 positivity is diagnostic of an Xp11 translocation RCC |
| 2/2006 | 43 years<br>Black male | Type 1 pRCC | Type 2 pRCC | Histology shows a tumour with a papillary growth pattern, with papillae lined by pseudostratified epithelium with high nucleolar grade and abundant eosinophilic cytoplasm. | Diagnosis changed based on two pathologist's opinions |
| 1/2007 | 54 years<br>White male | ccRCC | ccRCC with giant cells with emperipolesis | Male patient with histology showing a biphasic tumour with a clear cell component and multinucleated giant cells with emperipolesis | Morphological features compatible with the newly recognised entity |
| 5/2007 | 66years<br>White male | ccRCC | ccRCC with giant cells with emperipolesis | Male patient with tumour necrosis and histology showing a biphasic tumour with clear cell component and syncytial-type giant cells with emperipolesis | Morphological features compatible with the newly recognised entity |
| 4/2011 | 60 years<br>White female | ccRCC | Chromophobe RCC | Histology shows a tumour with a solid sheet-like growth pattern separated by vascular septae. The tumour cells have eosinophilic cytoplasm with irregular nuclei with coarse chromatin and perinuclear halos<br>Immunohistochemical stains:<br>CK7 – Diffusely positive<br>CD117 – Focally positive<br>Vimentin - Negative | Diagnosis changed based on immunohistochemical stains |

## DISCUSSION

One hundred and five cases were identified in this study which reviewed a 10 year period from 2005 – 2014, compared to 107 cases found by Claassen *et al*. [25] in a fifteen year period from 1996 – 2010, which was performed at the same institution. This increase in the number of cases correlates with international trends, as the global incidence of RCC has slowly been increasing over the last four decades [3]. The male:female ratio of RCC in our study is 1.2:1, which is slightly lower than the global ratio of 1.5:1 as well as the South African national ratio of 1.7:1 as reported by GLOBOCAN 2012 and the NCR of South Africa in 2014 [6, 7]. Interestingly we also noted a reversal in the male:female ratio during the last three years of our study with a female predominance of 1:1.5. This correlates with reported findings from studies performed in Nigeria between 2000 and 2015 [8]. Additional follow up will be required to determine whether this is a consistent finding.

The overall ASR for the Free State Province of 0.4 per 100 000 population is considerably lower than the global ASR of 4.4 per 100 000 population and the ASR for the rest of Africa of 1.2 per 100 000 population [6]. The ASR for males of 0.4 per 100 000 and 0.3 per 100 000 for females is also considerably lower than the global ASR of 6.0 per 100 000 and 3.1 per 100 000 for males and females respectively as well as the ASR of 2.0 per 100 000 and 1.0 per 100 000 for the South African population as reported by the NCR in 2014 [6, 7]. The difference in ASR between Africa and regions and countries such as Europe and the United States of America is well documented and possible reasons include poor reporting practices, underdiagnoses due to limited access to healthcare services as well as lifestyle and genetic factors [2, 11, 26, 27]. Except for data from the NCR of South Africa no other studies could be found that calculated the incidence of RCC in South Africa. Therefore we could not compare incidence rates for RCC in the different provinces/regions. Differences in the ASR between this study and the NCR data may partly be due to the fact that only patients seen at state sector health care facilities were included while the NCR data also includes private patients.

The mean age of the patients in our study is 55.1 years, which is very similar to the mean age of 58.8 years as reported by Claassen et al. in our institution in 2011. However, this is still older than the mean age of onset in the rest of Africa, where most patients present in the fifth decade [25, 28]. The mean age is also considerably younger than findings from Europe and North America where the peak incidence is in the seventh decade [29]. Furthermore, we reported a slightly higher percentage of cases in patients younger than 40 years of age with

9.5%, when compared with the 5% incidence as reported by Thompson *et al.* in 2008 and Suh *et al.* in 2009 [23, 30]. This trend was also reported by Ahmed *et al.* from Nigeria with 40% of their cases presenting below 40 years of age [31].

When comparing the age distribution of our cases in black and white patients, we found that black patients present on average a decade earlier than white patients, with RCC being significantly more common in white patients in the seventh decade. The older age at presentation in white patients is similar to studies from the United States of America and Europe, while the age of patients in studies performed in African countries is even lower with a peak incidence in the fifth decade for black patients [28, 29].

In this study, 58.1% of the patients is black, 37.1% is white and 4.8% is coloured. The 2011 census determined that 87.6% of the Free State population was black, while 8.7% was white and 3.1% was coloured [24]. It therefore appears that RCC is more common among white patients in our community. This is contrary to international literature that shows that RCC is more common in the black population or is at the most comparable between the two races [12–14, 32]. However, our data is limited as our department only receives specimens from state health care facilities and specimens from private health care facilities are submitted to private pathology firms.

Accurate histological subtyping of RCC is critical for appropriate management and treatment of patients with RCC. The development of targeted therapies and immunotherapy has placed greater emphasis on the precise subclassification of RCC. Current evidence indicates that clear cell, chromophobe and papillary RCCs have shown promising responses to these new treatments [33]. Clear cell RCC is the most common subtype seen in our study population with 58.1% of cases which is lower than the 65% to 70% reported in the literature [3, 10, 19, 34]. A study done in Nigeria in 2015 reported an even lower percentage of 47.8% [28]. Their study is however limited due the small sample size of 23 cases. With regards to previous South African studies, we reported slightly more cases than Singh et al. but less than Du Plessis et al. with 54% and 74% of cases respectively [35, 36].

Similar to international literature, pRCC was found to be the second most common subtype in our population. Our incidence of 20.9% is very similar to the 19% reported by both Singh et al. and Du Plessis et al. [35, 36] However, it is higher than the 10-15% incidence seen in western literature but substantially lower than the 34% reported by Atanda et al. in a

systematic review of RCC in Nigeria [8]. Although there were only 5 cases of chromophobe RCC in our study, the 4.8% of cases is similar to the 5-7% reported in the international literature[3, 10].

The distribution of histological subtype between the black and white populations showed statistically significant differences for pRCC and ccRCC. Papillary RCC was found to be four times more common in black patients compared to white patients, while clear cell RCC was found to be more common in white patients with 76.9% compared to 47.5% in black patients. This is consistent with reported literature [12, 21, 22].

We identified five cases of MiT family translocation RCCs and all five cases were diagnosed in black females (P=0.08) of which three were younger than 40 years of age. Translocation RCC is known to be the most common subtype in young adults and children with a mean adult patient age of 31 years [37]. No evidence of a racial association with this entity could be found in the literature. The only currently known risk factor associated with translocation RCC is a history of previous chemotherapy [38]. In addition, we found that white males were twice more likely to be diagnosed with RCC than white females, but that there was no difference in incidence between males and females in the black patients.

One patient had a clear cell papillary RCC. This is a new entity that was added to the 2016 WHO classification of RCC's. These tumours have morphological features that can overlap with ccRCC, pRCC and translocation RCC. Clear cell papillary RCC, however, behaves in an indolent manner and has a good prognosis. Therefore, it is important to differentiate them with accuracy from the above mentioned more aggressive subtypes [39].

We also identified two cases that fit the diagnostic criteria for a new entity called, ccRCC with giant cells with emperipolesis. This tumour is classified as an emerging or provisional new entity that will be included in the new WHO classification [20]. Both our cases were described in white male patients, one in the sixth and one in the seventh decade of life. This is a rare tumour with a limited number of published cases. The cases that have been described were found to be more common in men with a mean age of 58 years [40, 41]. Identification of this subtype is of clinical importance as it has an overall high grade appearance and behave aggressively with distant metastasis [20].

**Conclusion:**

To our knowledge this is the most comprehensive study on RCC performed in our region. Our study demonstrated that the incidence of RCC in our population is lower than reported in the rest of the world. The age distribution correlates closely with other African studies, with black patients presenting a decade earlier than white patients. In addition, our findings identified distinct age, sex and racial differences for the various RCC subtypes, which warrants further research.

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APPENDIX A

Letters of approval from the Health Sciences Research Ethics committee

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

IRB nr 00006340
REC Reference nr 230408-011
IORG0005187
FWA00012784

06 December 2017

MULLER LOUIS
DEPT OF ANATOMICAL PATHOLOGY
FACULTY OF HEALTH SCIENCES
UFS

Dear Muller Louis

**HSREC 164/2017 (UFS-HSD2017/1432)**
**PRINCIPAL INVESTIGATOR: MULLER LOUIS**
**PROJECT TITLE: RENAL CELL CARCINOMA DIAGNOSED BY THE DEPARTMENT OF ANATOMICAL PATHOLOGY AT THE UNIVERSITY OF THE FREE STATE, SOUTH AFRICA: A 10 YEAR HISTOPATHOLOGICAL REVIEW**

**APPROVED**

1. You are hereby kindly informed that the Health Sciences Research Ethics Committee (HSREC) approved this protocol at the meeting held on 05 December 2017.

*Dr D Goedhals recused herself from the meeting for the duration of the discussion and decision regarding this protocol.*

2. The Committee must be informed of any serious adverse event and/or termination of the study.
3. Any amendment, extension or other modifications to the protocol must be submitted to the HSREC for approval.
4. A progress report should be submitted within one year of approval and annually for long term studies.
5. A final report should be submitted at the completion of the study.
6. Kindly use the **HSREC NR** as reference in correspondence to the HSREC Secretariat.
7. The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services-(HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

Yours faithfully

PROF WJ STEINBERG
VICE CHAIR: HEALTH SCIENCES RESEARCH ETHICS COMMITTEE

Health Sciences Research Ethics Committee
Office of the Dean: Health Sciences
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UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

**Health Sciences Research Ethics Committee**

29-Jun-2020

Dear **Dr Louis Muller**

Ethics Number: UFS-HSD2017/1432

Ethics Clearance: **Renal Cell Carcinoma diagnosed by the Department of Anatomical Pathology at the University of the Free State, South Africa: a 10 year histopathological review**

Principal Investigator: **Dr Louis Muller**

Department: **Anatomical Pathology Department (Bloemfontein Campus)**

**SUBSEQUENT SUBMISSION APPROVED**

With reference to your recent submission for ethical clearance from the Health Sciences Research Ethics Committee. I am pleased to inform you on behalf of the HSREC that you have been granted ethical clearance for your request as stipulated below:

- The type of study will change from: Descriptive study to descriptive study with an analytical component
- I will use the data already collected to calculate the age-standardized incidence rate of Renal cell carcinoma in the state sector of the Free State province. To do this I will need to use the population data for the Free State province as reported by Census 2011. This will be added as a reference.
- I will also then do subgroup comparisons for categorical variables.The distribution of patients with RCC will then be compared in terms of race, histological subtype, age and gender to investigate subtype-specific associations between the groups. To do this I will use the data already collected. No new data will be collected for this purpose.
- My protocol needs to be amended. The method will show the change in study type.
- The statistical analysis will change to indicate that we will calculate incidence and that we will be using the population data from Census 2011.
- Further in the statistical analysis, I will add that subgroup comparisons of categorical variables will be done using chi-squared or Fisher's exact tests.

The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

For any questions or concerns, please feel free to contact HSREC Administration: 051-4017794/5 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this request for ethical clearance and we wish you continued success with your research.

Yours Sincerely

Dr. SM Le Grange
Chair : Health Sciences Research Ethics Committee

**Health Sciences Research Ethics Committee**
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APPENDIX B

Permission letter from the NHLS

NATIONAL HEALTH LABORATORY SERVICE

18 October 2017

TO:

Dr. Muller
Registrar
Anatomical Pathology

Dear Dr. Muller

RE: Your study for M.Med.: Renal Cell Carcinoma diagnosed by the Department of Anatomical Pathology at the University of the Free State, South Africa: a 10 year histopathological review

Permission is granted herewith for you to use the archived patient records on TrakCare, DISALAb or otherwise hardcopy-archived at Anatomical pathology, Universitas Academic Laboratories, for the abovementioned study.

It is noted that there are no additional costs associated with the research study with exception of paper and printing costs that are to be borne by the Department of Anatomical Pathology University entity.

Please ensure that the NHLS is recognised in all publications emanating from the research.

Wishing you all the best in your research.

Prof. H. Pieters
Business Manager / Associate Professor
NHLS Universitas Academic Laboratories

Tel: 051 4050500
Fax: 051 4050515

APPENDIX C

Copy of research protocol

# Renal Cell Carcinoma diagnosed by the Department of Anatomical Pathology at the University of the Free State, South Africa: a 10 year histopathological review

## RESEARCHERS


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3. Prof J Goedhals
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## INTRODUCTION

Renal cell carcinoma (RCC) connotes a group of neoplasms having a common origin from the epithelium of the renal tubules but having distinct morphologic and genetic features. They comprise a variety of distinct clinicopathological entities, each of which displays different morphological, immunohistochemical and molecular features. In the past decade, advances in our understanding of these features have led to an expansion in the number of distinct tumour entities that we currently recognize.

### Epidemiology

Approximately 2% of all new cancer cases worldwide originate in the kidney [1]. The incidence and mortality rates have been increasing in many countries, approximately 70% of the new cases occurred in countries with high and very high levels of socioeconomic development [2]. Globally, the incidence of renal cell carcinoma varies widely from region to region. The highest incidence rates are found in the Czech Republic, followed by the rest of eastern and western European countries, Italy, North America, Australia, and New Zealand and the lowest rates are in East Asian and African countries [2]. Although the reported incidence of RCC in Africa is also relatively low, African Americans in the United States have a higher incidence of RCC compared with the US white population [3,4]. This may well be multifactorial, and it is presumably related to the lower rates of imaging use and early RCC diagnosis in African countries.

RCC is approximately 50 percent more common in men compared with women and occurs predominantly in the sixth to eight decade of life [1]. RCC is unusual in patients under 40 years of age and rare in children [5]. The incidence has been increasing more rapidly in women than in men, as well as more rapidly in African Americans than in white Americans [6].

Obesity, smoking, hypertension or its treatment, end-stage renal disease/acquired cystic kidney disease and exposure to asbestos, petroleum products and heavy metals are currently being considered the highest risk factors associated with developing RCC. [7, 8, 9, 10, 11, 12]. No evidence thus far has been found to establish a significant relationship between the occurrence of RCC and the consumption of alcohol, coffee or analgesic use [13]

### Genetic susceptibility

Although most renal carcinomas are sporadic, 2-4% is associated with several distinctive hereditary cancer syndromes [14]. The risk of developing RCC is two to three times higher in individuals who have a first-degree relative with RCC [15]. Factors that favour a hereditary contribution in patients without a clear genetic disease include first degree relatives with a tumour, onset before the age of 40, and bilateral or multifocal disease [16].

### Pathogenesis

Because several hereditary RCC syndromes are known, genetic and epigenetic changes in the carcinogenesis of renal cancer are strongly suspected [17].

**1. The hypoxia inducible factors pathway in clear cell renal cell carcinoma and the von Hippel-Lindau syndrome** - The genetic association of RCC associated with the von Hippel-Lindau (VHL) gene with chromosomal loss in
3p25-26 was found in 1993. Mutations of the VHL gene were also found in 60–80% of sporadic RCC [18].

**2. The mesenchymal-epithelial transition (MET) pathway and hereditary papillary renal cell carcinoma** - The MET gene is located at chromosome 7q31. Gain-off- function mutations result in a consecutive activation of the receptor [19].

**3. The fumarate hydratase gene and the hereditary leiomyomatosis renal cell carcinoma** - Mutations in the fumarate hydratase gene (FH), located at chromosome 1q42, are found in patients with hereditary leiomyomatosis RCC [20].

**4. The folliculin gene and the Birt-Hogg-Dubé syndrome** - Birt-Hogg-Dubé (BHD) syndrome is characterised by loss of function mutations in the folliculin gene (FLCN) at chromosome 17p11.2. Renal tumours of BHD show variable histology like chromophobe RCC, ccRCC, oncocytoma, and oncocytic-chromophobe hybrid [20, 21].

**5. The phosphatidylinositol-3-kinase/Akt pathway -** Changes in the phosphatidylinositol-3-kinase/Akt pathway are known to be involved in the oncogenesis of many carcinomas [22]
**6. The mammalian target of rapamycin pathway -** MTOR is a multifunctional serine-threonine kinase that plays a central role in the regulation of cell growth, proliferation, apoptosis, and metabolism of the cell.

**7. Other genetic aberrations -** Constitutional chromosome 3 translocations are also found in patients with familial RCC. The tuberous sclerosis complex (TSC) is associated with inactivating mutations of TSC1 at 9q34 (hamartin) or of TSC2 at 16p13.3 (tuberin). TSC1 and TSC2 inhibit mTOR, and therefore these patients show an increased risk for RCCs [23, 24].

**8. Epigenetic alterations in renal cell carcinoma –** including DNA hypermethylation and histone modifications.

## Classification of Renal cell carcinomas

As the histological classification of RCC has developed, the terminology used for designating its subtypes has referred to various descriptive or characteristic features. Subtypes have been named on the basis of predominant cytoplasmic features and staining characteristics, architectural features, cell type and combinations of these features.

The classification of renal cell carcinomas is based on correlative cytogenetic, genetic and histological studies of both familial and sporadic tumours.

Classification of renal cell carcinomas according WHO – 2016 [2]:

- Clear cell carcinoma (most common)
- Multilocular cystic renal neoplasm of low malignant potential
- Papillary renal cell carcinoma (2nd most common)

- Hereditary leiomyomatosis and renal cell carcinoma-associated renal cell carcinoma
- Chromophobe renal cell carcinoma
- Collecting duct carcinoma
- Renal medullary carcinoma
- MiT family translocation renal cell carcinoma (Xp11 translocation)
- Succinate dehydrogenase-deficient renal cell carcinoma
- Mucinous tubular and spindle cell carcinoma
- Tubulocystic renal cell carcinoma
- Acquired cystic disease-associated renal cell carcinoma
- Clear cell papillary renal cell carcinoma
- Renal cell carcinoma, unclassified

## Morphologic features

On macroscopic examination, most RCC's are usually well circumscribed, centred on cortex, may bulge and distort kidney contour. They often extend into renal vein or vena cava. Renal sinus invasion is common in large tumours. Satellite nodules, haemorrhage, necrosis, calcification and cystic change may often be present. In a typical case of renal cell carcinoma the cut surface shows a solid, golden-yellow tumour, sharply separated from the surrounding tissues by a fibrous pseudocapsule.

Next follows the morphologic features of the most common RCCs.

**Clear cell RCC** — macroscopically, they may be solid or less commonly, cystic. It is usually unilateral and unicentric. The tumour usually has an expansile pushing growth pattern and is well demarcated from adjacent normal kidney. Microscopically, clear cell carcinoma displays a variety of growth patterns, most often forming sheets, nests, alveolar, acinar and microcystic structures, separated by an abundance of blood vessels. The classic clear cell of ccRCC has distinct cell membranes and clear cytoplasm. The nuclei show considerable variation in size, shape and nucleolar prominence [25].

**Papillary RCC** — macroscopically, they are typically well circumscribed and mostly confined to the renal parenchyma [26]. They may be multifocal, particularly in cases of hereditary papillary RCC [27]. A thick pseudocapsule is present in up to two thirds of cases [28, 29]. Microscopically, they are composed of varying proportions of papillary and tubular structures. The papillae are lined by a single layer of tumour cells that may sometimes appear pseudostratified [28]. Papillary RCC can be sub-classified into two morphologic variants [30]:

Type 1 - is composed of papillae covered by a single or double layer of small epithelial cells of low nuclear grade. Aggregates of foamy macrophages are commonly noted within the papillary cores.
Type 2 - tends to be larger than type 1 is of significantly higher nuclear grade.

**Chromophobe RCC** — histologically, they are composed of sheets of cells that are darker than clear cell carcinoma. They lack the abundant lipid and glycogen that is characteristic of most RCCs [31]. The tumour is intersected randomly by delicate to broad fibrous septa and blood vessels. Two types of tumour cells may be present, the large chromophobe cell mixed with smaller cells with less abundant cytoplasm. The larger cells classically have reticulated cytoplasm and a prominent cell border. The nuclei often have a distinct irregular nuclear

membrane with wrinkled (raisinoid) appearance with coarse chromatin, commonly binucleation and perinuclear haloes [31].

**Collecting duct RCC** - these tumours are rare, they tend to occur in younger patients and are frequently aggressive [32]. Macroscopically they are usually located centrally and localized to a medullary pyramid. They are grey-white and firm on cut surface with and indistinct tumour border. The majority of cases shows involvement of the adrenal gland, perirenal fat, renal sinus fat, renal pelvis, renal vein and regional lymph node involvement. Microscopically, they show a complex tubulopapillary architecture. The borders are ill defined with extensive infiltration of adjacent parenchyma and a prominent inflammatory cell infiltrate. Prominent stromal desmoplasia is a constant feature. The tumour cells are of high grade with prominent nucleoli [33].

**Renal Medullary carcinoma** - which is a highly aggressive variant of collecting duct carcinomas, is associated with the sickle cell trait and develops in young patients [34]. Macroscopically they typically occupy the renal medulla and are poorly circumscribed, lobulated and firm to rubbery with areas of haemorrhage and necrosis. Microscopically the most characteristic finding is a reticular or microcystic growth pattern. Tumour cells are typically pleomorphic, with large nuclei and prominent nucleoli. They may have a rhabdoid appearance with abundant neutrophils in the tumour. The majority shows mucin production. Sickled erythrocytes are present on most sections [25].

**MiT family translocation RCC** — A distinct variant of RCC, referred to as translocation carcinoma, is associated with fusion of the TFE3 gene to a number of other genes including ASPL and PRCC on chromosome Xp11.2 [35]. Translocation carcinoma tends to occur at a younger age compared with other RCCs [36]. Histologically they are diverse, often having papillary, alveolar and nested growth patterns with clear and eosinophilic cells and psammoma bodies. t(6;11) RCCs are rearranged carcinomas that are characteristically biphasic, with small cells clustered around basement membrane material (reminiscent of Call-Exner bodies in adult granulosa cell tumour) and larger epithelioid cells.

## Immunohistochemical markers for RCC

Diagnosis and classification of renal cell tumours are usually straightforward based on gross and microscopic examination. Immunohistochemistry however has been increasingly used in the workup of difficult cases. It is mainly used to verify histological subtypes and to distinguish RCC's from rare metastatic tumours to the kidney or to confirm the renal origin in a metastatic tumour.

The following immunohistochemical panel is recommended in the workup of challenging renal epithelial cell tumours: PAX8/PAX2, CD10, CK7, CAIX, AMACR, CD117, Ksp Cadherin, Cathepsin K, Vimentin, TFE3, HMB45, Melan A, 34BE12, p63, INI1, SDHB [37].

## South African data:

Very little information is available on the demography, prevalence and histopathological profile of RCCs in a South African population. The only information that could be found was by Claassen et al [38]. Their study stretched over a 15 year period from 1996 to 2010. They found that black patients presented with renal cell cancer at a younger age (mean age 54.2 years) compared to white patients (mean age 70 years). They also found that patients with

HIV presented at a younger age (mean 32 years) compared to patients that tested negative for HIV (mean 62 years).

## AIMS

The aim of the study is to determine the profile of RCC's in the public sector that presented to the Department of Anatomical Pathology at the University of the Free State.

Objectives:

1. To determine the number of cases of RCC seen over a ten year period from January 2005 to December 2014 by the Department of Anatomical Pathology, University of the Free State and NHLS.
2. To determine the age-standardized incidence rate for the state sector in the Free State province.
3. To evaluate the demographic features of patients diagnosed with RCC.
4. To determine the histological spectrum (subtypes) of RCC's that was diagnosed.

## METHODOLOGY

### Study design

A retrospective descriptive study with analytical component will be performed. All cases processed and histologically confirmed as renal cell carcinoma in the ten year period from 1 January 2005 to 31 December 2014 at Universitas Academic Hospital and NHLS Bloemfontein, will be included in this study. Cases will be identified by performing a computerized SNOMED search of the NHLS electronic databases. The DISA system will be searched for the years 2005 to 2014 and the Labtrack system will be searched for the year 2014. The department of Anatomical Pathology at the University of the Free State and the NHLS provides histology services to all public hospitals and clinics in the Free State Province and occasionally also provide the service to hospitals from the North West and Northern Cape province.

### Study participants

Inclusion criteria:

- All renal cell carcinomas seen and diagnosed by the Anatomical Pathologists at the department of Anatomical Pathology at the University of the Free State.

Exclusion criteria:

- All repeat specimens. (In cases already diagnosed on biopsy for which we then received a resection specimen, only the one diagnosis will be included)
- All cases with missing slides and wax blocks.

We estimate that approximately 100 cases will meet the inclusion criteria.

## Measurements

Once the cases have been identified, the slides will be retrieved from the departmental archives and then be reviewed by Dr Muller. If the cases meet the inclusion criteria, they will be reviewed by Dr van der Westhuizen together with Dr Muller in terms of specific subtype, based on characteristic histological features and immunohistochemical stains performed. The specific subtype of renal cell carcinoma and the demographic data, including the patient's age, gender and race will be noted which will be obtained from the pathology reports. The data will then be captured on a data sheet for statistical analysis.

The age-standardized incidence rate (ASR) will then be calculated only for the patients from the Free State province. This will reflect the incidence for the state sector only. The distribution of patients with RCC will then be compared in terms of race, histological subtype, age and gender to investigate subtype-specific associations between the groups.

A pilot study of the first ten cases will be done to test the data sheet. These cases will also be included in the study.

## PILOT STUDY

The pilot study will be executed after approval has been received from the Health Sciences Research Ethics Committee. The researchers will use a process of simple random sampling to select 10 cases from each of the years between 2005 and 2014. A number will be allocated to each case to ensure that the data remains anonymous. These cases will be processed using the data sheet form in Appendix 1, to ensure that the selected headings used on the data sheet are adequate. Once the data has been collected any necessary changes will be made to the data sheet before the main study is initiated. The results of the pilot study will be included in the main study sample.

## METHODOLOGICAL AND MEASUREMENT ERRORS

It is assumed that race cannot be purely defined and remains debatable, but different racial backgrounds and cultural differences are well documented. In this study, race will be classified according to the information provided by the patient on admission to hospital. All cases where the diagnosis was changed to one of the newly recognised entities will be reviewed by Prof Goedhals. All data will be entered onto the data sheet by Dr Muller and will then be reviewed by Dr van der Westhuizen to check for incorrect data entry.

## STATISTICAL ANALYSIS

Analysis of the data collected will be performed by the researchers and the Department of Bio-Statistics at the University of the Free State. Data will be captured on an Excel worksheet and will be analysed using categorical and continuous variables. The data will be expressed as frequencies, mean, median and range. Age-standardized incidence rates will be calculated using the Free State province's population data from the South African census as performed in 2011 (39). Subgroup comparisons of categorical variables will be done using chi-squared or Fisher's exact tests.

## PROPOSED TIME SCHEDULE

The study should take approximately 18 months to complete once approval has been obtained from the Health Sciences Research Ethics Committee.

Submission for Ethics approval: October 2017
December 2017 – Pilot study
January - December 2018: Identification and review of cases and data collection
January - June 2019: Analysis and write up

## BUDGET

Paper and printing costs: Approximately R500 - will be paid for by the Department of Anatomical Pathology's research entity.

## ETHICAL CONSIDERATIONS

The study protocol will be submitted to the Health Sciences Research Ethics Committee at the University of the Free State. A report on conclusion of the research will be submitted to the Ethics Committee.

All data will be treated as confidential. New unique study numbers will be assigned to each case to replace the hospital numbers and names of the patients. All precautions will be undertaken to ensure patient confidentiality as well as privacy of the research data.

Data collection and data analysis will be performed using only the unique study number in order to protect the participant's identities. The data file will be stored by the researcher in the Department of Anatomical Pathology, at the University of the Free State.

No individual patient consent will be obtained. Permission for the study will be obtained from Dr J Naicker, the Head of the School for Pathology at the University of the Free State and NHLS.

As only data from the NHLS pathology reports will be used it will not be necessary to obtain permission from the Free State Department of Health.

## Proposed outcome

The proposed project will be completed in fulfilment of the requirements for a MMed degree for Dr L Muller under the supervision of Dr G van der Westhuizen. The results will be submitted for publication in a peer reviewed journal.

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APPENDIX D

Data collection form

| Study number | Year of biopsy | Age | Race | Sex | Type of specimen | Original Diagnosis | Immunohistochemical stain results | Diagnosis after review |
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APPENDIX E

Instructions for authors African Journal of Urology

# African Journal of Urology

## Author Guidelines

The *African Journal of Urology* welcomes original papers, case reports and letters to the editor from any country of the world, particularly from Africa. Review articles are usually commissioned, but the Editorial Board are happy to discuss potential articles with authors who would like to contribute.

Mailing Address: All correspondence should be sent to the following email address: eman_gawad@hotmail.com

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Authorship: All authors must give signed consent to publication.

Review Process: All manuscripts received are subject to review by at least 2 internationally renowned experts on the subject. Authors will receive the reviewers ‘comments and their reply will be transferred back to the reviewers.

Format: Articles are accepted in English or French language. They should be carefully reviewed for accuracy of typing, spelling and grammar before they are submitted, since these mistakes might delay the acceptance of the article. They should be written in double spacing. The computer program used should be MS Word, and the articles should be sent as Word documents. Authors should retain a copy of the article for references.

Organization: Original articles on clinical and scientific aspects of urology and its associated specialities should be organized as follows:
The title page should give the following information: (a) title of article (b) names and initials of authors (c) institution to which the work should be attributed. (d) 1 - 5 key words should be typed at the bottom of the page (e) name and full postal address, telephone, fax number and Email address of the author to whom the reviewers‘ comments and requests for reprints should be sent (f) running title. The title should be concise and clear and should not contain abbreviations.

The abstract should consist of a brief summary of the article and should be subdivided into objective, patients/material and methods, results, conclusion. The abstract should be self-explanatory, without reference to the text. Abbreviations may be included, provided they are defined in the abstract as well as in the main text.

The introduction should be short and include both a brief review of the data in the literature which are strictly related to the subject and express the exact aim of the work.

Material/Patients and Methods: The patients' characteristics and the technique(s) applied should be described in detail. The statistical analysis method should be exactly defined and its reference should be mentioned.
Results: The results of the work should be presented in detail. The number of patients should be followed by the percentage. Results that are presented in tables should not be repeated in the Results section.

The Discussion should be limited to the reported findings and their implications. Case reports should be preceded by a short abstract. The case report itself should include a brief description of the case and a comment section discussing the salient features of the case in the context of the world literature.

References: The author is responsible for the accuracy of his references. Every author is kindly requested to check his references via the pubmed website www.ncbi.nlm.nih.gov/entrez before submitting the articles. References should conform to the Vancouver style and should be numbered consecutively in the order in which they appear (and not listed alphabetically). They should be indicated by Arabic numerals in parentheses. Only the first six authors should be listed. If there are more than six then the first six should be listed followed by et al. Note that journal titles are abbreviated in accordance with *Index Medicus.*

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3. References to a chapter in a book (or paper in a Proceedings): Tabatabaei S, McDougal WS. Invasive carcinoma of the penis: Management and prognosis. In: Richie JP, D'Amico A, editors. Urologic Oncology, Philadelphia: ELSEVIER Saunders; 2005. p. 710-22.

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Tables: The tables should be self-explanatory and should supplement, not duplicate, the information in the text. Tables should be numbered consecutively with Arabic numerals. Each table should have a concise heading and should be comprehensible without reference to the text. Care should be taken that the numbers and percentages in the tables are exactly the same as those mentioned in the abstract and the text.

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APPENDIX F

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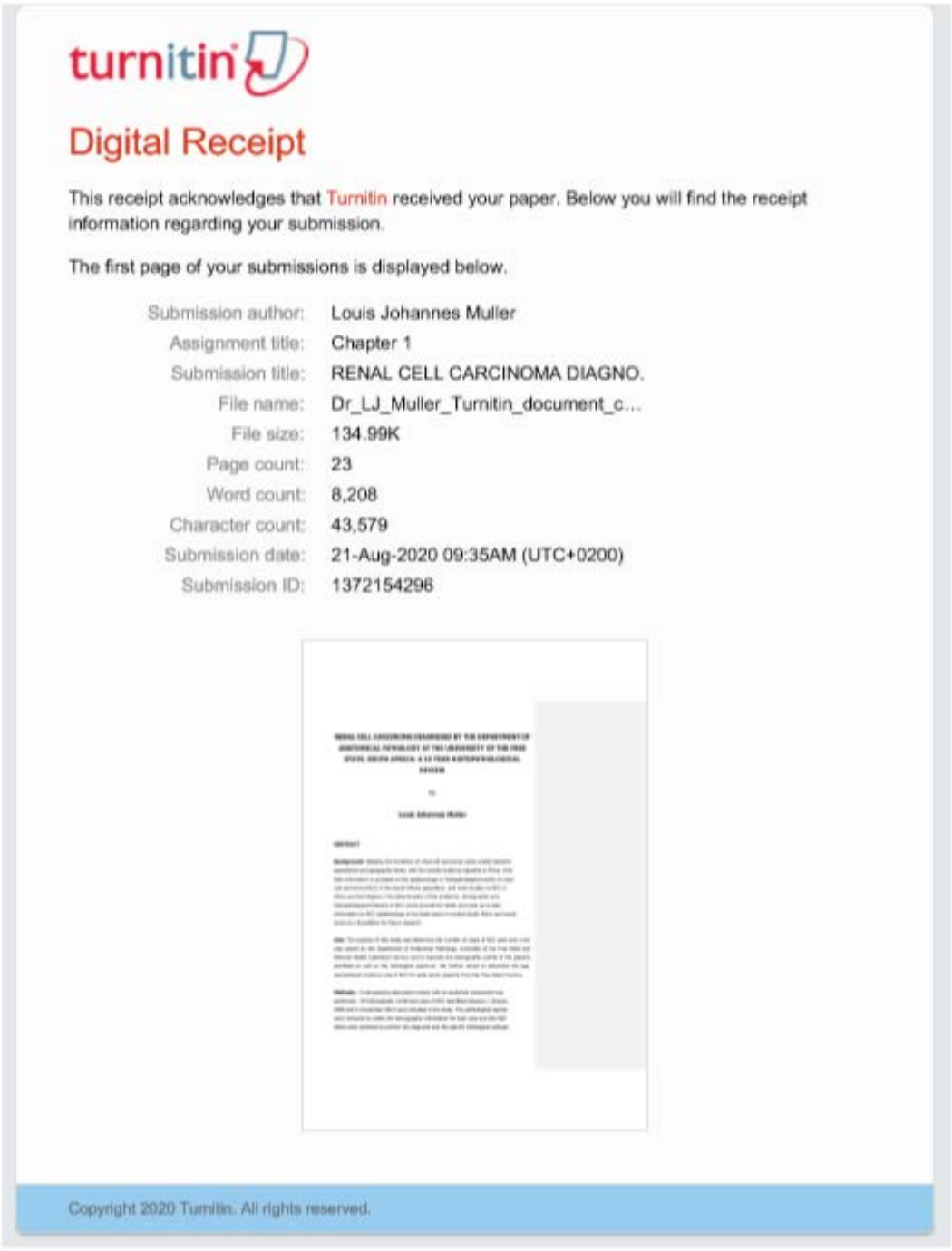

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# RENAL CELL CARCINOMA DIAGNOSED BY THE DEPARTMENT OF ANATOMICAL PATHOLOGY AT THE UNIVERSITY OF THE FREE STATE, SOUTH AFRICA: A 10 YEAR HISTOPATHOLOGICAL REVIEW

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