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Evaluation of Survivin Expression in Renal Cell Carcinoma by Immunohistochemistry and Studying its Correlation with Clinicopathological Features and Prognosis

A Thesis Submitted in Partial Fulfillment of the Requirement for the Master Degree in Pathology.

By

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Dedication

I'm pleased to dedicate this work to my parents for their care and support.

Also, I am happy to express my gratitude to my husband, Dr. Walid Alagouri, my son Mohammed, and my daughter Kanzy for their patience and endless love and support.

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Abstract

Background. The aim of the current study is to cast further light on the issues related to prognostication of renal cell carcinoma (RCC) and assessing the expression of Survivin in a subset of primary RCC and determine its relation to different clinicopathological features and disease free survival in patients from Eastern Libya .

Patients and methods. The present series consisted of tissue samples obtained from 37 Libyan patients with stage I, II, III, or IV Renal cell carcinoma during 2003-2012. Archival paraffin-embedded samples obtained from Benghazi University Laboratories. Survivin expression was assessed by immunohistochemistry using an automated staining system. Different grading systems were tested for expression of Survivin.

Results. There was no significant correlation between Survivin expression and gender, age, histological grade, distance metastasis, lymph node involvement, perinephric fat and capsular invasion, status at end point and recurrence. However, expression of Survivin was significantly associated with venous invasion (Tumor thrombus) ($P< 0.042$), larger tumor size ($P< 0.051$), higher primary T classification ($p< 0.013$), advanced tumor stage ($p< 0.033$), and borderline association ($p< 0.068$) with Tumor location. In univariate (Kaplan-Meier) survival analysis, Survivin expression showed a borderline association ($P< 0.089$) with disease-free survival(DFS).

Conclusion: The present study shows statistically significant correlation between Survivin expression and venous invasion (tumor thrombus), larger tumor size, advanced tumor stage. Also associated significantly with primary tumor classification (pT1, pT2 VS pT3; $P< 0.013$), shows a borderline association with tumor location. Survivin expression in RCC may identify patients at risk of a more aggressive disease and a worse prognosis. further investigations, on a larger and more heterogeneous population, should be carried out to validate and extend our results.

Key words: Survivin expression, renal cell carcinoma, IHC, prognosis, survival analysis.

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List of abbrevations

ADPKD	Autosomal dominant polycystic kidney disease
AJCC	American Joint Committee on Cancer
APTT	Activated partial thromboplastin time
AUA	American Urological Association
BDC	Bellini duct carcinoma
BCR	Benghazi cancer registry
BMI	Body mass index
BHD	Birt-Hogg-Dube syndrome
B7H1	B7 homolog 1\ family of the immunoglobulin
BIRC5	Baculoviral inhibitor of apoptosis repeat-containing 5gene
CT	Computed tomography
C-IAPl	Cellular IAP 1
C-IAP2	Cellular IAP 2
Cp-IAP	Cydia pomonella IAP
CcRCC	Clear cell renal cell carcinoma
chRCC	Chromophobe renal cell carcinoma

CAIX	Carbonic anhydrase 9
CBC	Complete blood cell count
CSS	Cancer specific survival
DNA	Deoxyribonucleic acid
DSS	Disease-specific survival
ESR	Erythrocyte sedimentation rate
GFR	Glomerular filtration rate
HPRC	Hereditary papillary renal carcinoma
HIF-1a	Hypoxia induced factor1 alpha
IHC	Immunohistochemistry
IMP3	Insulin-like growth factor II mRNA-binding protein
IAP	Inhibitor of apoptosis protein
IFN-α	Interferon-alpha
IL-2	Interleukin-2
IVC	Inferior vena cava
Ki 67	Proliferation factor
LFTs	Liver function tests

mRCC	metastatic renal cell carcinoma
MtDNA	mitochondrial DNA
MRI	Magnetic resonance imaging
mTOR	mammalian target of rapamycin
MRNA	messenger ribonucleic acid
MSKCC	Memorial Sloan-Kettering Cancer Institute
MM	Molecular marker
NSAIDs	Non-steroidal anti-inflammatory drugs
NCI	National cancer institute
NCCN	National Comprehensive Cancer Network.
NAIP	Neuronal apoptosis inhibitory protein
PT	Prothrombin time
P53	Tumor suppressor gene\protein p53
PRCCs	Papillary renal cell carcinoma
PFS	Progression-free survival
Op-IAP	Orgyia pseudotsugata IAP
RR	Relative risk

RCC	Renal cell carcinoma
SEER	Surveillance, Epidemiology, and End Results
TNM	Tumor node metastasis classification
UICC	Union International contra Cancer
VEGF	Vascular endothelial growth factor
VHL	Von Hippel-Lindau syndrome\ gene
WHO	World Health Organization
XIAP	X-chromosome-linked IAP

Chapter I

Introduction
and
aims of the study

Introduction

Renal cell carcinoma (RCC) also called hypernephroma. These tumours are derived from the renal tubular epithelium; hence they are predominantly located in the cortex (Kumar, 2007). RCC represents about 3% of all newly diagnosed visceral cancers in the United States of America and accounts 85% of renal cancer in adults. There are approximately 30,000 new cases per year, and 12,000 deaths from the disease (Kumar, 2010). The tumours are often among all ethnic groups and geographic areas, with highest incidence reported in northern Europe and North America, and lowest incidence in Asian countries, central and South America (Myoad, 2001).

In United States of America, in 2010 approximately 58,000 people were diagnosed and about 13,000 died from RCC (Jemal et al., 2010). Worldwide, there were an estimated 270,000 cases and 116,000 deaths in 2008 (Ferlay et al., 2010). In a previous study conducted in Eastern Libya, a total of 23 renal cancers were diagnosed in Benghazi cancer registry (BCR) during 2004, with predominance in male (16 male and 7 female). overall, cancer of kidneys and other urinary organs except urinary bladder account for 2% of all cancer in BCR with crude rate 2.07/100000 in male and 0.91/100000 in female (El Mistiri, 2004). RCC occurs predominantly in the 6^{th} to 8^{th} decades of life, with median age at diagnosis about 64 years of age, according to 2003 to 2007 National cancer institute (NCI), Surveillance, Epidemiology, and End Results (SEER) it is unusual in patients under 40 years of age (Siemer et al., 2006; Thompson et al., 2008). RCC is rare in children (Cook et al., 2006).

Risk factors. A number of environmental and clinical factors have been implicated in the etiology of RCC. These include smoking, hypertension, occupational exposure to toxic compounds, obesity, acquired cystic disease of the kidney (typically associated with dialysis), analgesic abuse nephropathy, and genetic predisposition (Mandel et al., 1995). Classification of RCC is based on correlative cytogenetic, genetic and histologic studies of both familial and sporadic tumours. According to WHO review in 2004, RCC classified into, clear cell (multilocular cystic) renal cell carcinoma, papillary carcinoma, chromophobe renal carcinoma, collecting duct carcinoma, medullary, mucinous tubular and spindle cell, associated with XP11.2 translocation/TFE 3 gene fusion, unclassified (Eble et al., 2004).

Apoptosis is a pathway of cell death that induced by tightly regulated suicide program in which cell, destined to die (Kumar, 2010). Disruption in such process as to impair the ability of the cell to undergo normal apoptosis. This results in a cell that lives past its "use-by-date" and is able to replicate and pass on any faulty machinery to its progeny, increasing the likelihood of the cell becoming cancerous or diseased. Apoptosis has been proposed to play role not only in cancer onset and progression, but also in sustained decrease in tumour cell sensitivity to chemotherapy (Hickman 1992; Thompson 1995). Delicate balance between pro-apoptotic and anti-apoptotic mechanism determines whether cell death signals can activate the execution of apoptotic program, in this balance, pro-apoptotic protein promote apoptosis, and anti-apoptotic protein inhibit apoptosis **(**Yunbo et al., 2003).

Survivin a novel member of IAP family, reduces the susceptibility of the tumour cell to pro-apoptotic stimuli thereby promoting tumour cell survival during tumour progression and treatment with anticancer drugs (Mahotka et al., 2002). The Survivin protein functions to inhibit caspase activation thereby leading to negative regulation of apoptosis. This has been shown by disruption of Survivin induction pathway leading to increase in apoptosis and decrease in tumour growth (Sah, 2006). Two novel splice variants of Survivin have been identified, Survivin delta-Ex3 (lacking exon 3), Survivin-2B (retaining a part of intron 2 as a cryptic exon). The role of the novel isoforms in the regulation of apoptosis was assessed experimentally showing conservation of anti-apoptotic properties for Survivin delta-Ex3 and a markedly reduced anti-apoptotic potential for Survivin-2B. Survivin variants generated by alternative splicing in human RCCs are intrinsically involved in the complex regulation of apoptosis (Mahotka et al., 1999).

Survivin, an important inhibitor of apoptosis has been found to play an important role in the initiation, progression, and chemo-radio resistance of human malignancies. In addition, patients with high Survivin levels had a significantly shorter overall survival than those with low levels the expression of Survivin protein was an independent prognostic factor for RCC patients. Recently, studies show Survivin knockdown could inhibit growth and enhance in *vivo* radio-sensitivity of RCC cell line by inducing apoptosis enhancement.

The status of Survivin protein expression may be an independent factor for predicting the prognosis of RCC patients and tumor-specific Survivin knockdown combined with radiotherapy will be a potential strategy for RCC therapy (Lei et al., 2010).

Aims of the study

1. Evaluation of Survivin expression in RCC in patients from Eatern Libya by using the immunohistochemical technique.
2. Correlation of Survivin expression with clinicopathological features in patients from Eatern Libya.
3. Evaluation of prognostic role of Survivin in RCC in patients from Eatern Libya.

Chapter II

Review of the literature

Kidneys

The kidney is a structurally complex organ that has evolved to carry out a number of important functions, excretion of the waste products of metabolism, regulation of body water and salt, maintenance of appropriate acid balance, and secretion of a variety of hormones and autacoids.

Embryology of the kidney

The development of the kidney proceeds through a series of successive phases, the pronephros, mesonephros, and metanephros (Fig. 2-1).

Pronephros

It develops in the cervical region of the embryo. During approximately day 22 of human gestation, the paired pronephroi appear towards the cranial end of the intermediate mesoderm. In this region, epithelial cells arrange themselves in a series of tubules called nephrotomes and join laterally with the pronephric duct. This duct is fully contained within the embryo and thus, cannot excrete filtered material outside the embryo. Therefore the pronephros is considered nonfunctional in mammals.

Mesonephros

The development of the pronephric duct proceeds in a cranial-to-caudal direction. As it elongates caudally, the pronephric duct induces nearby intermediate mesoderm in the thoracolumbar area to become epithelial tubules called mesonephric tubules. Each mesonephric tubule receives a blood supply from a branch of the aorta, ending in a capillary tuft analogous to the glomerulus of the definitive nephron. The mesonephric tubule forms a capsule around the capillary tuft, allowing for filtration of blood. This filtrate flows through the mesonephric tubule and is drained into the continuation of the pronephric duct, now called the mesonephric duct or Wolffian duct. The nephrotomes of the pronephros degenerate while the mesonephric duct extends towards the most caudal end of the embryo, ultimately attaching to the cloaca. The mammalian mesonephros is similar to the kidneys of aquatic amphibians and fishes.

Metanephros

During the fifth week of gestation, the mesonephric duct develops an outpouching, the ureteric bud, near its attachment to the cloaca. This bud, also called the metanephrogenic diverticulum, grows posteriorly and towards the head of the embryo. The elongated stalk of the ureteric bud, called the metanephric duct, later forms the ureter. As the cranial end of the bud extends into the intermediate mesoderm, it undergoes a series of branchings to form the collecting duct system of the kidney. It also forms the major and minor calyces and the renal pelvis. The portion of undifferentiated intermediate mesoderm in contact with the tips of the branching ureteric bud is known as the metanephrogenic blastema. Signals released from the ureteric bud induce the differentiation of the metanephrogenic blastema into the renal tubules. As the renal tubules grow, they come into contact and join with connecting tubules of the collecting duct system, forming a continuous passage for flow from the renal tubule to the collecting duct. Simultaneously, precursors of vascular endothelial cells begin to take their position at the tips of the renal tubules. These cells differentiate into the cells of the definitive glomerulus. In humans, all of the branches of the ureteric bud and the nephronic units have been formed by 32 to 36 weeks of gestation. However, these structures are not yet mature, and will continue to mature after birth. Once matured, humans have an estimated one million nephrons (approximately 500,000 per kidney) or more (Bruce, 2004).

Migration

After inducing the metanephric mesenchyme the lower portions of the nephric duct will migrate caudally and connect with the bladder, thereby forming the ureters. The ureters will carry urine from the kidneys to the bladder for excretion from the fetus into the amniotic sac. As the fetus develops, the torso elongates and the kidneys rotate and migrate upwards within the abdomen which causes the length of the ureters to increase (Bruce, 2004).

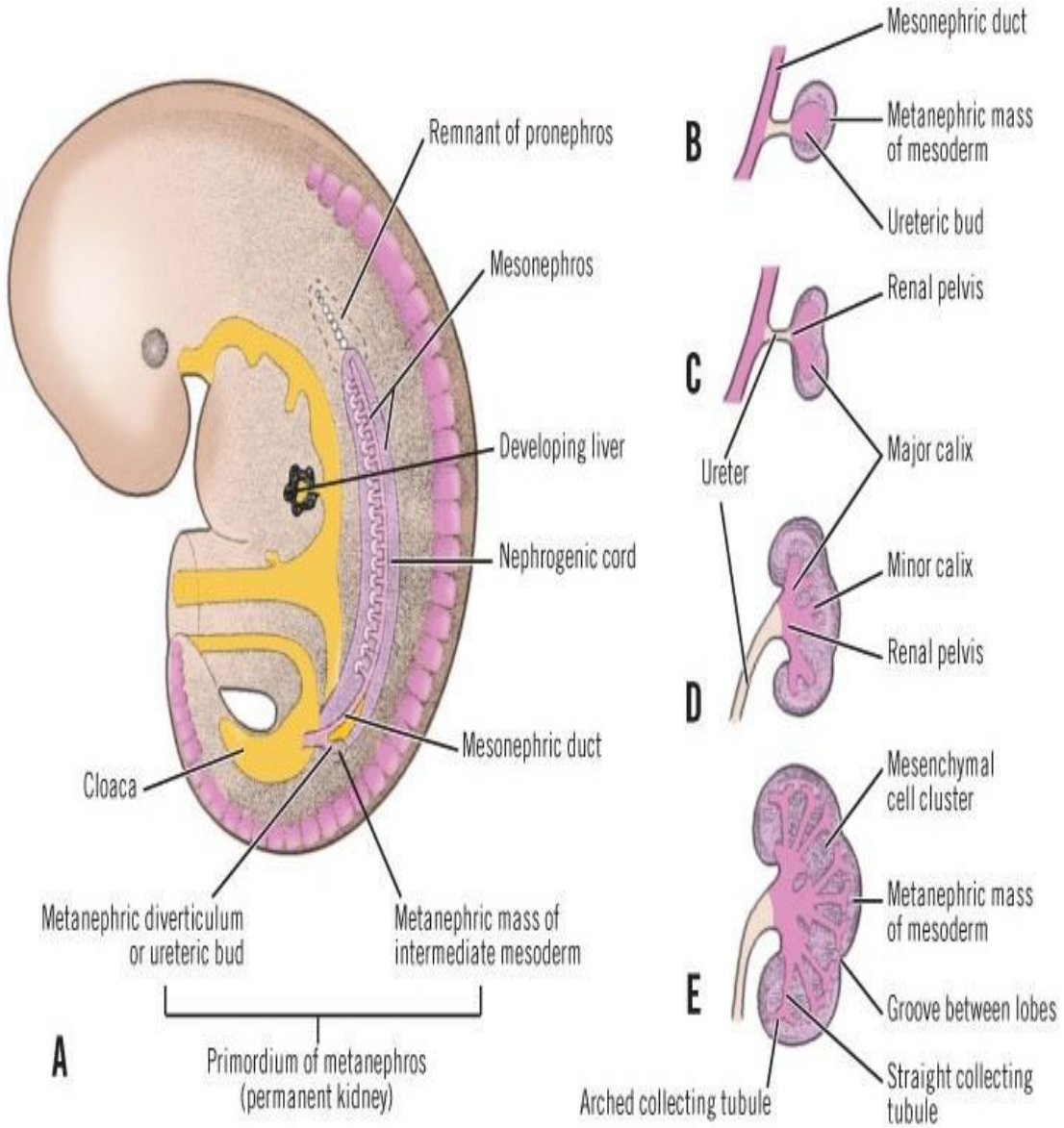


Fig. 2-1 Renal emberology web.uni-plovdiv.bg

Anatomy of the kidney

The Kidneys are situated in the posterior part of the abdomen, one on either side of the vertebral column, behind the peritoneum, and surrounded by a mass of fat and loose areolar tissue. Their upper extremities are on a level with the upper border of the twelfth thoracic vertebra, their lower extremities on a level with the third lumbar. The right kidney is usually slightly lower than the left, probably on account of the vicinity of the liver. The long axis of each kidney is directed downward and lateralward; the transverse axis backward and lateralward. Each kidney has a convex and concave surface. The concave surface, the renal hilum, is the point at which the renal artery enters the organ, and the renal vein and ureter leave. The kidney is surrounded by tough fibrous tissue, the renal capsule, which is itself surrounded by perinephric fat, renal fascia (of Gerota) and paranephric fat. The anterior border of these tissues is the peritoneum, while the posterior border is the transversalis fascia. The superior border of the right kidney is adjacent to the liver, and the spleen, for the left kidney. Therefore, both move down on inhalation (Drake, 2008). Grossly, the kidneys are bean-shaped structures and weigh about 150 g in the male and about 135 g in the female. They are typically 10-12 cm in length, 5-7 cm in width, and 2-3 cm in thickness. (Wein et al., 2007).

Superiorly, kidneys have the suprarenal (adrenal) glands sit adjacent to the upper pole of each kidney. On the right side, the second part of the duodenum (descending portion) abut the medial aspect of the kidney. On the left side, the greater curvature of the stomach can drape over the superomedial aspect of the kidney, and the tail of the pancreas may extend to overlie the renal hilum. The spleen is located anterior to the upper pole and is connected by the splenorenal (lienorenal) ligaments. Inferiorly to these organs, the colon typically rests anteriorly to the kidneys on both sides. Posteriorly, the diaphragm covers the upper third of each kidney, with the 12th rib most commonly crossing the upper pole. The kidneys sit over the psoas medially and the quadratus lumborum muscles laterally (Fig. 2-2), (David et al., 2011).

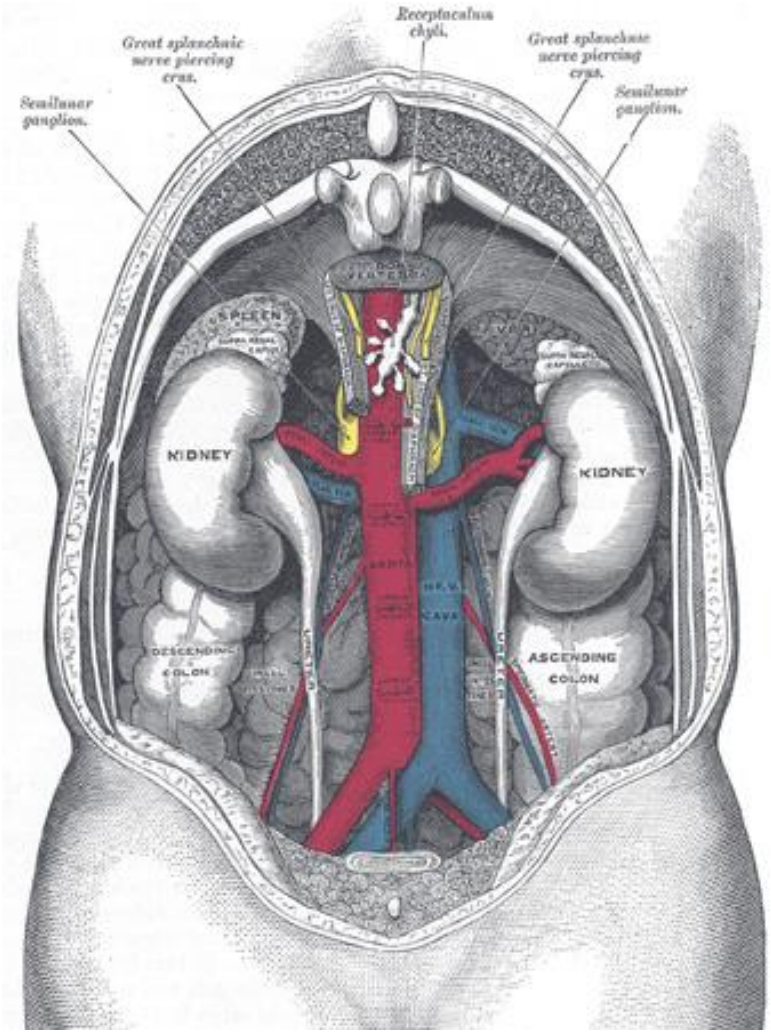


Fig. 2-2 Picture shows relationship between kidneys and adjacent structure (Drake, 2008)

Renal blood supply

The kidneys receive blood from the renal arteries, left and right, which branch directly from the abdominal aorta. Despite their relatively small size, the kidneys receive approximately 20% of the cardiac output (Walter et al., 2004). Each renal artery branches into segmental arteries, dividing further into interlobar arteries which penetrate the renal capsule and extend through the renal columns between the renal pyramids (Fig. 2-3).

The interlobar arteries then supply blood to the arcuate arteries that run through the boundary of the cortex and the medulla. Each arcuate artery supplies several interlobular arteries that feed into the afferent arterioles that supply the glomeruli. The interstitum (or interstitium) is the functional space in the kidney beneath the individual filters (glomeruli) which are rich in blood vessels. The interstitum absorbs fluid recovered from urine. Various conditions can lead to scarring and congestion of this area, which can cause kidney dysfunction and failure. After filtration occurs the blood moves through a small network of venules that converge into interlobular veins. As with the arteriole distribution the veins follow the same pattern, the interlobular provide blood to the arcuate veins then back to the interlobar veins which come to form the renal vein exiting the kidney for transfusion for blood. renal vein, which emerges from the kidney at the hilum and opens into the inferior vena cava; the left vein is longer than the right, and crosses in front of the abdominal aorta (Drake, 2010).

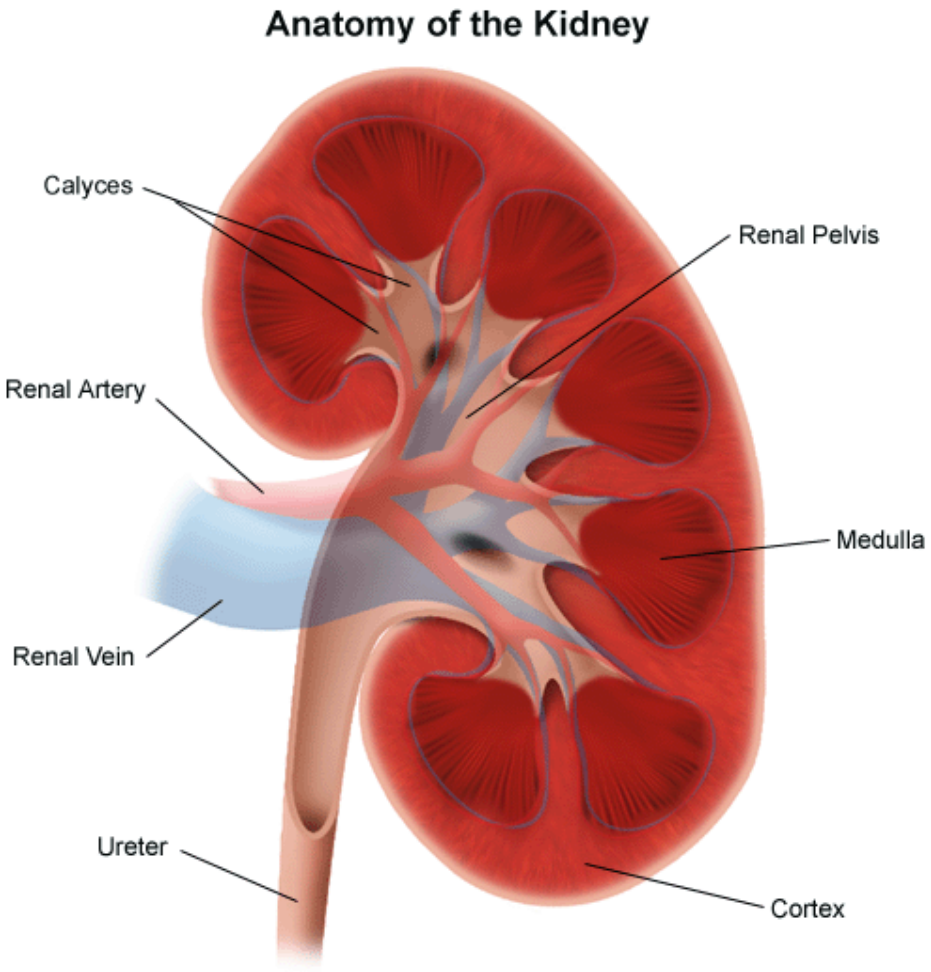


Fig. 2-3 From illustration of the anatomy of the kidney. htt\\lpch.org

Renal lymphatics

The lymphatic drainage parallels the venous drainage system. After leaving the renal hilum, the left primary lymphatic drainage is into the left lateral aortic lymph nodes, including nodes anterior and posterior to the aorta between the inferior mesenteric artery and the diaphragm. On the right, it drains into the right lateral caval lymph nodes (Wein et al., 2007).

Nerves of the kidney

The nerves of the kidney, although small, are about 15 in number. They have small ganglia developed upon them, and are derived from the renal plexus, which is formed by branches from the celiac plexus, the lower and outer part of the celiac ganglion and aortic plexus, and from the lesser and lowest splanchnic nerves. They accompany the renal artery and its branches, and are distributed to the blood vessels and to the cells of the urinary tubules (Drake, 2008).

Renal histology

The substance, or parenchyma, of the kidney is divided into two major structures:

1. Renal cortex, superficial part, and

2. Renal medulla, deep part.

Grossly, these structures have 8 to 18 cone-shaped renal lobes, each containing renal cortex surrounding a portion of medulla called a renal pyramid (of Malpighi). Between the renal pyramids are projections of cortex called renal columns (of Bertin). Nephrons, the urine-producing functional units of the kidney, span the cortex and medulla. The initial filtering portion of a nephron is the renal corpuscle, located in the cortex, which is followed by a renal tubule that passes from the cortex deep into the medullary pyramids. Renal papilla the apex of renal pyramid forms the renal papilla which contains ducts of Bellini (the largest of the collecting ducts). Part of the renal cortex, a medullary ray is a collection of renal tubules that drain into a single collecting duct. The tip, or papilla, of each pyramid empties urine into a minor calyx; minor calyces empty into major calyces, and major calyces empty into the renal pelvis, which becomes the ureter. The basic unit of the kidney is the nephron, with each kidney in humans containing approximately 1.0 to 1.3 million nephrons. Each nephron consists of a glomerulus (Fig. 2-4), which is a tuft of capillaries interposed between two arterioles (the afferent and efferent arterioles), and a series of tubules lined by a continuous layer of epithelial cells (Walter, 2004).

It contain the following cell types, mesangial cells, endothelial cells, podocytes, parietal cells. Glomerulus mean diameter, 200 μm Juxtamedullary glomeruli and glomeruli of solitary kidney are larger. The glomerular “barrier consists of: Capillary endothelium with fenestrations (70-100nm in diameter) without diaphragms, negative charged due to podocalyxin, glomerular basement membrane (240- 340nm thickness) divided in lamina rara interna, lamina densa, lamina rara externa (both laminae electron lucent and negatively charged), podocytes with primary and secondary foot processes .

Permeability depends on electrical charge, molecular size and configuration (George, 2011). The glomeruli are located in the outer part of the kidney, called the cortex, whereas the tubules are present in both the cortex and the inner part of the kidney, the

medulla. The initial step in the excretory function of the nephron is the formation of an ultrafiltrate of plasma across the glomerulus. This fluid then passes through the tubules and is modified in two ways: by reabsorption and by secretion. Reabsorption refers to the removal of a substance from the filtrate, whereas secretion refers to the addition of a substance to the filtrate. Fluid filtered across the glomerulus enters Bowman's space and then the proximal tubule. The proximal tubule: is composed anatomically of an initial convoluted segment and a later straight segment, the pars recta, which enters the outer medulla.

The loop of Henle begins abruptly at the end of the pars recta. It generally includes a thin descending limb and thin and thick segments of the ascending limb. The hairpin configuration of the loop of Henle plays a major role in the excretion of ahyper osmotic urine. It is important to note that the length of the loops of Henle is not uniform Approximately 40% of nephrons have short loops which penetrate only the outer medulla or may even turn around in the cortex; these short loops lack a thin ascending limb, The remaining 60% have long loops that course through the medulla and may extend down to the papilla (the innermost portion of the medulla). The length of the loops is largely determined by the cortical location of the glomerulu, glomeruli in the outer cortex (about 30%) have only short loops. Those in the juxtamedullary region (about 10%) have only long loops and those in the midcortex may have either short or long loops. The thick ascending limb also has a cortical segment which returns to the region of the parent glomerulus. It is in this area, where the tubule approaches the afferent glomerular arteriole, that the specialized tubular cells of the macula densa are located.

The juxtaglomerular cells of the afferent arteriole and the macula densa constitute the juxtaglomerular apparatus, which plays a central role in renin secretion. After the macula densa, there are three cortical segments the distal convoluted tubule, the connecting segment (previously considered part of the late distal tubule), and the cortical collecting tubule. The connecting segments of many nephrons drain into a single collecting tubule. Fluid leaving the cortical collecting tubule flows into the medullary collecting tubule and then drains sequentially into the calyces, the renal pelvis, the ureters, and the bladder (Walter, 2004).

Renal interstitium

The renal interstitial compartment, situated between basement membranes of epithelia and vessels, contains two contiguous cellular networks. One network is formed by interstitial fibroblasts, the second one by dendritic cells. Both are in intimate contact with each other. Fibroblasts are interconnected by junctions and connected to basement membranes of vessels and tubules by focal adhesions. Fibroblasts constitute the “skeleton” of the kidney. The fibroblasts in the interstitium provide the “skeleton” of the tissue and maintain the three-dimensional architecture of the tissue. The interstitium is necessarily involved in all intrarenal exchange processes since the reabsorption and secretion of fluid and solutes implicates a transit across the interstitial compartment. Cortical fibroblasts play a role in the production of regulatory substances, such as extracellular adenosine and erythropoietin (Brigitte et al., 2008).

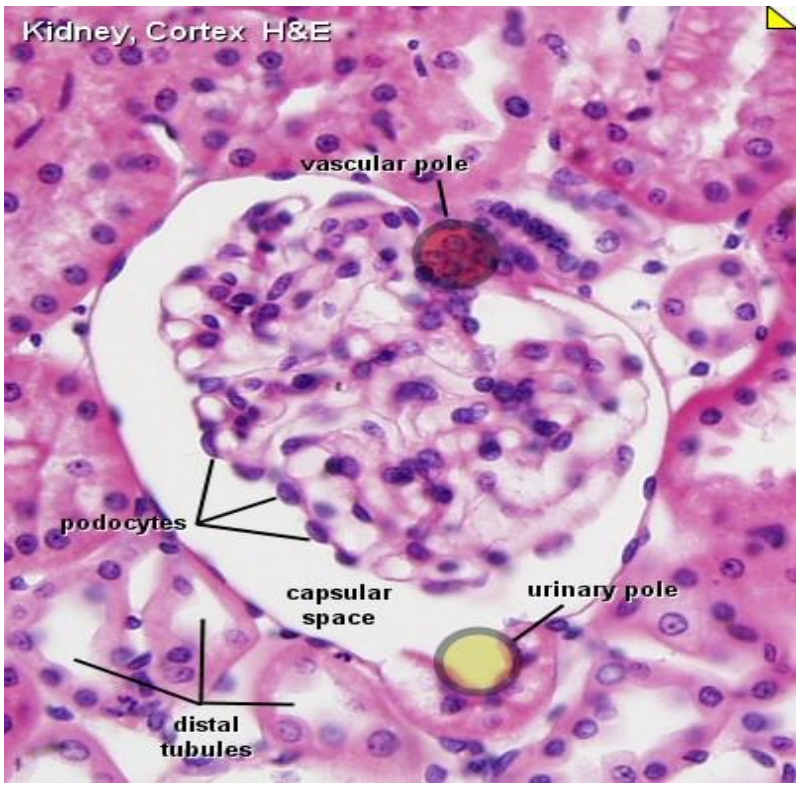


Fig. 2-4 Renal histology from http\\ php.med.unsw.edu.au

Epidemiology of renal cell carcinoma

Globally, the incidence of RCC varies widely from region to region, with the highest rates observed in the Czech Republic and in North America (Chow, 2010). Approximately 273,000 new cases of kidney cancer are diagnosed worldwide each year, representing approximately 2% of all cancers (Ferlay et al., 2010). RCC accounts for 2% to 3% of all malignant diseases in adults. It is the seventh most common cancer in men and the ninth most common in women. In the United States, there are approximately 65,000 new cases each year and about 13,500 deaths from RCC annually (Siegel et al., 2012). The incidence of RCC in the US has increased over time. Between 1992 and 2005, the incidence rose by 1.8% and 2.1% among white men and white women, respectively, and by 2.1% and 1.7% among black men and black women, respectively (Stafford et al., 2008).

RCC accounts for approximately 90% of all renal malignancies. Despite advances in diagnosis, especially improved imaging techniques and the incidental diagnosis of many tumours with imaging tests for unrelated complaints, about 20–30% of all patients are diagnosed with metastatic renal cell carcinoma (mRCC) (Gupta et al., 2008). In addition, another 20% of patients undergoing nephrectomy will have a relapse and develop mRCC during follow-up (Athar et al., 2008). Incidence of RCC seems to be substantially lower among Asians, both in most Asian countries and in the United States, suggesting a higher risk of RCC among whites compared to Asians. While The incidence is highest among African Americans in the United States (Chow et al., 2008; Ferlay et al., 2010). The lowest incidences have been reported from African countries (Karim-Kos et al., 2008). Racial disparities in incidence may be attributable to differences in frequency of diagnostic imaging, access to health care, genetic background, and prevalence of lifestyle or environmental risk factors, but it is almost impossible to distinguish among these (Ljungberg et al., 2011).

In Europe, during 2006, it was estimated that there were 63,300 new case of RCC have been diagnosed (Ferlay et al., 2007). In most of Europe, the incidence of kidney cancer has decreased or stabilized over the past decade, perhaps in part because of reduced tobacco smoking in men. Mortality from kidney cancer has also declined in most of Europe, principally in Scandinavia and other western European countries. In men, the mortality rate per 100,000 populations decline from 4.8 in 1990-1994 to 4.1

in 2000-2004; in women, the rate of decline pattern of kidney cancer incidence and mortality in Europe (Levi et al., 2008). In United Kingdom (UK), during 2009, there were 9,286 new cases of kidney cancer in the UK. 5,706 (61%) in men and 3,580 (39%) in women, giving a male: female ratio of 16:10. (Cancer research UK 2011).

In Libya, cancer of kidney (including cancers of renal pelvis) comprises 2% of all cancer patients. The incidence is remarkably less as compared to US and European rates. It has been found more common in males compare to females with ratio 2.3:1.9 with median age of 69 years of age in male and 55 years of age in female (Abuageila et al., 2006).

Gender and age

In older studies, RCC was at least twice as frequent in men as women. However, more recent data suggest that this gap has slightly narrowed (Stafford et al., 2008). RCC occurs predominantly in the sixth to eighth decade of life with median age at diagnosis around 64 years of age, according to 2003 to 2007 National cancer institute (NCI's), Surveillance, Epidemiology, and End Results (SEER) Cancer Statistics Review; it is unusual in patients under 40 years of age, and rare in children (Siemer et al., 2006; Thompson et al., 2008). In the UK (2007-2009), an average 62% of kidney cancer cases were diagnosed in people aged 65 years and over, but around three-quarters (74%) of cases were in people aged >60 (Kidney cancer incidence statistic, cancer research UK 2009).

Trends over time

Increases in kidney cancer incidence have been reported in many different countries around the world. There has been some debate as to how much this is due to the introduction of new imaging methods, such as ultrasound and computed tomography (CT), which leads to the incidental detection of asymptomatic disease (Nguyen et al., 2006).

Risk factors

A number of environmental and clinical factors have been implicated in the etiology of RCC. These include smoking, hypertension, occupational exposure to toxic compounds, obesity, acquired cystic disease of the kidney (typically associated with dialysis), analgesic abuse nephropathy, and genetic predisposition.

Smoking

Cigarette smoking increases the probability of developing RCC approximately twofold, thereby possibly contributing to its development in nearly one-third of cases (Hunt et al., 2005). Pipe and cigar smokers are also more susceptible. Cigarette smoking is an independent risk factor for advanced RCC. Heavier smoking increases the likelihood of advanced disease. Durable smoking cessation attenuated the risk of advanced disease. Given that cigarette smoking is among the few modifiable risk factors for RCC (Tsivian et al., 2011). In other study shows that a history of smoking was associated with worse pathologic features and survival outcomes, and with increased risk of having mutated p53 (Kroeger et al., 2011).

Hypertension

Hypertension predisposes to RCC development. This seems to be independent of anti-hypertensive medications or obesity, both closely correlated with hypertension itself. The underlying biological explanations linking hypertension to RCC remain largely unknown (Weikert et al., 2008). Hypertension is a risk factor for renal cancer among both blacks and whites, and might explain a substantial portion of the racial disparity in renal cancer incidence. Preventing and controlling hypertension might reduce renal cancer incidence, adding to the known benefits of blood pressure control for heart disease and stroke reduction, particularly among blacks (Colt et al., 2011).

Diabetes mellitus

There is an evidence that the incidence of solid tumors is markedly increased in patients with diabetes mellitus including RCC, diabetic RCC patients have a predominance of localized, small clear cell RCC. In addition, females with a history of RCC have a higher frequency of diabetes compared to males (Samy et al., 2012). Other study shows that the history of diabetes mellitus has been associated with a

modest increase in the risk of RCC. This may be mediated through an increase in the incidence of hypertension (Setiawan et al., 2007).

Occupational exposure

Occupational exposure to toxic compounds, such as cadmium, asbestos, and petroleum by-products, has been associated with an increased risk of RCC. In one international multicenter study of over 1700 patients with RCCs and 2300 controls, an increased risk of cancer was observed in those exposed to asbestos (relative risk [RR] 1.4), cadmium (RR 2.0), and gasoline (RR 1.6) (Mandel et al., 1995). Cadmium workers who smoke may have a particularly high incidence of RCC (Kolonel et al., 1976). Kidney cancer is generally not considered an occupational disease, but researchers have found an association with certain occupational exposures. Some studies have suggested that people who are exposed to cadmium (a metal often found in batteries) at work may be at a higher risk of developing kidney cancer (Canadian Cancer Society 2012).

Obesity

Obesity may be a particular risk factor in both men and women. There appears to be a direct correlation between increased body weight and an enhanced risk of developing RCC. The global rise in obesity may be contributing to the increased RCC incidence (Pischon et al., 2006). A meta-analysis of prospective studies provided evidence for an association between body mass index (BMI) and risk of RCC with summary risk estimates (per 5 kg/m^2 increase in BMI) of 1.24 in men and 1.34 in women. The results suggested a somewhat stronger association in women than in men but, overall, evidence on sex-specific differences in the association between BMI and RCC risk is not conclusive (Renehan et al., 2008). In other study, the incidence of RCC in obese people (BMI>29 kg/m2) is double that of normal individuals and about 50% increased if overweight (BMI 25-30 kg/m2). One quarter of kidney cancers in both sexes are attributable to excess weight (Bergstrom et al., 2001). Studies investigating body fat distribution suggested an increased risk of RCC with increasing waist-to-hip ratio, (Pischon et al., 2006; Adams et al., 2008).

Acquired cystic disease of the kidney

This disease affects one third or more of long-term (≥3 yr) hemodialysis patients and that approximately 20% of those with ACKD will have renal cell carcinoma, representing a prevalence of approximately 5% (Chapman et al., 2007). Age, male gender, and duration of dialysis are primary risk factors. It may occur less frequently in those who are on peritoneal dialysis and may regress after transplantation (Lien, 1993). Its pathogenesis is not understood but may relate to the activation of proto-oncogenes, which may also be responsible for the subsequent development of renal cell carcinoma (Oya et al., 2005). Transplantation carries risk for cancer, more so as the years of exposure to the immunosuppressive agents' occur. Kidney transplant recipients in the USA have been shown to carry a 15-fold risk for kidney cancer (which would include non–renal cell cancers as well) in the first 3 yr after transplantation when compared with the general US population and a 39% higher risk for developing kidney cancer in those years in comparison with transplant candidates who are still on the waiting list (Kasiske et al., 2004).

Polycystic kidney disease

The prevalence rate of RCC of 8.3% in patients with Autosomal dominant polycystic kidney disease (ADPKD) and Chronic Renal Failure. It increased to 12% in their patient population after one year of dialysis or kidney transplantation. Additionally, the propensity of these malignancies to present bilaterally and at a younger age compared to the general population also suggests an elevated risk in this patient population (Hajj et al., 2009).

Analgesic abuse nephropathy

The prolonged ingestion of analgesic combinations, particularly compounds containing phenacetin (of which acetaminophen is a major metabolite) and aspirin, can lead to chronic renal failure. Such patients are at increased risk for renal pelvic and urothelial tumors. Epidemiologic studies have demonstrated an increased risk for RCC with heavy use of aspirin (Gago-Dominguez et al., 1999) and non-steroidal anti-inflammatory drugs (NSAIDs), (Sorensen et al., 2003), and acetaminophen (Derby et al., 1996).

Genetic factors

Inherited renal cell cancer is known to occur in a number of familial cancer syndromes, most notably the von Hippel-Lindau (VHL) syndrome. VHL disease is an inherited, autosomal dominant syndrome manifested by a variety of benign and malignant tumors, including clear cell carcinoma of the kidney. This syndrome is characterized by alterations in the *VHL* gene and predisposition to a number of diseases among family members, including the clear cell subtype of renal cell cancer. Only a very small proportion of renal cell cancer patients are known to occur in families with these rare syndromes, although the exact percentage is difficult to pinpoint (Kaelin et al., 2007). Other familial syndrome includes; Hereditary papillary renal carcinoma (HPRC) is a familial cancer syndrome in which affected individuals are at risk for the development of type 1 papillary RCCs (Zbar et al., 1994). HPRC is a highly penetrant, autosomal dominant condition. Both early and late onset forms of HPRC have been described (Dharmawardana et al., 2004; Schmidt et al., 2004). Genetic linkage analyses found that the HPRC gene (the MET protooncogene) is located on the long arm of chromosome 7 (Schmidt et al., 1997). Birt-Hogg-Dube (BHD) syndrome is an inherited syndrome in which affected individuals are at risk for the development of bilateral, multifocal kidney cancer, as well as various dermatologic and pulmonary lesions (Menko et al., 2009). Tuberous sclerosis complex. Less than 5% of patients with the disease develop RCC (Neumann et al., 2002). The disease-associated RCC tumors occurred at a younger age than sporadic tumors and occurred primarily in women (Bjornsson et al., 1996). However, sporadic renal cell cancers have been shown to have a familial predisposition, with a recent meta-analysis showing a greater than twofold risk among individuals having a first-degree relative diagnosed with kidney cancer (Clague et al., 2009).

RCC patients were found to have shorter telomere length in blood DNA compared to control subjects, and the association appeared to be modified by cigarette smoking (Wu et al., 2003). Low mitochondrial DNA (mtDNA) content in peripheral blood lymphocytes also was associated with elevated risks of renal cell cancer in a dose response manner (Xing et al., 2008). Although mtDNA content was significantly lower among smokers than non-smokers, smoking did not modify the association between mtDNA and renal cell cancer risk. The findings with telomere length and mtDNA have yet to be confirmed, preferably in larger studies with prospectively

collected genomic DNA samples. Studies of signature tumor DNA alterations may also provide clues to relevant environmental carcinogen exposures (Shiao et al., 2009).

Vitamin D maintains calcium homeostasis and has been shown to play a role in cell proliferation and progression to a number of cancers (Bouillon et al., 2006). Recently, several studies examined eight vitamin D pathway genes with complete genomic coverage, and found increased risk associated with variant haplotypes of the vitamin D receptor gene (Karami et al., 2009).

Cytotoxic chemotherapy

The use of cytotoxic chemotherapy in childhood for malignancies, autoimmune disorders, or bone marrow transplant conditioning has been associated with the subsequent development of translocation RCC (Argani et al., 2006).

Alcohol

Lower risk with alcohol consumption has been reported for cancers of thyroid and kidney (Allen et al., 2009). Moderate consumption of alcohol is associated with a decreased risk of RCC in both men and women (Lew et al., 2011).

Chronic hepatitis C infection

An epidemiologic study of over 67,000 patients found that chronic infection with hepatitis C virus was associated with a significantly increased risk of RCC (hazard ratio 1.77, after correcting for age, ethnicity, gender, and the presence of chronic kidney disease (Gordon et al., 2010).

High fat diet

High-fat and high-protein diets as well as sugar-and fat-rich confectioneries might be risk factors for renal cell cancer (Kiren et al., 2002). On other hand, multiple studies suggest that fruit and vegetable consumption was associated with a lower risk of RCC (Lee et al., 2009).

Dialysis

The risk of developing RCC has been estimated to be 30 times greater in dialysis patients with acquired polycystic disease of the kidney than in the general population (Truong et al., 1995). Other factors: Additional clinical factors that may be associated are, unopposed estrogen therapy (Lindblad et al., 1995; Kabat et al., 2007), prior radiation therapy (Vogelzang et al., 1998).

Classification of renal cell carcinoma

The average RCC diameter is approximately six cm. Although lesions smaller than three cm were previously thought to represent benign adenomas, it is now clear that even small tumors frequently represent carcinomas (Bosniak et al., 1995; Schlomer et al., 2006). As a result, the distinction between a malignant and a benign growth based upon size alone is no longer made. Instead, basic histologic criteria are used to discriminate between a malignant or benign growth. Thus, all solid renal masses require resection or biopsy for accurate diagnosis unless the presence of a metastatic lesion can be established by biopsy. Previously, RCCs were classified by cell type and growth pattern (Richie et al., 1981).

This classification has recently changed to more accurately reflect the morphology, growth pattern, cell of origin, histochemical, and molecular basis of the different types of adenocarcinomas (Tannenbaum 1971; Thoenes et al., 1986; Störkel et al, 1995).

WHO classification of RCC (Eble et al., 2004)

- Clear cell renal cell carcinoma
- Multilocular clear cell renal cell carcinoma
- Papillary renal cell carcinoma
- Chromophobe renal cell carcinoma
- Carcinoma of the collecting ducts of Bellini
- Renal medulary carcinoma
- Xp11 translocation carcinomas
- Carcinoma associated with neuroblastoma
- Mucinous tubular and spindle cell carcinoma
- Renal cell carcinoma unclassified

Currently, RCC histologic subtypes are classified according to the Union International Contra Cancer (UICC), and the American Joint Committee on Cancer (AJCC) recommendations, 2010. This classification is based on the Heidelberg classification system (Kovacs et al., 1997), which categorizes RCCs as clear cell, papillary, chromophobe, collecting duct, and unclassified RCC subtypes, the American Cancer Society, UICC, AJCC 2010, they classify RCC as seen in Table. 2-1.

Table. 2-1 AJCC 2010 classification of renal cell neoplasm

Benign	**Malignant**
Oncocytoma	Clear cell (conventional) renal cell carcinoma
Papillary (chromophil) adenoma	Papillary (chromophil) renal cell carcinoma
Metanephric (embryonal) adenoma	Chromophobe renal cell carcinoma
Metanephric adenofibroma	Collecting duct carcinoma
	Medullary carcinoma
	Mucinous tubular and spindle cell carcinoma
	Renal cell carcinoma, unclassified
Tumors of undetermined malignant potential	
Multilocular cystic renal cell carcinoma	

Clear cell renal cell carcinoma (ccRCC)

Clear cell RCC is a malignant neoplasm composed of cells with clear or eosinophilic cytoplasm within a delicate vascular network. The term "granular cell RCC"was used for many years for renal cell carcinomas with eosinophilic cytoplasm and high nuclear grade. Some renal neoplasms of this morphology are now included among the clear cell type, but similar appearing cells occur in other tumour types, and so the term"granular cell RCC" should no longer be used (Storkel et al., 1997). Clear cell RCC is the most common type, accounting for 70% to 80% of renal cell cancers (Sean, 2012).

Macroscopic picture

Clear cell carcinomas arise most likely from proximal tubular epithelium, and usually occur as solitary unilateral lesions. Orange/yellow (from lipid), usually upper pole, well circumscribed (“pushing” borders), hemorrhage, necrosis and calcification are common (Fig. 2-5), resulting in heterogeneous or variegated cut surface. There is frequent involvement of renal vein and renal sinus (particularly for large tumors). Soft fleshy areas may indicate sarcomatous component of the tumour. It may undergo cystic degeneration, may be multifocal; bilateral in 1% (usually with VHL or tuberous sclerosis), (Sean, 2012).

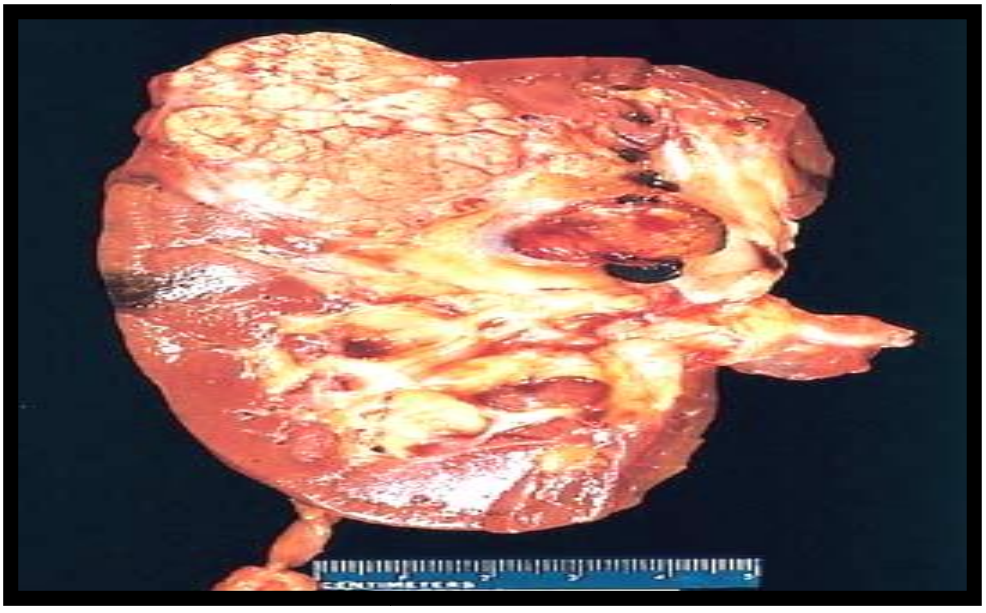


Fig. 2-5 Macroscopic appearance of clear cell renal cell carcinoma (Kumar, 2010)

Microscopic picture

Compact, tubulocystic, alveolar or rarely papillary architecture of cells with clear cytoplasm (from glycogen/lipid), distinct but delicate cell boundaries; cell size is twice normal epithelial tubule cell (Fig. 2-6). Cytoplasm contains glassy hyaline globules (multiple/small or single/large) and myospherulosis; usually nuclear grade 2 or higher. Occasionally there is irregular central area of edematous stroma. May have angioleiomyomatous features (Kuhn et al., 2006), smooth muscle stroma (Shannon et al., 2009). Some tumors may have pseudopapillary architecture due to high grade changes and loss of cell cohesion (Gobbo et al., 2008).

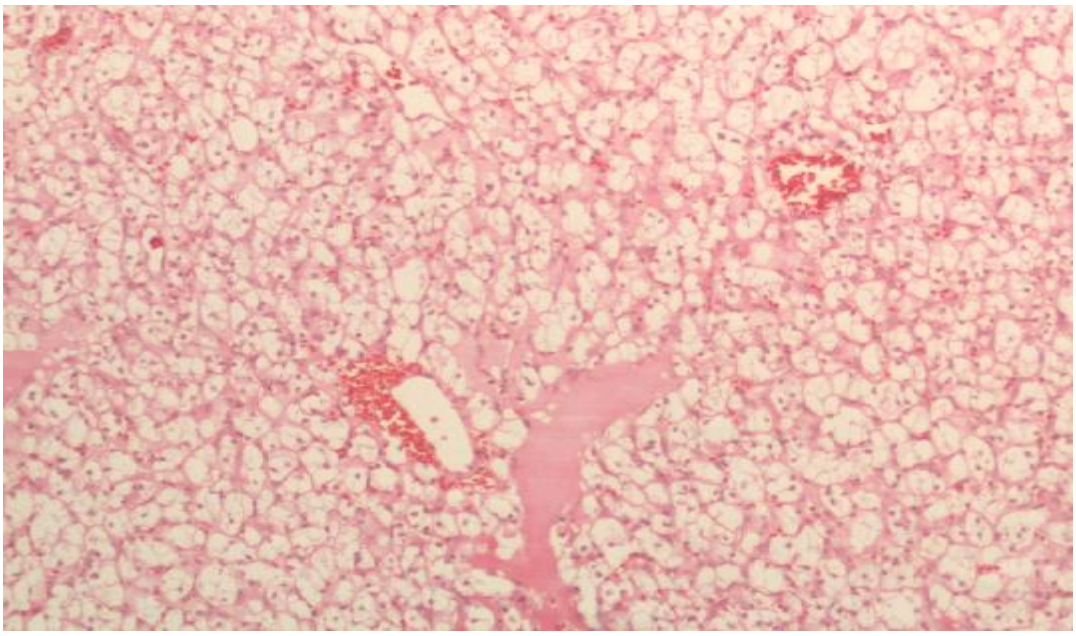


Fig. 2-6 Microscopic appearance of clear cell renal cell carcinoma H&E X 10
(Dept of Pathol. Benghazi University, Benghazi, Libya)

Papillary renal cell carcinoma (pRCCs)

A malignant renal parenchymal tumour with a papillary or tubulopapillary architecture. PRCC is the second most frequent RCC subtype, accounting for approximately 13%–15% of all known RCC lesions. Patients present in the third to eighth decades of life. As is true for all other cell types, the majority of pRCCs are discovered incidentally during work-up of unrelated conditions. The male-to-female ratio ranges from 2:1 to 3.9:1. Although most pRCCs are unilateral, pRCC is the most common multifocal or bilateral renal tumor (Reuter et al., 2006). Two morphologic types of pRCC with different clinical behavior. Type 1 tumors have papillae covered by a single layer of cuboidal or low columnar cells with scanty cytoplasm and low-grade nuclei. Type 2 tumors are of a higher nuclear grade and contain more than one layer of cells with abundant eosinophilic cytoplasm. Type 2 tumors generally carry a worse prognosis than do type 1 tumors (Delahunt et al., 2001). Most pRCCs are sporadic. However, there are a few familial forms. The majority of sporadic pRCCs are characterized by trisomy of chromosomes 7 and 17, as well as loss of chromosome Y in males (Reuter et al., 2006). Hereditary papillary renal cell cancer syndrome, hereditary leiomyomatosis and RCC syndrome, and occasionally BHD syndrome are associated with papillary renal cell cancers.

Macroscopic picture

Grossly, tumour has thick capsule with reactive changes and hemorrhage, red/brown (from hemorrhage); multifocal (80% of tumors), occasionally bilateral; mean seven cm, Tissue “pours out" of kidney pRCC looks necrotic but microscopically less necrosis than expected (Sean, 2012), (Fig. 2-7).

Microscopic pictutre

pRCC appears well-circumscribed, often with distinct fibrous capsule, papillary or tubulopapillary in every case, have papillary fibrovascular cores that may be edematous and look cystic. Papillae may be long and solidly packed (Fig. 2-8). Foamy macrophages in papillary cores and intracellular hemosiderin are sensitive/specific features to pRCC, Papillae are composed of columnar/cuboidal cells with finely granular cytoplasm, lower grade nuclei, longitudinal nuclear grooves in low grade cases; often tubular dysplasia

may have glassy hyaline globules, variable psammoma bodies, neutrophils and necrosis. Focally clear cell areas may be present (Klatte et al., 2011).

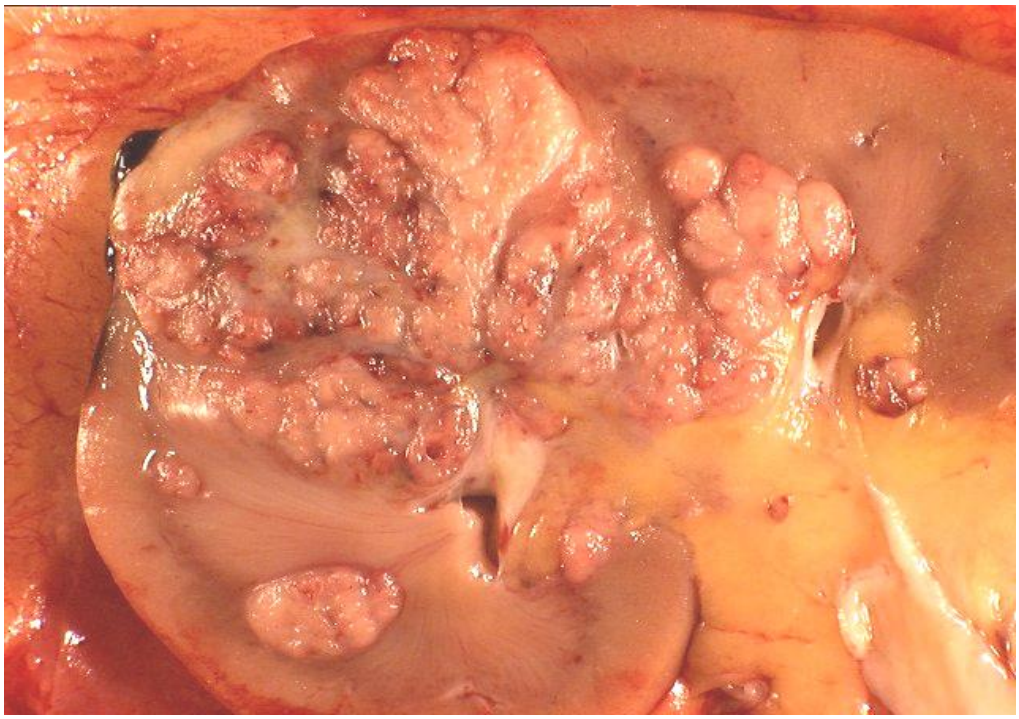


Fig. 2-7 Macroscopic appearance of papillary renal cell carcinoma (Sean, 2012)

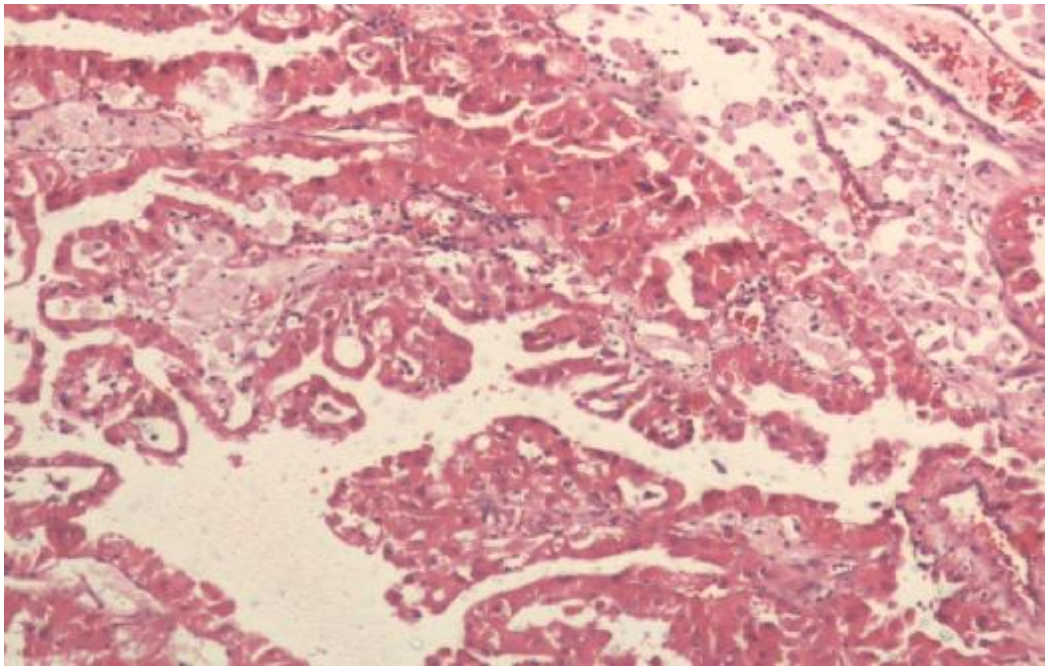


Fig. 2-8 Microscopic appearance of papillary renal cell carcinoma H&E X 10

(Dept of Pathol. Benghazi University, Benghazi, Libya)

Chromophobe renal cell carcinoma (chRCC)

chRCC comprises 5–10% of the total cases of RCC. It represents approximately 3000–6000 of new cases of the 61 000 expected new RCC cases in 2011 in the USA (Siegel et al., 2011). The mean age of occurrence is reported in the fifth decade, with a range of 27–86 years, more commonly observed in women (52%) than in men (48%). Most of the cases are diagnosed in stage I or II. Renal vein invasion is seen in approximately 5% of cases and incidence of metastatic disease is 6–7%. The most common sites of metastases are liver (39%) and lung (36%). Typically, chRCC presents as a large solitary solid compact mass without necrosis or calcification (Zini et al., 2008).

Macroscopic picture

chRCC are well-circumscribed solid neoplasms and highly lobulated. The surface appears homogeneously beige, light tan, brown, mahogany brown or yellow (Amin et al., 2008). The median tumor size is 6.0 cm, which is larger than other subtypes (Papanikolaou et al., 2004).

Microscopic picture

The growth pattern is solid, at times tubulocystic, with broad fibrotic septa. Two types of tumor cells might be present in varying proportions. The first type; pale cells are large, polygonal cells with abundant transparent cytoplasm and prominent cell membranes (Fig. 2-9), (Prasad et al., 2006). Typically, they are admixed with a second population of smaller cells with granular and eosinophilic cytoplasm. The nuclei of both are irregular. Binucleation and perinuclear halos are common (Stec et al, 2009).

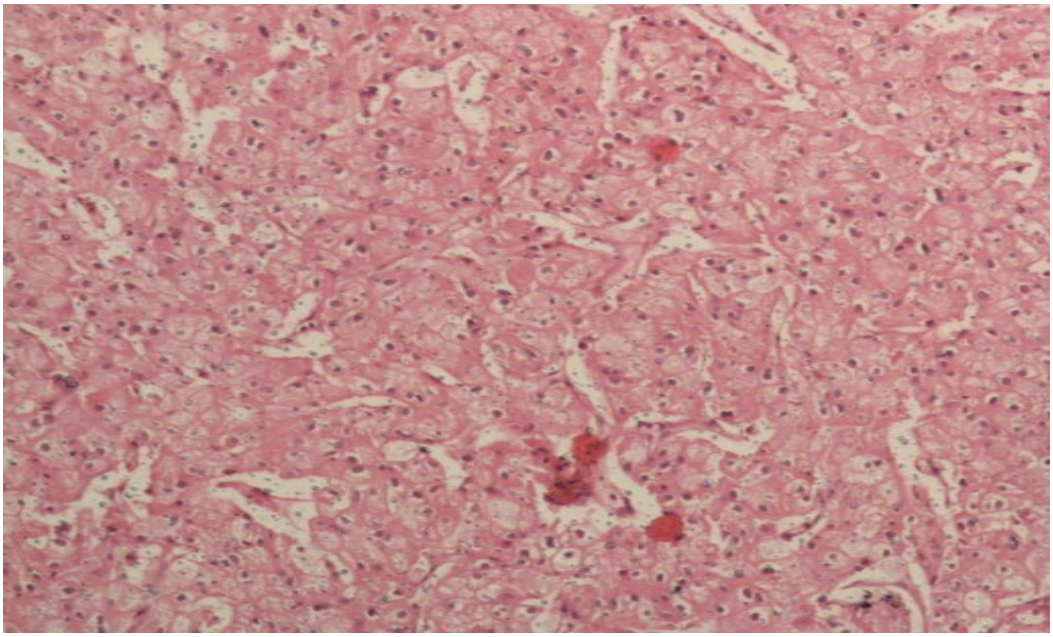


Fig. 2-9 Microscopic appearance of chromophobe renal cell carcinoma H&E X 20 (Dept of Pathol. Benghazi University, Benghazi, Libya)

Collecting duct carcinoma

Bellini duct carcinoma (BDC), also known as and tubulocystic carcinoma (Amin et al., 2009). It is a type of kidney cancer that originates in the duct of Bellini of the kidney. It is rare, accounting for 1-3% of all kidney cancers (Fakhrai et al., 2005). Previously, due to its location. BDC was commonly diagnosed as renal cell carcinoma or a subtype of renal cell carcinoma. However, BDC does not respond well to chemotherapy drugs used for RCC, and progresses and spreads more quickly. One small study reporting 20% survival at 2 years (Méjean et al., 2003). It has been

reported to occur together with urothelial carcinoma of the bladder leading to the suspicion that these cancers are related. (Hsiao et al., 2008; Okuda et al., 2008).

BDC can be identified based on gross, microscopic, histochemical, and immunohistochemical features. Macroscopically, BDCs are often located at the confluence of the medulla and renal pelvis, and show a characteristic gray-white-tan color, with absence of foci of necrosis and hemorrhage (Histologically, BDC presents a tubulo-papillary morphology, often accompanied by desmoplasia, atypia in collecting ducts, and intratubular spread (Kennedy et al., 1990).

Clinical manifestations

RCC may remain clinically occult for most of its course. The classic triad of flank pain, hematuria, and flank mass is uncommon (10%) and is indicative of advanced disease. Twenty-five to thirty percent of patients are asymptomatic, and their renal cell carcinomas are found on incidental radiologic study. The most common presentations include hematuria (40%), flank pain (40%), and a palpable mass in the flank or abdomen (25%). Other signs and symptoms include weight loss (33%), fever (20%), hypertension (20%), hypercalcemia (5%), night sweats, malaise, and a varicocele, usually left sided, due to obstruction of the testicular vein (2% of males). RCC is a unique tumor because of the frequent occurrence of paraneoplastic syndromes, including hypercalcemia, erythrocytosis, and nonmetastatic hepatic dysfunction (ie, Stauffer syndrome). Polyneuromyopathy, amyloidosis, anemia, fever, cachexia, weight loss, dermatomyositis, increased erythrocyte sedimentation rate (ESR), and hypertension are also associated with renal cell carcinoma. Cytokine release by tumor (eg, interleukin (IL)-6), erythropoietin and nitric oxide cause these paraneoplastic conditions. Resolution of symptoms or biochemical abnormalities may follow successful treatment of the primary tumor or metastatic foci. Approximately 30% of patients with RCC present with metastatic disease. The physical examination should include a thorough evaluation for metastatic disease, particularly in the following organs: Lung (75%), soft tissues (36%), bone (20%) liver (18%) cutaneous sites (8%), and central nervous system (8%). The presence of a varicocele and findings of paraneoplastic syndromes should raise clinical suspicion for this diagnosis. (Curti et al., 2012).

Diagnosis of renal cell carcinoma

The following are initial laboratory studies in the evaluation of suspected RCC: Urine analysis, complete blood cell (CBC) count with differential, electrolytes, renal profile, liver function tests (LFTs) (aspartate aminotransferase and alanine aminotransferase), calcium, erythrocyte sedimentation rate (ESR), prothrombin time (PT), activated partialthromboplastin time (aPTT).

Other tests are made as indicated by the patient's presenting symptoms (Curti et al., 2012)

Radiology and imaging

Patients with unexplained hematuria, or other symptoms, signs, or findings suggestive of a possible RCC, must undergo radiographic evaluation for the presence of a renal mass. The usual first test is abdominal ultrasound or CT. Although ultrasonography is less sensitive than CT in detecting a renal mass, it is useful to distinguish a simple benign cyst from a more complex cyst or a solid tumor (Atkins et al., 2011). The goals of radiologic imaging are to detect and stage the primary tumor. In most institutions, CT is the main imaging technique for the evaluation of the intraabdominal component of renal tumors. In some specific instances, such as allergy to iodinated contrast medium, magnetic resonance imaging (MRI) and sonography can provide complementary information (Broome et al., 2007). Contrast-enhanced CT scanning has become the imaging procedure of choice for diagnosis and staging of renal cell cancer and has virtually replaced excretory urography and renal ultrasonography. In most cases, CT imaging can differentiate cystic masses from solid masses and supplies information about lymph node, renal vein, and inferior vena cava involvement (Curti et al., 2012).

The 2009 American Urological Association (AUA) guidelines for the management of the clinical T1 renal mass recommends a high-quality cross-sectional CT or MRI, first without and then with intravenous contrast if renal function is adequate. The objective is to rule out angiomyolipoma, evaluate for locally invasive features, study the involved anatomy, and determine status of the uninvolved kidney and its vasculature

(Campbell et al., 2009). MRI may be useful when ultrasonography and CT are nondiagnostic and/or radiographic contrast cannot be administered because of allergy or poor renal function. MRI may be particularly valuable if a tumor is present to identify the presence and/or extent of involvement of the collecting system and/or inferior vena cava (Atkins et al., 2011).

Dynamic, contrast-enhance MRI may also be useful in determining histology, with clear cell carcinoma showing greater signal intensity change in the corticomedullary and nephrographic phase than either papillary or chromophobe carcinomas (Sun et al., 2009). A bone scan is recommended for patients with bone pain or an elevated alkaline phosphatase level (National Comprehensive Cancer Network. NCCN 2011).

Grading of renal Cell Carcinoma

Renal cell carcinoma can be graded according to several systems that use 2, 3, or 4 grades of malignancy. The 4-tiered nuclear grading system originally proposed by (Fuhrman et al., 1982) has been the most widely used and will be presented here. In the Fuhrman grading system, the tissue is examined at low magnification and the most anaplastic area is identified for grading. The grading takes into account the size and shape of nuclei, the chromatin pattern, and the presence of nucleoli (Table. 2-2) as follows (Ivan et al., 2007)

• ***Grade 1.*** The tumor cells have uniform, round, small nuclei (<10μm) comparable to the nuclei of lymphocytes. The chromatin is condensed, and the nucleoli are not visible (Fig. 2-10).

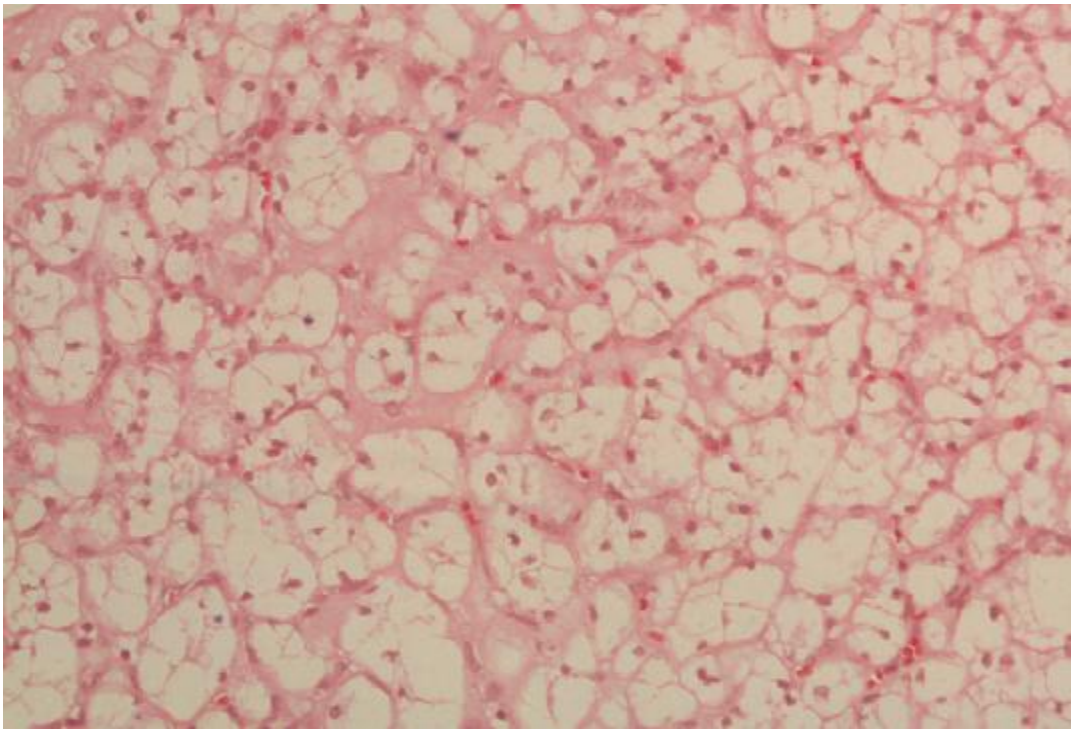


Fig. 2-10 Fuhrman grade 1 tumor has small condensed nuclei H&E X 40 (Dept of Pathol. Benghazi University, Benghazi, Libya)

• ***Grade 2*.** The cells have somewhat larger, round vesicular nuclei (15μm), with finely dispersed chromatin. The nucleoli are not present or are not clearly visible at low magnification (Fig. 2-11).

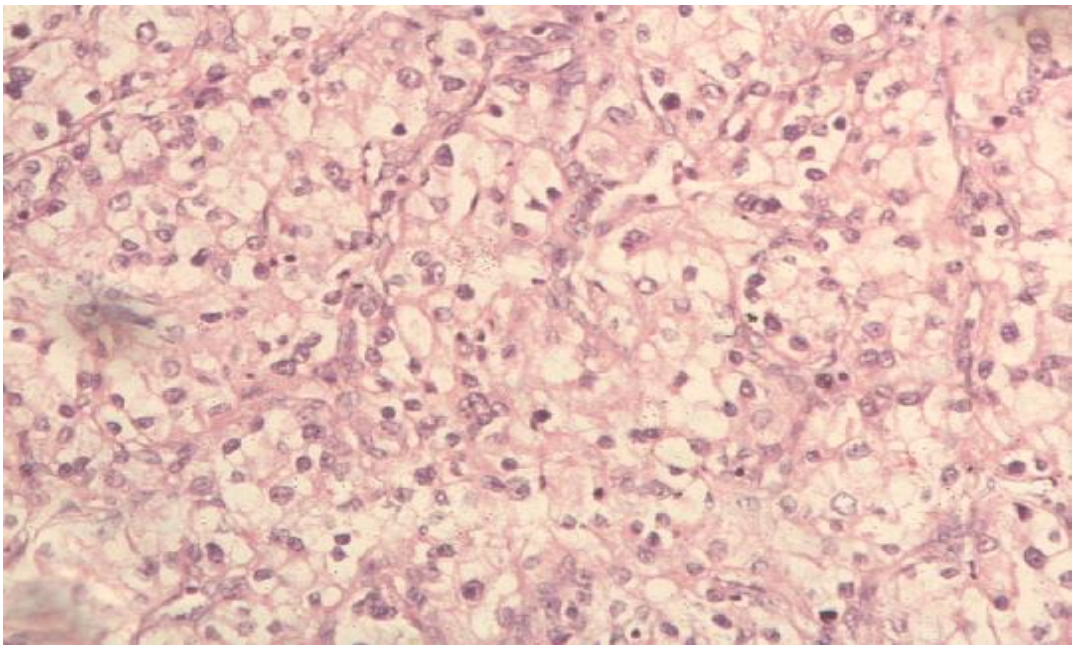


Fig. 2-11 Fuhrman grade 2 tumor shows small vesicular nuclei, which contain no obvious nucleoli H&E X 40 (Dept of Pathol. Benghazi University, Benghazi, Libya)

• ***Grade 3*.** These cells have still larger nuclei (>20μm), which are round or oval and contain finely dispersed chromatin. The nucleoli are easily seen at low magnification (Fig. 2-12).

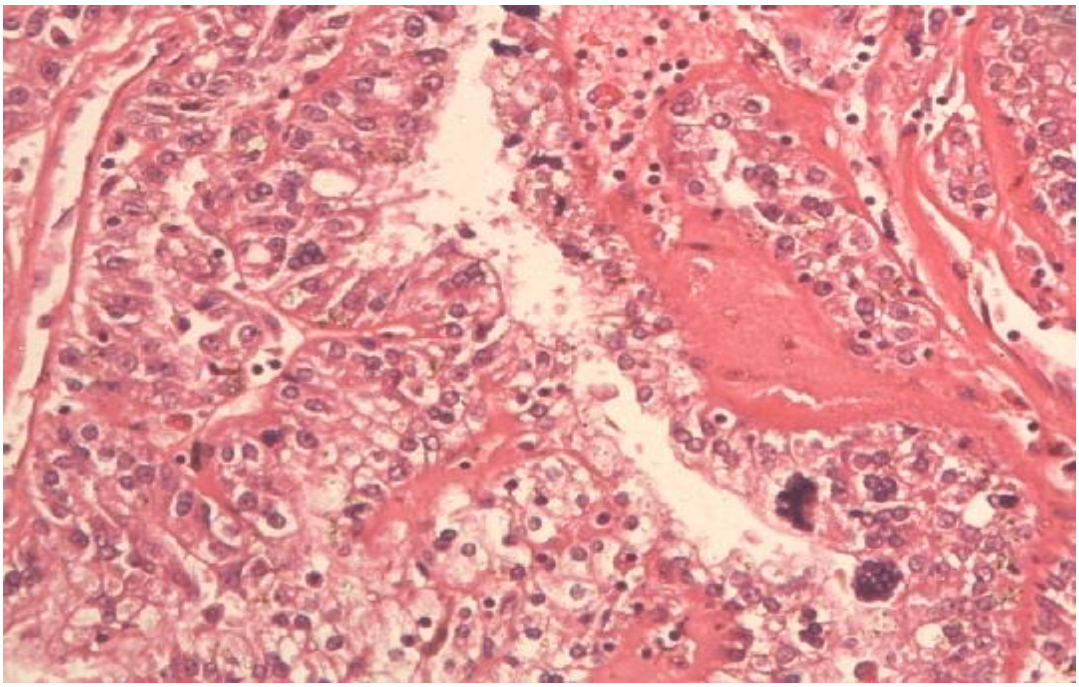


Fig. 2-12 Fuhrman grade 3 tumor has enlarged vesicular nuclei, which contain obvious nucleoli H&E X 40 (Dept of Pathol. Benghazi University, Benghazi, Libya)

- ***Grade 4.*** The cells display irregularly shaped, hyperchromatic large nuclei (>20μm) that vary in size and shape.The chromatin is irregularly distributed, and the nucleoli are large (“macronucleoli”) (Fig. 2-13).

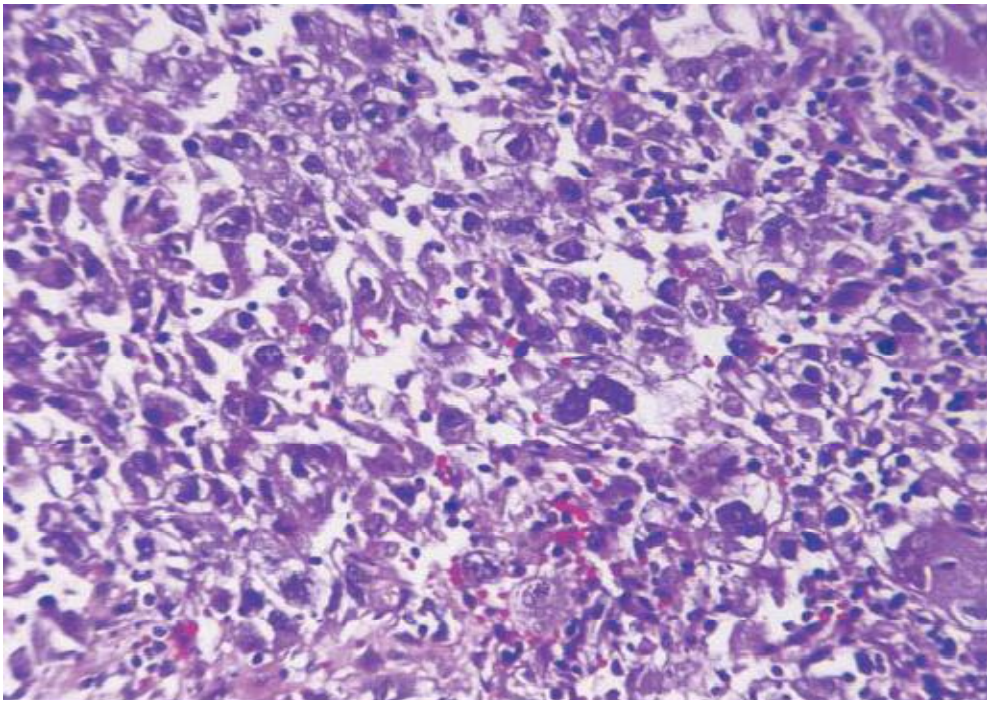


Fig. 2-13 Fuhrman grade 4 tumor nuclei show pleomorphism and appear hyperchromatic H&E X 20 (Ivan, 2007)

Grade 1 renal cell cancers have cell nuclei that differ very little from normal kidney cell nuclei. These cancers usually grow and spread slowly and tend to have a good prognosis. At the other extreme, grade 4 renal cell cancer nuclei look quite different from normal kidney cell nuclei and have a worse prognosis.

Table. 2-2 Fuhrman grading of renal cell carcinoma, (Murphy et al., 2004)

Grade	Nuclear size	Nuclear shape	Chromatin pattern	Nucleoli
1	<10μm	Round, uniform	Condensed	Not evident
2	15μm	Round, uniform	Finely granular,dispersed	Rudimentary, not seen at low magnification
3	20μm	Round or oval, Slightlyvariable	Coarsely granular	Clearly visible at low magnification
4	>20μm	Pleomorphic, Multilobated	Hyperchromatic and clumped	Larg macronucleoli

Staging of renal cell carcinoma

The staging system for renal cell cancer is based on the degree of tumor spread beyond the kidney (Robson et al., 1969; Bassil et al., 1985; Golimbu et al., 1986). Involvement of blood vessels may not be a poor prognostic sign if the tumor is otherwise confined to the substance of the kidney. Abnormal liver function test results may be caused by a paraneoplastic syndrome that is reversible with tumor removal, and these types of results do not necessarily represent metastatic disease. Except when CT examination is equivocal or when iodinated contrast material is contraindicated, CT scanning is as good as or better than MRI for detecting renal masses (Consensus conference. Magnetic resonance imaging (1988).

Currently, the TNM classification, which defines local extension of the primary tumor (T), involvement of regional lymph nodes (N), and presence of distant metastases (M), is globally accepted for the staging of diverse solid tumors, including renal cell carcinoma (RCC), (Frank et al., 2005; Ficarra, et al., 2005).

Table. 2-3. TNM staging classification system of renal cell carcinoma.

The American Joint Committee on Cancer has designated staging by tumor node metastasis (TNM) classification to define RCC (Edge et al., 2010).

Tumor size	
TX	No evidence of primary tumor.
T1	Tumor ≤7 cm in greatest dimension, limited to the kidney.
T1a	Tumor ≤4 cm in greatest dimension, limited to the kidney.
T1b	Tumor >4 cm but not >7 cm in greatest dimension, limited to the kidney.
T2	Tumor >7 cm in greatest dimension, limited to the kidney.
T2a	Tumor >7 cm but ≤10 cm in greatest dimension, limited to the kidney.
T2b	Tumor >10 cm, limited to the kidney.
T3	Tumor extends into major veins or perinephric tissues but not into the ipsilateral adrenal gland and not beyond Gerota fascia.
T3a	Tumor grossly extends into the renal vein or its segmental (muscle containing) branches, or tumor invades perirenal and/or renal sinus fat but not beyond Gerota fascia.
T3b	Tumor grossly extends into the vena cava below the diaphragm.
T3c	Tumor grossly extends into the vena cava above the diaphragm or invades the wall of the vena cava.
T4	Tumor invades beyond Gerota fascia (including contiguous extension into the ipsilateral adrenal gland).
Regional Lymph Nodes (N)	
NX	Regional lymph nodes cannot be assessed.
N0	No regional lymph node metastasis.
N1	Metastases in regional lymph node(s).
Distant Metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis.

Table. 2-4. Anatomic stage of TNM classification

Anatomic stage/prognostic groups			
Stage	T	N	M
I	T1	N0	M0
II	T2	N0	M0
III	T1 or T2	N1	M0
	T3	N0 or N1	M0
IV	T4	Any N	M0
	Any T	Any N	M1

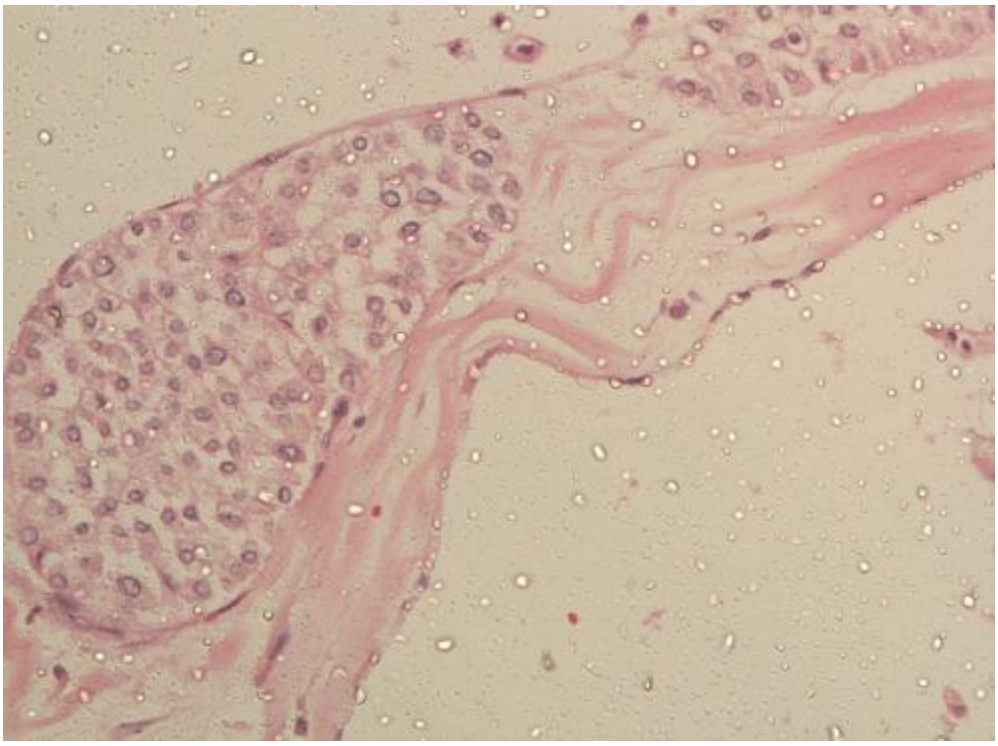


Fig. 2-14 Capsular invasion H&E X40 (Dept of Pathol. Benghazi University, Benghazi, Libya)

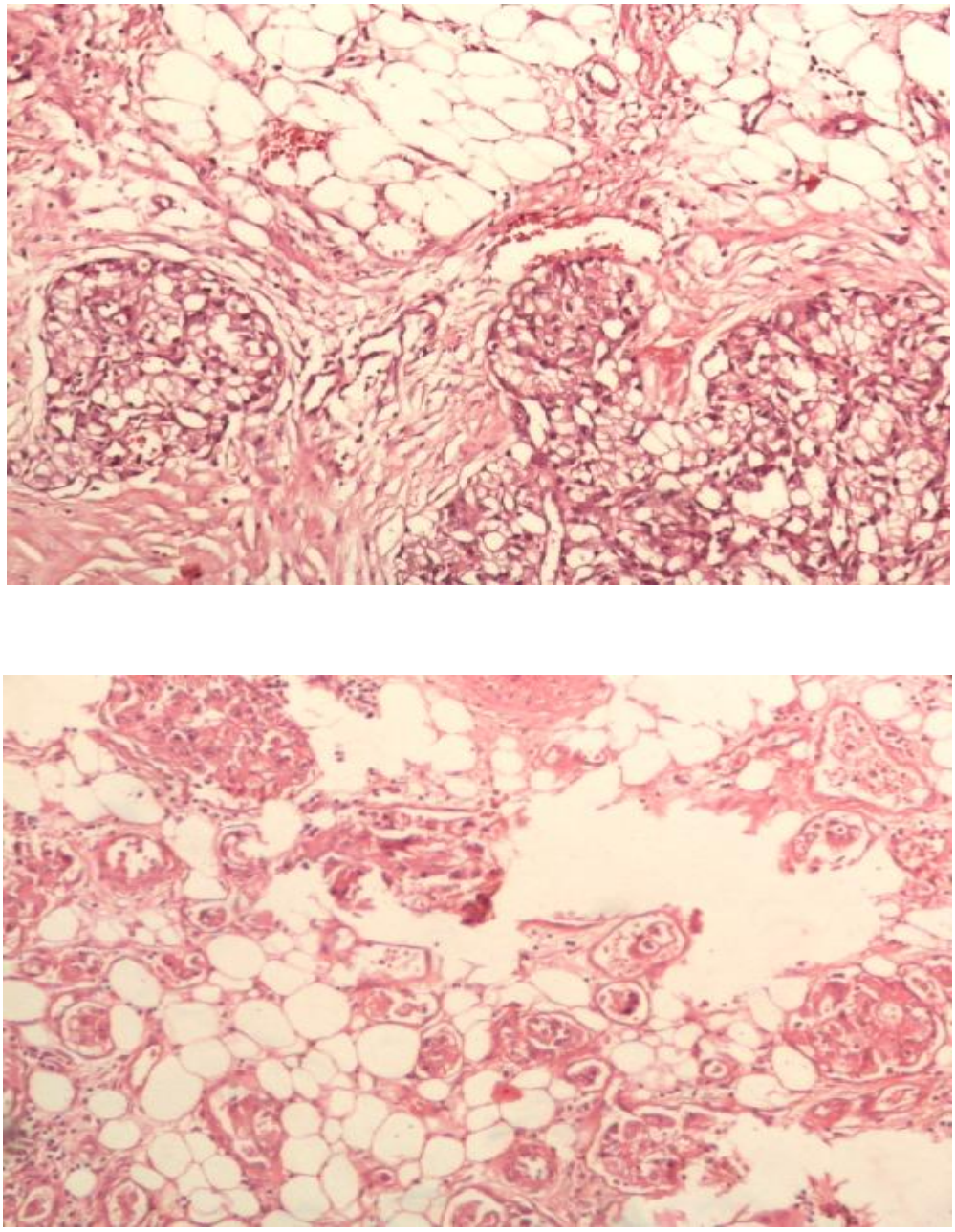
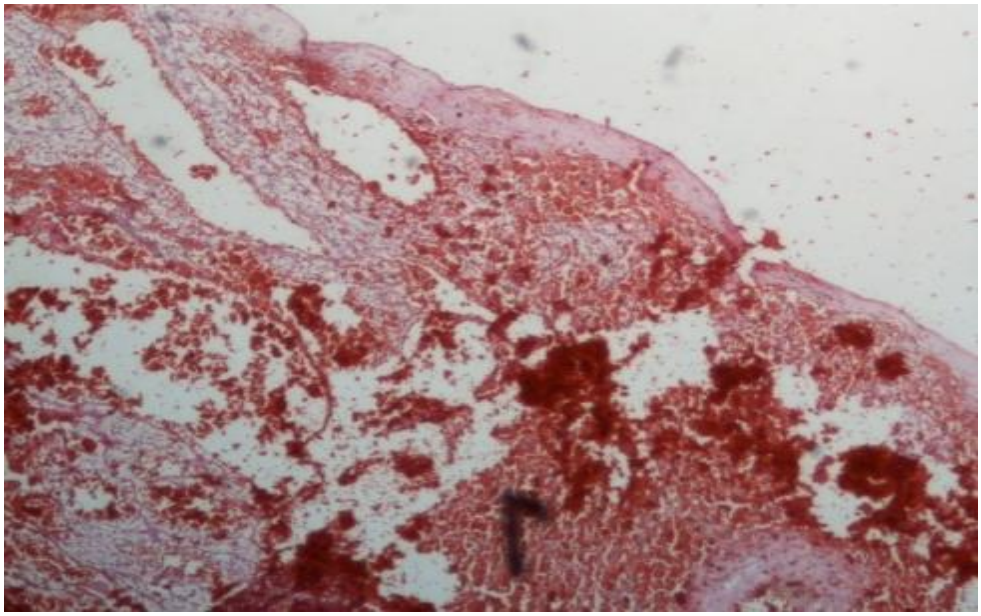


Fig. 2-15 Perinephric fat invasion H&E X40 (Dept of Pathol. Benghazi University, Benghazi, Libya)



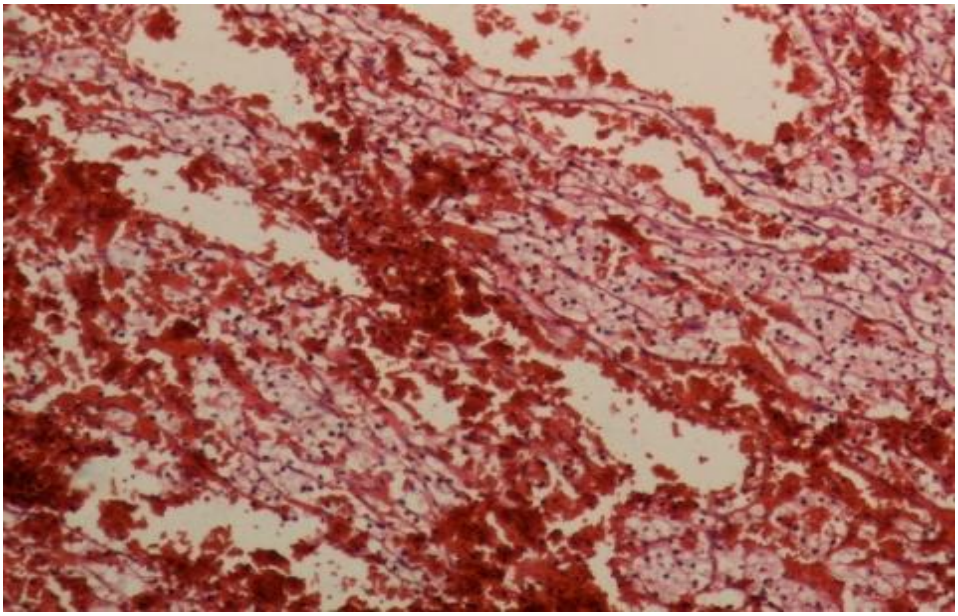


Fig. 2-16 Vascular invasion H&E X10 (Dept of Pathol. Benghazi University, Benghazi, Libya)

Treatment of renal cell carcinoma

The therapeutic approach to RCC is guided by the probability of cure, which is related directly to the stage or degree of tumor dissemination (Simon et al., 2000; Linehan et al., 2001; Jonasch et al., 2006). Surgical resection remains the only known effective treatment for localized renal cell carcinoma, and it also is used for palliation in metastatic disease. About 25-30% of patients have metastatic disease at diagnosis, and fewer than 5% have solitary metastasis. Surgical resection is recommended in selected patients with metastatic renal carcinoma. This procedure may not be curative in all patients but may produce some long-term survival. The possibility of disease-free survival increases after resection of primary tumor and isolated metastasis excision (Curti et al., 2012). The treatment options for RCC are surgery, radiation therapy (palliative), targeted therapy (bevacizumab, sunitinib, sorafenib, everolimus, temsirolimus), biological therapy (immunotherapy), and combinations of these (Wein et al., 2012).

Surgery is curative in the majority of patients without metastatic RCC and is therefore the preferred treatment for patients with stages I, II, and III disease. Treatment can involve either a radical nephrectomy (removal of kidney, ipsilateral adrenal gland, and Gerota's fascia) or a variety of renal-sparing approaches (partial nephrectomy or ablative techniques) in carefully selected patients, depending upon the extent of disease (Atkins et al., 2011). The role of adrenalectomy at the time of nephrectomy has also been questioned. In a review of 351 patients who underwent radical nephrectomy from 1998 to 2008, Tsui et al., (2000) concluded that adrenal involvement is not likely with localized early stage RCC, and thus, adrenalectomy is not necessary, particularly when the CT scan is negative (Tsui et al., 2000).

Stage I

For tumors ≤4 cm in diameter, surgical excision by partial nephrectomy (nephron sparing surgery) is recommended. The surgical approach can be either open or laparoscopic, depending on surgeon preference, and tumor size and location. The objective of surgery is to spare as many functioning nephrons as possible and to preserve renal function while excising the tumor. Numerous studies have shown that patients who have small tumors (< 4 cm) with a healthy contralateral kidney treated

with partial nephrectomy will have much higher likelihood of maintaining an acceptable glomerular filtration rate (GFR) over 45 ml/minute when compared to radical nephrectomy patients (36% vs. 5%) (Huang et al., 2006). Survival was better for patients undergoing partial nephrectomy compared to patients treated with radical nephrectomy (85% vs. 78%) for tumors between 4 to 7 cm ($p = 0.01$). This increased survival is likely attributable to better renal function in patients with partial nephrectomy (Weight et al., 2010). Other nephron-sparing approaches, such as radiofrequency ablation, cryoablation, and even active surveillance, may be acceptable alternatives to radical nephrectomy for carefully selected patients who are compliant with regular follow-up visits. Guidelines developed by an AUA panel exist for the management of the clinical Stage 1 renal mass (AUA, 2009).

Laparoscopic nephrectomy, hand-assisted laparoscopic nephrectomy, and robotic-assisted nephrectomy are less invasive procedures than the traditional open radical nephrectomy and are reasonable alternatives to open radical nephrectomy in T1 and T2 tumors. These procedures incur less morbidity, and are associated with less blood loss and a shorter recovery time (Burgess et al., 2007). Radical nephrectomy is recommended only if the tumor is not amenable to partial nephrectomy (Namita et al., 2012). In patients with decreased life expectancy─or those considered to be at high risk during surgery options include active surveillance and thermal ablation (cryoablation or radiofrequency ablation). Although distant recurrence-free survival rates are comparable, thermal ablation has been associated with an increased risk of local recurrence compared with conventional surgery (Kunkle et al., 2008; Campbell et al., 2009). For 4- to 7-cm tumors, partial nephrectomy, if feasible, or radical nephrectomy is the standard of care (Namita et al., 2012).

Stages II and III

Locally advanced tumors are managed with radical nephrectomy, which can require resection of adjacent organs, and with tumor thrombectomy from the renal vein and possibly the inferior vena cava (Rini et al., 2009). Between 40% and 60% of patients can be cured with such an aggressive surgical approach (Blute et al., 2004; Margulis et al., 2007). After surgical excision, up to 30% of patients with localized tumors experience relapse. The lung is the most common site of distant recurrence, seen in 50% to 60% of patients. The median time to relapse after surgery is approximately

two years, with most relapses occurring within five years. Interferon alpha and high-dose interleukin-2 (IL-2) have been tested as adjuvant treatments following resection of stage I and II kidney cancer. However, no benefit has been seen in randomized trials (Clark et al., 2003; Messing et al., 2003). NCCN Kidney Cancer Panel has recommended that patients be seen every six months for the first two years after surgery and annually thereafter. Each visit should include a history, physical examination, and comprehensive metabolic panel (eg, blood urea nitrogen, serum creatinine, calcium levels, LDH, and liver function tests). Abdominal and chest imaging studies should be done approximately two to six months after surgery and as clinically indicated thereafter **(**NCCN, 2012).

Stage IV

Cytoreductive nephrectomy before systemic therapy is recommended in patients with a surgically resectable primary tumor (Flanigan et al., 2004). Cytoreductive nephrectomy is a removal of the primary tumor (cytoreductive or debulking nephrectomy) is useful prior to initiating immunotherapy in some patients who present with advanced RCC. Patients with mRCC who undergo cytoreductive nephrectomy prior to treatment with vascular endothelial growth factor (VEGF) targeted therapy may have longer overall survival than patients who do not have surgery (Tuma et al., 2010). Metastasectomy of a solitary metastasis is recommended in selected patients with good performance status. A large retrospective analysis from a single institution revealed improved cancer-specific survival advantage, even with metastasectomy of more than one lesion. The study also revealed increased risk of death due to renal cell carcinoma in patients who did not undergo surgical resection of metastasis (Alt et al., 2011). Selected patients older than 70 years who have asymptomatic renal masses and slow growth documented on serial imaging may be observed. A retrospective, single institution review of 51 patients showed no metastatic spread with a median follow-up of almost 6 years; only 2 patients required surgical intervention for local progression or symptoms (Haramis et al., 2011). Elderly patients and those with significant comorbidity may not be candidates for surgical resection (Santos et al., 2008). Although nonsurgical procedures (cryoablation, RFA) may be useful, most small tumors grow slowly and do not become symptomatic or metastasize (Rendon et al., 2000; Lamb et al., 2004; Bosniak

et al., 1995; Volpe et al., 2004; Wehle et al., 2004). A study by Alt et al found that complete resection of multiple RCC metastases may be associated with long-term survival (Alt et al., 2011). Another study by Zagoria et al found that radiofrequency ablation can result in durable oncological control in patients with RCC that are smaller than 4 cm who are poor surgical candidates (Zagoria et al., 2011).

Adjuvant therapy

Efforts to improve on the results with surgery alone for localized RCC include adjuvant therapy, with either immunotherapy or molecularly targeted agents.

Cytokine therapy

Cytokine therapy with interferon-alpha (IFN-α) or interleukin-2 (IL-2) has been shown to induce objective responses, and interferon-alpha appears to have a modest impact on survival in selected patients. Interferon-alpha has approximately a 15% objective response rate in appropriately selected individuals (Coppin et al., 2005). High-dose IL-2 produces a similar overall response rate to IFN-α, but approximately 5% of patients had durable complete remissions (Rosenberg et al., 1987; Fisher et al., 1988; Weiss et al., 1992; Rosenberg et al., 1994; Fyfe et al., 1995)

Molecular targeted therapy

In recent years several targeted therapies have become available for first- and second-line use. These include sorafenib, sunitinib, bevacizumab (plus IFN-α), temsirolimus, everolimus, and, most recently, pazopanib. This expanded list of treatment options arose from molecular biological research that revealed aberrant signal transduction activities in RCC, enabling the identification of specific molecular targets for therapy. Molecular-targeted therapies have better efficacy and tolerability than cytokine therapy, and many are administered orally. The superior outcomes achieved with molecular-targeted agents are prompting investigators to reconsider overall survival. In mRCC trials, progression-free survival has become a popular primary endpoint and has served as the basis of approval for several targeted therapies (Thomas, 2011).

1. Anti-angiogenic (VEGF). Sunitinib, sorafenib, pazopanib are used for blocking the intracellular domain of the VEGF receptor and a monoclonal antibody (eg,

bevacizumab) to bind circulating VEGF and prevent its activating the VEGF receptor (Atkins et al., 2005).

2. mTOR inhibitors (mammalian target of rapamycin).Temsirolimus, an inhibitor of mTOR, significantly increased survival of poor-prognosis patients in a phase III trial when used as the initial treatment for patients with advanced disease (Hudes et al., 2007). Everolimus, another inhibitor of mTOR, significantly increased progression-free survival compared to placebo in a phase III trial in patients who have progressed after treatment with a VEGF-targeted agent (Motzer et al., 2008).

Chemotherapy

RCC is refractory to most chemotherapeutic agents because of multidrug resistance mediated by p -glycoprotein. Normal renal proximal tubules and RCC both express high levels of p -glycoprotein. Calcium channel blockers or other drugs that interfere with the function of p -glycoprotein can diminish resistance to vinblastine and anthracycline in human renal cell carcinoma cell lines (Curti et al., 2012). A phase 2 trial of weekly intravenous (IV) gemcitabine (600 mg/m^2 on days 1, 8, and 15) with continuous infusion fluorouracil (150 mg/m^2/d for 21 d in 28-d cycle) in patients with mRCC produced a partial response rate of 17%. No complete responses were noted. Eighty percent of patients had multiple metastases, and 83% had received previous treatment. The mean progression-free survival duration of 28.7 weeks was significantly longer than that of historic controls (Rini et al., 2000).

Hormonal agents

Progestational agents have been extensively evaluated in patients with advanced RCC, but do not appear to have antitumor activity. Medroxyprogesterone is the most widely studied. Despite occasional reports of responses, a review of medroxyprogesterone treatment concluded that RCCs are neither hormone-dependent nor hormone-responsive (Kjaer., 1988). Although some patients with severe anorexia may derive symptomatic benefit from hormonal therapy. There is no evidence that other hormonal agents (eg, androgens, antiestrogens, or combinations of hormones and chemotherapy) are any more effective (Stahl et al., 1992; Oh et al., 2002; Atkins, 2011).

Prognostic factors in renal cell carcinoma

RCC is a malignancy with an adverse prognosis for the majority of the patients. Despite that an increasing number of patients incidentally is diagnosed, still around 25–30 % of patients with new diagnosed disease already have metastatic disease. Of the remaining patients with nonmetastatic disease, about 30–40 % will progress with distant metastases or local recurrent RCC. Thus, approximately 50–60 % of the patients who are clinically diagnosed will die because of progressive disease (Thrasher et al., 1993; Atkins, 2011). Investigators have attempted to identify pathological and morphological features within tumors that correlate with survival in patients with RCC. Tumor stage remains the most important factor predictive of survival in RCC (Skinner et al., 1971; Golimbu et al., 1986).

In addition to, tumor size (Medeiros et al., 1988), histological pattern, cell type (Golimbu et al., 1986), nuclear grade (Medeiros et al., 1988), DNA content (Ljungberg et al., 1986), and nuclear morphometry (Murphy et al., 1986), have been reported as prognostic surrogates for survival. Additionally, variables such as performance status (Thrasher et al., 1993), weight loss, time to progression, number and type of metastases (Citterio et al., 1997), vascular invasion (Van Poppel et al., 1997), and several laboratory values, e.g. haemoglobin level, ESR and alkaline phosphatase levels, have been studied in relation to prognosis (Citterio et al., 1997). Increasing knowledge of cytogenetic abnormalities and the role of oncogenes and tumor suppressor genes in RCC is critical, but the study of molecular mechanisms underlying RCC is still in its infancy. Future studies will have to provide information on the implications for the prognosis of patients with RCC (Van Brussel et al., 1999)

Clinicopathological prognostic factors

1. *Anatomic extent of tumor*

TNM staging system

TNM staging system is used to assess the anatomic extent of disease. The anatomic extent of disease is the most consistent factor that influences prognosis in patients with RCC.

Stage I/II: Patients with stage I (T1N0) RCC have a five-year survival rate over 90% in most contemporary series. The survival rate may be slightly lower for patients with stage II (T2N0) disease, with reported five-year survival rates ranging from 75 to 95 percent. Patients with stage I or II RCC that invades the urinary collecting system appear to have a significantly worse prognosis (Verhoestet al., 2009; Atkins, 2011). In a multivariate analysis of a series of 1124 cases of RCC, the 10-year survival rates for patients with T1 or T2 primary lesions that invaded the urinary collecting system was 43 and 41%, respectively (Atkins, 2011).

Stage III: The reported five-year survival rate for patients with stage III RCC (T1N1M0, T2N1M0, T3N0M0, or T3N1M0) who undergo nephrectomy ranges from 59 to 70%. There are conflicting data about whether extension into the perinephric fat (T3a) alone adversely affects prognosis. Among patients with T3a disease, the size of the primary tumor remains a prognostic factor (ten-year survival rates of 77, 54, and 46 % for tumors <4, 4 to 7, and >7 cm, respectively) (Siddiqui et al., 2007). Patients with involvement of the renal vein or inferior vena cava are classified as stage III. Although some studies have not identified an adverse impact of renal vein involvement on prognosis (Waters et al., 1979; Ficarra et al., 2001; Atkins, 2011). In addition, invasion of the urine collecting system also appears to be a prognostic factor in patients with stage III RCC. Multiple studies found that patients with urine collecting system invasion had significantly worse disease-specific and overall survival compared with those without invasion (Anderson et al., 2011).

Stage IV: The median survival for patients with stage IV disease (T4 primary tumor, N2 involvement, or distant metastases) is 16 to 20 months in contemporary reports,

and the five-year survival rate is less than 10% for patients with distant metastases (Mekhail et al., 2005).

Renal capsule invasion

Renal capsular invasion is associated with a worse stage in localized RCC. Patients with Stage pT2 RCC and capsular invasion appear to have a worse prognosis than those with equivalently staged RCC without capsular invasion. The 5-year disease-specific survival rate for patients with no capsular invasion versus with capsular invasion was 91.8% versus 84.3%, respectively (Gab et al., 2006).

Perinepheric fat invasion

Patients with perinephric plus sinus fat invasion had worse cancer specific survival than those with perinephric or sinus fat invasion alone (Kresowik et al., 2010). Perinephric and renal sinus fat invasion are classified as pT3a RCC. Patients with renal sinus fat invasion were 63% more likely to die of RCC compared with those with perinephric fat invasion. Results indicate that clear cell tumors invading the renal sinus fat are more aggressive than tumors with perinephric fat involvement (Thompson et al., 2005).

Nodal involvement

Nodal involvement is one of the main factors influencing the prognosis of patient with cancer and RCC in not exception. Life expectancy decrease considerably when lymph nodes metastasis are present, with overall five-years survival rates of 11%-35% reported (Minervini et al., 2001; Pantuck et al., 2003).

Vascular invasion

Involvement of the renal vein or/and the inferior vena cava (IVC) has been reported in 4%-10% of patients (Zisman et al., 2003; Haferkamp et al., 2007). When it occurs without evidence of lymph node involvement or distant metastasis, surgery offers the only potential cure (Ciancio et al., 2007). Mean while, there are several reports of larger series of patients who underwent radical surgery for RCC with inferior vena

caval involvement, with reported 5-year survival rates of 34% to 72% (Staehler et al., 2000; Tanaka et al., 2008).

2. *Histopathology*

Tumor type

Whether the tumor subtype (ie, clear cell versus papillary or chromophobe carcinoma) affects prognosis is controversial. Multiple studies failed to identify a prognostic difference when TNM stage, histologic grade, and performance status were considered advanced study (Patard et al., 2005). In contrary other studies from the Mayo Clinic and from Memorial Sloan-Kettering Cancer Institute (MSKCC), both found that patients with clear cell histology had significantly poorer cancer-specific survival (Teloken, 2009; Leibovich, 2010). Many studies suggest that chRCC has a significantly better survival than conventional and papillary RCC, which have an intermediate prognosis (Amin et al., 2002; Beck et al., 2004). In PRCC, the five year survivals for all stages range from 49% to 84% (Comiter et al., 1998). Up to 70% of PRCC are intrarenal at diagnosis (Amato et al., 1992; Lager et al., 1995) and type 1 tumours are usually of lower stage and grade than type 2 tumours (Delahunt et al., 1997; Moch et al., 2000). Longer survivals have been demonstrated for type 1 when compared with type 2 PRCC on both univariate and multivariate analysis that included both tumour stage and grade (Delahunt et al., 2001). The typical collecting duct carcinomas have a poor prognosis with many being metastatic at presentation. About two thirds of patients die of their disease within two years of diagnosis (Srigley et al., 1998), sarcomatoid clear cell carcinomas, and renal medullary carcinomas, are considered more aggressive and are associated with a shorter survival (Golshayan et al., 2009).

Tumor grade

Histologic grade is an independent factor correlating with survival (Novara et al, 2007; Rioux-Leclercq et al., 2007). Multiple systems are used to grade RCC, of which the Fuhrman's grade is the most widely used In one report, the five-year survival rate based upon tumor grade was 89, 65, and 46% for tumors of histologic grade 1, 2, and 3 to 4, respectively (Tsui et al., 2000).

3. *Clinical factors*

In addition to the anatomic extent of disease, clinical factors can influence survival. Negative prognostic signs include a poor performance status, the presence of symptoms and/or paraneoplastic syndromes (eg, anemia, hypercalcemia, hepatopathy, thrombocytosis, fever, weight loss), and obesity. Although younger patients (ie, 20 to 40 years old) are more likely to be symptomatic at presentation, their outcome may be slightly better due to a lower incidence of nodal involvement (Siemer et al., 2006). Other factors as age, sex and race do not influence the clinical course of the disease. Although younger patients will lose more years of expected survival, there is no difference in actual survival time compared to older patients (Abou El Fettouh et al., 2002). Among women, age is an independent prognostic factor of disease-specific survival (DSS) with the risk of RCC-specific death increasing by 1% with each year increase in age. As a group, women present with less advanced tumors, leading to a 19% reduced risk of RCC-specific death compared with men. This survival difference is present only in patients aged <60 years, but disappears in older patients. Age is an independent prognostic factor in women but not men (Pantuck et al., 2008).

Molecular prognostic factors

Renal cell carcinoma–associated tumor markers

The use of tissue microarrays permits the evaluation of the histologic and immunohistochemical features of multiple tumors simultaneously (Junker et al., 2000; Takahashi et al., 2001). This technology can achieve rapid molecular profiling and may identify definitive prognostic indicators for RCC (Gonzalgo et al., 2004; Kosari et al., 2005; Jones et al., 2005)

Although none of these factors has an established role independent of stage, some markers have shown promise as prognostic markers in patients with clear cell RCC. In particular, lack of B7H1 and B7H4 expression in patients has been strong predictors of overall survival in patients without metastases (Yao et al., 2002; Shvarts et al., 2005; Thompson et al., 2006).

1. Von Hippel–Lindau gene

The understanding of the role of the von Hippel–Lindau (VHL) tumor suppressor gene in RCC has been one of the landmarks for the considerations about angiogenic pathways. It is inactivated in almost all RCC in patients with VHL syndrome. Importantly, this tumor-suppressor gene on chromosome 3p was found to be also inactivatedin about 70% of sporadic ccRCC, resulting in deficient protein isoforms pVHL19 andpVHL30. One well-studied consequence of the deficientVHL proteins is an impaired degradation of hypoxia induced factor 1alpha (HIF-1a), which accumulates even under nonhypoxic conditions (Struckmann et al., 2008). However, the entire range of the regulative mechanisms controlled by pVHL goes far beyond this and includes regulation of cell-cycle arrest via p53 or deposition of extracellular matrix, closely linked to neoangiogenesis and tissue invasion (Frew et al., 2008). The VHL gene's complex position might also explain why the prognostic role of VHL alterations is divergent. Multivariate analysis of sex, age, grade, symptoms, that VHL mutation or hypermethylation strongly related to a better PFS and a CSS for stage I–III ccRCC(Yao et al., 2002). In another study reported that only ''loss-of-function'' mutations of VHL are associated with worse prognosis in univariate analyses, while tumor grade, stage, microvessel density, and tumor-cell proliferation were not associated with VHL mutations. They concluded that the regulation of angiogenesis and proliferation of RCC might not be directly influenced by VHL mutations (Schraml et al., 2002). As for the therapeutically predictive value, VHL mutations or promoter methylations seem to have a modest positive correlation to anti– VEGF–targeted therapy response, with a described objective response rate of 48% compared to 35% in patients with no VHL mutation or methylation (Rini et al, 2006).

2. *Hypoxia-induced factor 1 alpha*

HIF-1α accumulates either in hypoxic cell conditions or when the pVHL is deficient. In a study, somatic mutations of the VHL gene were detected only in HIF-1α overexpressing ccRCC. Consequently, an increased expression of HIF-1a was found in 24 of 32 ccRCC tumors (75%), but only in three of eight non-ccRCC tumors. Moreover, none of the HIF-1a–negative ccRCCs displayed a VHL mutation

(Wiesener et al., 2001). Similar to VHL mutation, the prognostic value of an HIF-1a over-expression is also controversial. In a Western blot analyses of 66 ccRCCs, showed that a high level of HIF-1a protein expression to be an independent, favorable, prognostic factor (Lidgren et al., 2005). In one study, high levels of immunohistochemical expression of HIF-1α were associated with a significantly worse survival compared to tumors with lower levels of HIF-1α (Klatte et al., 2007). However, at least one other study has suggested that patients with tumors that express HIF-1α have a better prognosis than those with tumors that only express HIF-2α (Gordan et al., 2008). Furthermore, despite the fact that HIF-1a acts via transcriptional regulation of a number of factors involved in the downstream regulation of angiogenesis, glucose metabolism, and stimulation of growth factors, its complex intracellular signaling includes also the induction of apoptosis by stabilizing wild type p53, but it cannot interact with mutated p53 (An et al., 1998). Therefore, the p53 status might bias the results of the prognostic ability of HIF-1a, although p53 mutations are rare in ccRC. And finally, the excellent CSS rate of patients with early stage, nonmetastatic tumors after surgery could simply overexpose effects of HIF expression on survival. The proportion of N+ or M1 tumors in the Lidgren et al., article is not explicitly mentioned. However, 76 of 176 ccRCCs investigated were stage 1 or stage 2 (Lidgren et al., 2006).

3. Vascular endothelial growth factor (VEGF)

VEGF production is significantly increased in RCC with VHL gene alterations and raised HIF-1α protein expressions. Furthermore, it is associated with a more aggressive tumor phenotype (Na et al., 2003). Several groups could show that a raised VEGF expression is a significant predictor for outcome, and in some studies showed this correlation even using multivariate analyses together with stage and grade (Paradis et al., 2000; Yildiz et al., 2004; Jacobsen et al., 2004). With the close relationship between VHL and HIF- 1 α, one might expect a raised VEGF expression to be an exclusive feature of ccRCC. However, a study of 300 RCCs demonstrated no difference between the RCC types (Jacobsen et al., 2004).

Reported elevated VEGF expression in 29% of ccRCCs and in 67% of papRCCs. (Yildiz et al., 2008).

4. Grawitz 250 or carbonic anhydrase 9

As early as 1986 Oosterwijk described Grawitz 250 (G250) as an RCC-specific antibody (Oosterwijk et al., 1986) high CAIX expression levels in primary tumors, as well as in resected lung metastases, were associated with improved prognosis in advanced ccRCC (Bui et al., 2003; Tennstedt et al., 2008). CAIX identified as an independent predictor of survival, even when analyzed together with stage, grade, nodal status, metastatic status, and performance status (Bui et al, 2004). This result was in a large analysis of 730 patients that also found low CAIX expression univariately associated with increased risk of RCC death (risk ratio: 1.65), but not at multivariate analysis (Leibovich et al., 2007). Beside the ccRCC specificity and the prognostic value of CAIX, it seems to predict outcome of therapy with interleukin 2, with more responding patients having high CAIX expressing tumors compared with nonresponders (78% vs 51%) (Atkins et al., 2005).

5. *Mammalian target of rapamycin*

mTOR pathway has been shown to be upregulated in many human cancers. As for RCC, it symbolizes the second major pathway of today's targeted therapy options, with a proven efficiency of the mTOR inhibitors temsirolimus (Hudes et al., 2006). And everolimus (Motzer et al., 2008). The literature about the prognostic role of mTOR as an MM is sparse. In a very recent study, reported positive cytoplasmatic mTOR staining in metastatic specimens to be correlated with improved cancer specific survival (CSS) in 132 specimens (Youssif et al., 2008).

Generic molecular marker

1. p53

Overall, p53 positivity seems to be a rare event in RCC (Sejima et al., 1999), and is probably more frequent in metastases than in primary tumors. As for the different RCC types, p53 over-expression was found to be more frequent in non-ccRCCs, and especially in papRCC. However, with regard to the predictive value of a positive p53 staining, the literature is again divided. A reason for this could be the heterogeneous p53 staining within tumors (Zigeuner et al., 2004).

2. Ki 67

Ki 67 has been described as a multivariate independent negative predictor of overall survival (Rioux-Leclercq et al., 2000; Bui et al., 2004) and progression free survival (Shvarts et al., 2005). Immunohistochemical detection of CAIX and the proliferative marker Ki 67 has also been shown to correlate with prognosis. In a series of 224 patients with clear cell RCC, multivariate analysis found that low levels of CAIX expression and high levels of Ki 67 were associated with asignificantly worse prognosis (Bui et al., 2004). A combination of these two parameters was useful in separating patients into good, intermediate, and poor prognosis subsets. The role of CAIX expression by the primary tumor as a predictor of responsiveness to immunotherapy remains uncertain (Atkins et al., 2011).

3. Insulin-like growth factor II mRNA-binding protein

The oncofetal RNA-binding protein IMP3 (insulin-like growth factor II mRNA-binding protein) is assumed to regulate transcription of insulin-like growth factor II mRNA. Its reappearance after embryogenesis has been observed in a number of other solid tumors to be a negative predictor. For RCC, there are validated data (Hoffmann et al., 2008), that IMP correlate with higher stage, grade, sarcomatoid differentiation and decreased CSS. Moreover, there is a profound correlation with decreased PFS in localized tumors with a 4–17-fold lesser probability for a metastasis-free survival, both in ccRCC (Jiang et al., 2006), as in papRCC, and in chRCC (Jiang et al., 2008).

Survivin

Survivin is a member of the inhibitor of apoptosis (IAP) family and has been found to play an important role in the initiation, progression, and chemoradioresistance of human malignancies (Lei et al., 2010). Survivin, also called baculoviral inhibitor of apoptosis repeat-containing 5 or BIRC5, is a protein that, in humans, is encoded by the BIRC5 gene (Altieri, 1994). Survivin is a member of the IAP family of antiapoptotic proteins. It is shown to be conserved in function across evolution as homologues of the protein are found both in vertebrates and invertebrates. The first members of the IAPs identified were from the baculovirus IAPs, Cp-IAP and Op-IAP, which bind to and inhibit caspases as a mechanism that contributes to its efficient infection and replication cycle in the host. Later, five more human IAPs that included XIAP, c-IAPl, C-IAP2, NAIP, and survivin were discovered. Survivin, like the others, was discovered by its structural homology to IAP family of proteins in human B-cell lymphoma. The human IAPs, XIAP, c-IAPl, C-IAP2 have been shown to bind to caspase-3 and -7, which are the effector caspases in the signaling pathway of apoptosis. It is not known with absolute certainty though, how the IAPs inhibit apoptosis mechanistically at the molecular level. A common feature that is present in all IAPs in the presence of a BIR (Baculovirus IAP Repeat, a ~70 amino acid motif) in one to three copies. It was shown that knocking out BIR2 from XIAP was enough to cause a loss of function in terms of XIAPs ability to inhibit caspases. This gives the implication that it is within these BIR motifs that contains the anti-apoptotic function of these IAPs. Survivin's one BIR domain shows a similar sequence compared to that of XIAP's BIR domains (Tamm et al., 1998).

Isoforms

The single Survivin gene can give rise to four different alternatively spliced transcripts (Caldas et al., 2005). Survivin, has a three-intron–four-exon structure in both the mouse and human. Survivin-2B, which has an insertion of an alternative exon 2. Survivin-Delta-Ex-3, which has exon 3 removed. The removal of exon 3 results in a frame shift that generates a unique carboxyl terminus with a new function. This new function may involve a nuclear localization signal. Moreover, a mitochondrial

localization signal is also generated. Survivin-3B, which has an insertion of an alternative exon 3.

Structure

A structural feature common to all IAP family proteins is that they all contain at least one baculoviral IAP repeat (BIR) domain characterized by aconserved zinc coordinating Cys/His motif at the N-terminal half of the protein.

Survivin is distinguished from other IAP family members in that it has only one BIR domain. The mice and human BIR domain of Survivin are very similar structurally except for two differences that may affect function variability. The human Survivin also contains an elongated C-terminal helix comprising 42 amino acids. Survivin is 16.5 kDa large and is the smallest member of the IAP family.

X-ray crystallography has shown two molecules of human Survivin coming together to form a bowtie-shape dimer through a hydrophobic interface. This interface includes N-terminal residues 6-10 just before the BIR domain region and the 10 residue region connecting the BIR domain to the C-terminal helix (Shi, 2000).

Function

The Survivin protein functions to inhibit caspase activation, thereby leading to negative regulation of apoptosis or programmed cell death. This has been shown by disruption of Survivin induction pathways leading to increase in apoptosis and decrease in tumour growth. The Survivin protein is expressed highly in most human tumours and fetal tissue, but is completely absent in terminally differentiated cells **(**Sah et al., 2006).

Mechanism of action

Survivin inhibits both Bax and Fas-induced apoptotic pathways (Tamm et al., 1998). Survivin does not inhibit by mechanism of preventing Bax or Fas protein from being made into fully functional proteins. Therefore, Survivin should be acting somewhere downstream of the Bax or Fas signaling pathway to inhibit apoptosis through these pathways. Further evidence to support the idea that Survivin blocks apoptosis by

directly inhibiting caspases, in a study 293 cells were transfected with either overexposed caspase-3 or -7 encoding plasmid and with Survivin, they showed that Survivin inhibited processing of these two caspases into their active forms. While Survivin has been shown to bind to only the active forms of these caspases, it is likely here that Survivin inhibits the active forms of the caspases resulting from cleaving and activating more of its own proforms. Thus, Survivin acts possibly by preventing such a cascade of cleavage and activation amplification from happening resulting in decreased apoptosis. In similar manner, looking at the mitochondrial pathway of apoptosis, cytochrome *c* was transiently expressed in 293 cells to look at the inhibitory effects survivin had on this pathway. Although the details are not here, Survivin was shown to also inhibit cytochrome *c* and caspase-8-induced activation of caspases (Tamm et al., 1998).

Role in cancer

Survivin is known to be expressed in most tumour cell types but it is absent in normal non-malignant cells. Survivin protein is most highly expressed in lung and breast cancer cell lines and least expressed in renal cancers (Tamm et al., 1998). Wild-type p53 has been shown to repress Survivin expression at the mRNA level. P53's normal function is to regulate genes that control apoptosis. As Survivin is a known inhibitor of apoptosis, it can be implied that p53 repression of Survivin is one mechanism by which cells can undergo apoptosis upon induction by apoptotic stimuli or signals. When Survivin is over-expressed in the cell lines mentioned in the previous paragraph, apoptotic response from DNA-damaging agent Adriamycin decreased in a dose-dependent manner. This suggests that down-regulation of Survivin by p53 is important for p53-mediated apoptotic pathway to successfully result in apoptosis. It is known that a defining characteristic of most tumors is the over-expression of Survivin and the complete loss of wild-type p53 (Mirza et al., 2002).

Prognostic significance of Survivin in renal cell carcinoma

The expression of Survivin gene could be reduced in RCC cell line and survivin knockdown could inhibit growth and enhance in *vivo* radiosensitivity of RCC cell line by inducing apoptosis enhancement. Taken together, the status of Survivin protein expression may be an independent factor for predicting the prognosis of RCC patients

and tumor-specific Survivin knockdown combined with radiotherapy will be a potential strategy for RCC therapy (Lei et al., 2010). Survivin expression was significantly associated with poorly differentiated, advanced stages and more aggressive ccRCCs. Patients with low Survivin expression had statistically significant better survival rates than patients with high Survivin expression (Zamparese et al., 2008). A significant increase in Survivin expression was associated with increased T stage, increased tumor grade, and low recurrence-free survival. The results of this study suggest that Survivin-mediated inhibition of apoptosis is associated with progression and recurrence of RCC. Thus, Survivin is a useful independent prognostic marker for this condition (Byun et al., 2007).

The 5-year cancer-specific survival rate was 43.0% for patients with high Survivin expression and 87.2% for patients with low Survivin expression. Survivin expression is an independent predictor of ccRCC progression and death from RCC. Thus, survivin has the potential to offer additional prognostic information and to provide a novel target for the development of new adjuvant therapies (Parker et al., 2006). The results of previous study suggest that Survivin-mediated inhibition of apoptosis is associated with progression and recurrence of renal cell carcinoma. Thus, Survivin is a useful independent prognostic marker for this condition (Seok-Soo Byun et al., 2007).

Chapter III

Patients and methods

Patients and methods

Clinicopathological features and follow up

The records of all newly diagnosed Renal cell carcinoma cases between 2003 to Jan. 2012 based on availability of representative paraffin blocks were retrieved from the files Department of the Histopathology, Benghazi University, Benghazi, Libya. 37 Libyan patients, were diagnosed with RCC. For each patient obtained the following information: age, gender, diagnosis, grading, staging. the patient files and hospital information systems, were obtained then reviewed to collect more information about type of initial surgical procedure, and the anatomic site of tumour at initial presentation, date of initial diagnosis.

One independent pathologist confirmed all histological diagnoses and the following histopathological features were recorded included primary tumor (T1, T2, T3 or T4), tumour type (Clear cell RCC, Papillary RCC and Chromophobe RCC), tumour grade, lymphovascular permeation, capsular and perinephric fat invasion and number of lymph nodes examined.

All the patients were followed up until death or when last seen alive at their clinical visit (Oct. 2012) with the FU-time of months (range: 9-119 month). The duration of follow-up and the outcomes at the end of follow-up were determined for each patient from hospital and clinic charts.

The clinical and histopathological data of each patient were collected and entered into a computer database according to the criteria of tumour–node–metastasis (TNM) classification of the International Union against Cancer (Sobin et al., 2009). The histological garde of tumors was determined according to the Fuhrman garding system. The key clinicopathological data of the patients are summarized in Table. 3-1.

Table. 3-1. Clinicopathological characteristics of the patients with RCC

Characteristic	*No. of patients (%)*
Gender	
Male	*23 (62%)*
Female	*14 (38%)*
Age (yrs)	
≤ 55 years	*20 (54%)*
> 55 years	*17 (46%)*
Primary tumor status	
T1	*14 (38%)*
T2	*15 (40%)*
T3	*8 (22%)*
T4	*0 (0%)*
LN involvement[1]	
No	*15 (83%)*
Yes	*3 (17%)*
Metastasis	
No	*22 (59%)*
Yes	*7 (19%)*
Unknown	*8 (22%)*
Stage	
I	*15 (40%)*
II	*13 (35%)*
III	*4 (11%)*
IV	*5 (14%)*
Histological grade[2]	
G 1	*2 (6%)*
G 2	*26 (72%)*
G 3	*8(22%)*
Localization	
Rt. Kidney	*18 (49%)*
Lt. Kidney	*19 (51%)*

Table. 3-1. (continued)	
tumor thrombus	
Yes	***7 (19%)***
No	***30 (81%)***
Primary tumor size	
≤ 5cm	***9 (24%)***
>5cm	***28 (76%)***
Histological subtypes	
Clear cell RCC	***29 (78%)***
Papillary RCC	***6 (17%)***
Chromophobe RCC	***2 (5%)***
Perinephric fat invasion[3]	
No	***34 (94%)***
Yes	***2 (6%)***
Capsular invasion	
No	***33 (89%)***
Yes	***4 (11%)***
Recurrence during the follow-up	
Yes	***11 (30%)***
No	***26 (70%)***
Status at the end of follow-up	
Alive	***15 (40.6%)***
Died	***8 (21.6%)***
Missed	***14 (37.8%)***
Respond to treatment	
No	***12 (32%)***
Yes	***25 (68%)***

1 There were no data about the number of the lymph node in some cases.

2 In G4, there was only one case, which was sarcomatoid RCC. It was excluded.

3 In one case, perinephric fat invasion can not be assessed, because no perinephric fat tissue was observed.

Survivin Immunostaining

Formalin-fixed, paraffin-embedded primary RCC tissue was obtained from 37 patients. Sections were cut serially at 5µm for immunohistochemical (IHC) analysis by using microtone. IHC analysis was done using the automatic system (BenchMark XT, Ventana Medical System, Inc. Tucson, Arizona, USA). This fully automated processing of code-labeled slides included baking of the slides, solvent free deparaffinization, antigen retrieval in a cell conditioning buffer CCI (Mild: 36 minutes conditioning, and standard: 60 minutes conditioning), incubation with Rabbit monoclonal anti-Survivin antibody, 2.0ml ready-to-use from Spring Bioscience (clone: SP79, Catalog No: M3794, 6920 Koll Center Parkway, CA 94566, USA), Dilution 1: 50 for 32 min, at 37ºC. Application of I-View™ DAB Detection Kit (Lot no. B05860AZ), which, includes: I-View DAB HRP, I-View DAB Inhibitor, I-View DAB Biotin, I-View DAB H2O2, and I-View DAB Copper. Counterstaining with haematoxylin II (C00758) was done for 4 minutes, and post-counterstaining with blueing reagent (B11129) was done for 4 minutes as well. After staining, the sections was dehydrated in ethanol, cleared in xylene, and covered with Mountex and cover slips.

Evaluation of Survivin staining

Survivin staining was evaluated using regular light microscope at the magnification of x40, blinded by the information on tumour grade, stage or clinical outcome. Nuclear and cytoplasmic staining was evaluated separately. Three different grading (A, B, and C) systems were applied to assess the patterns of Survivin expression in tumor cells. In system A, the staining was graded into four categories: (0) no expression (no detectable staining), (1) weak staining, (2) moderate staining, and (3) strong staining intensity. In system B, staining was graded in two categories: (1) no/weak expression and (2) moderate/strong expression.

Finally, in system C, Survivin expression was categorized simply as negative or positive. All three systems were statistically tested, and the negative/positive grading (C) seemed to provide the most meaningful correlates of Survivin with the clinically relevant data.

In calculating the staining indexes, cytoplasmic and nuclear index, the intensity of staining and the fraction of positively stained cells were taken into account using the following formula:-

$$\mathbf{I = 0 \times f0 + 1 \times f1 + 2 \times f2 + 3 \times f3}$$

Where **I** is the staining index, and f0–f3 are the fractions of the cells showing a defined level of staining intensity (from 0 to 3). Theoretically, the index could vary between 0 and 3(Buhmeida et al., 2008; Elzagheid et al., 2012).

Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics (IBM Company, NY, USA) software packages (IBM PASW Statistics for Windows, version 19). Frequency tables were analysed using the Chi-square test, with likelihood ratio (LR) or Fischer□s exact test being used to assess the significance of the correlation between the categorical variables. Odds Ratios and their 95% Confidence Intervals (95%CI) were calculated where appropriate, using the exact method. Difference in the means of continuous variables was analysed using non-parametric tests (Mann-Whitney or Kruskal-Wallis) and multiple independent samples, respectively. Analysis of variance (ANOVA) was only used deriving the mean values (and their 95%CI) of each individual stratum. Univariate survival analysis for the outcome measure (DSS, DFS) was based on Kaplan-Meier method, with log-rank (Mantel-Cox) comparison test. In all tests, the values p□0.05 were regarded statistically significant.

Chapter IV

Results

Results

Clinicopathological results

Among 37 cases of RCC in the current study, 23 (62 %) of patients were males, and 14 (38 %) were females, with mean age of 55 years at the time of diagnosis. The youngest patient was 28 years old and the eldest was 90 year old. In the present study 17 (45.9 %) of cases, their age were above 55years and 20 (54.1%) were below 55y.

Out of 37 cases in the study, 19 (51.4%) had left kidney RCC, while 18 (48.6%) were right sided RCC. Regarding tumor size we found that, 28 (75.7%) of patients having tumor size >5 cm in diameter at time of diagnosis, while other 9 (24.3%) patients having tumor size ≤5 cm in diameter. Furthermore, 30 cases (81.1%) showed no evidence of vascular invasion, while only 7 cases (18.9) showed evidence of vascular invasion under microscope. According to TNM staging system, 15(40 %) cases were diagnosed at stage I, (Fig. 4-1).

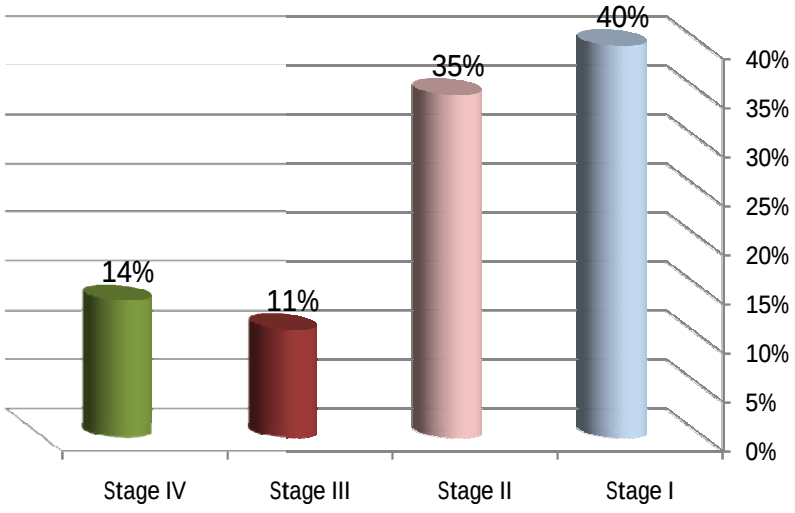


Fig. 4-1 Distribution of RCC cases according to TNM staging

Among 37 cases in the current study, 72.2% of cases were diagnosed at grade II, (Fig. 4-2).

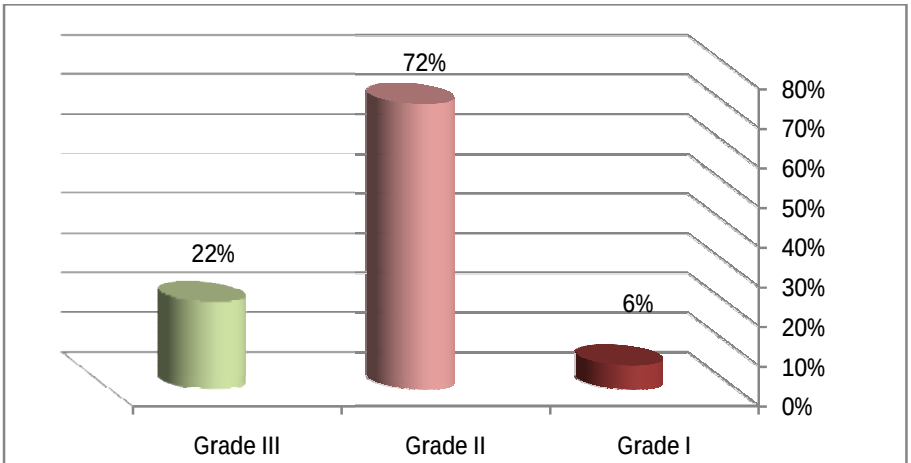


Fig.4-2 Distribution of RCC cases according to histopathological grade

Twenty two (59.5%) of all patient were free from metastasis at time of diagnosis, (Fig.4-3).

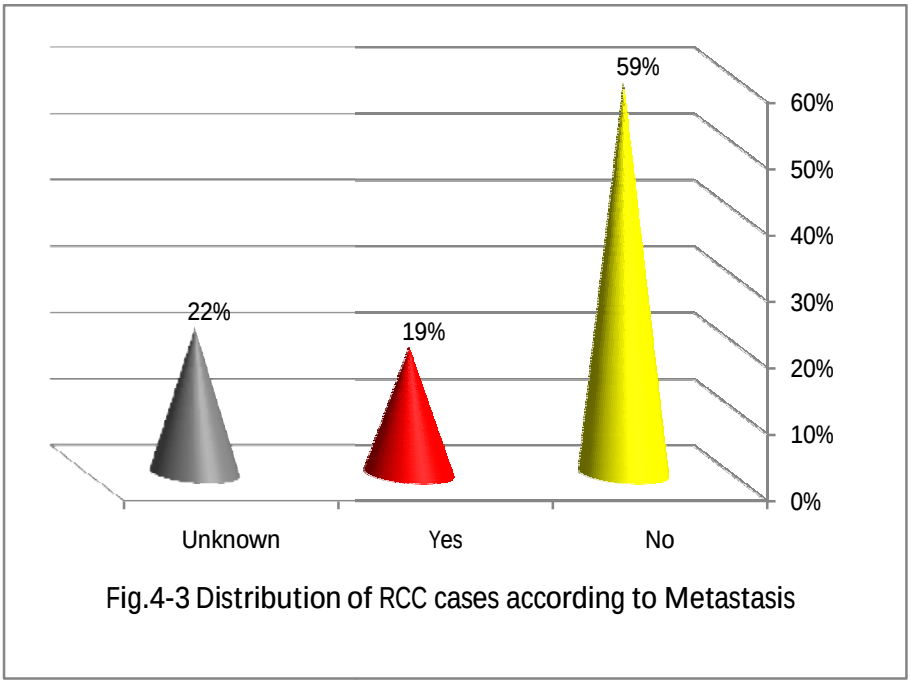


Fig.4-3 Distribution of RCC cases according to Metastasis

With patient follow up we found that, 26 (70.3%) cases there were no recurrence after radical nephrectomy, while 11 (29.7%) cases experience recurrence after radical nephrectomy.

During collection of our data, 15 (40.6 %) cases were alive, (Fig. 4-4).

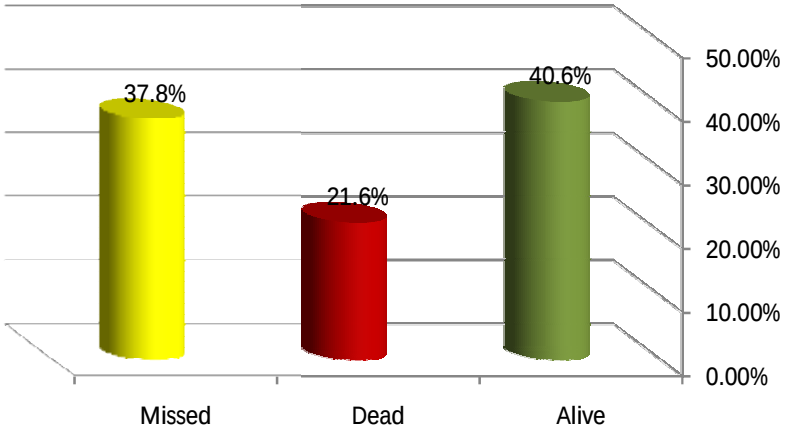


Fig.4-4 Distribution of RCC cases according to status at the end of follow up

Description of Survivin expression pattern

The intracellular localization of Survivin in tumor cells was predominantly nuclear and only weakly cytoplasmic, so the nuclear and cytoplasmic percentage of immunostaining were included in the statistical analysis. Examples of the staining patterns of Survivin are illustrated in figures 4-5, 4-6,4-7and 4-8.

Of the 37 tumors, 28 (76%) were considered positive (Fig. 4-5, 4-6, 4-7). whereas 9 (24%) were considered negative (staining intensity 0), (Fig. 4-8).

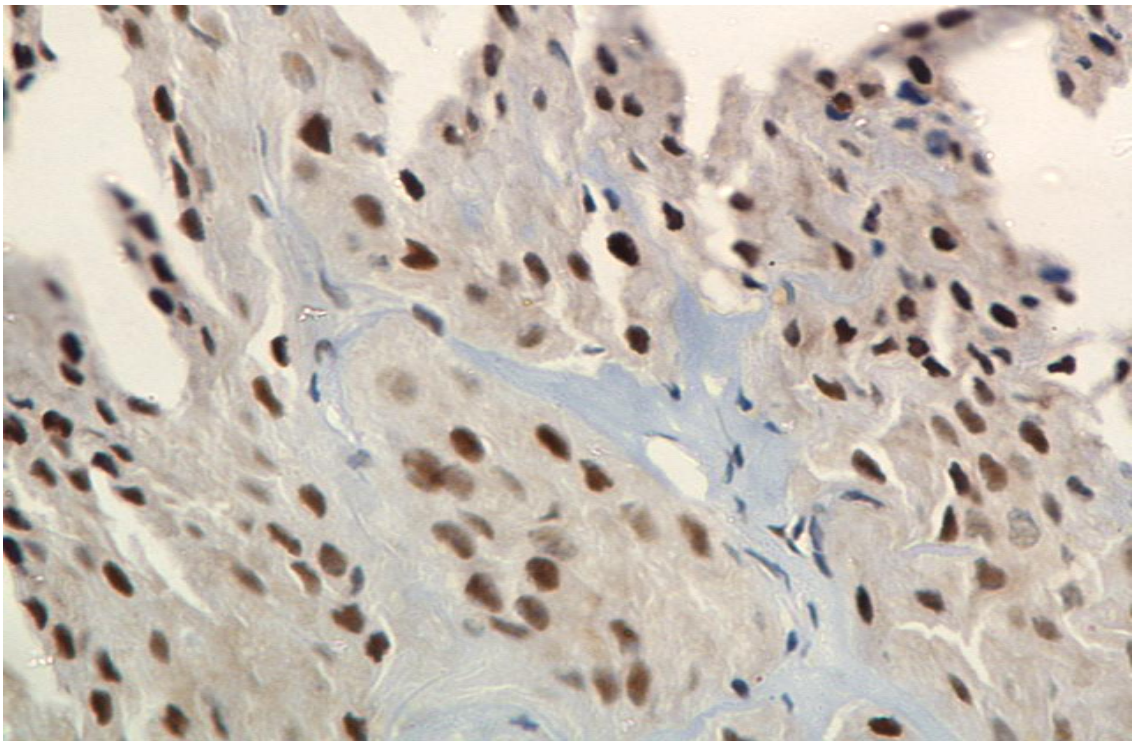


Fig. 4-5 Strong nuclear Survivin expression in RCC (X40) (Dept of Pathol. Benghazi University, Benghazi, Libya)

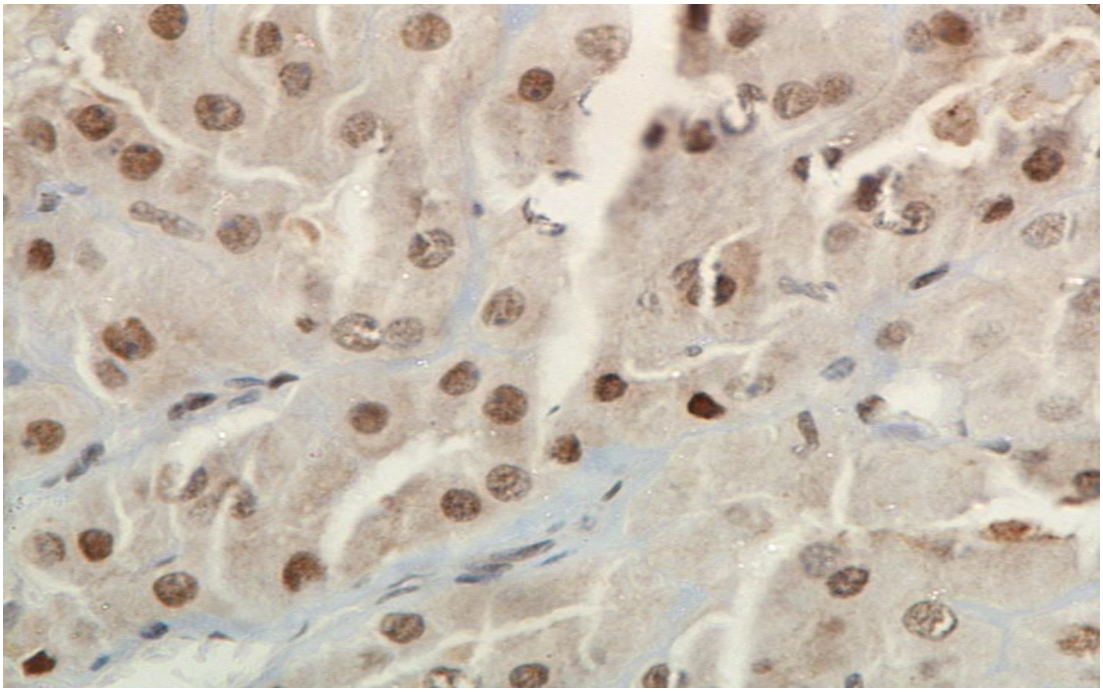


Fig. 4-6 Moderate nuclear Survivin expression in RCC (X40) (Dept of Pathol. Benghazi University, Benghazi, Libya)

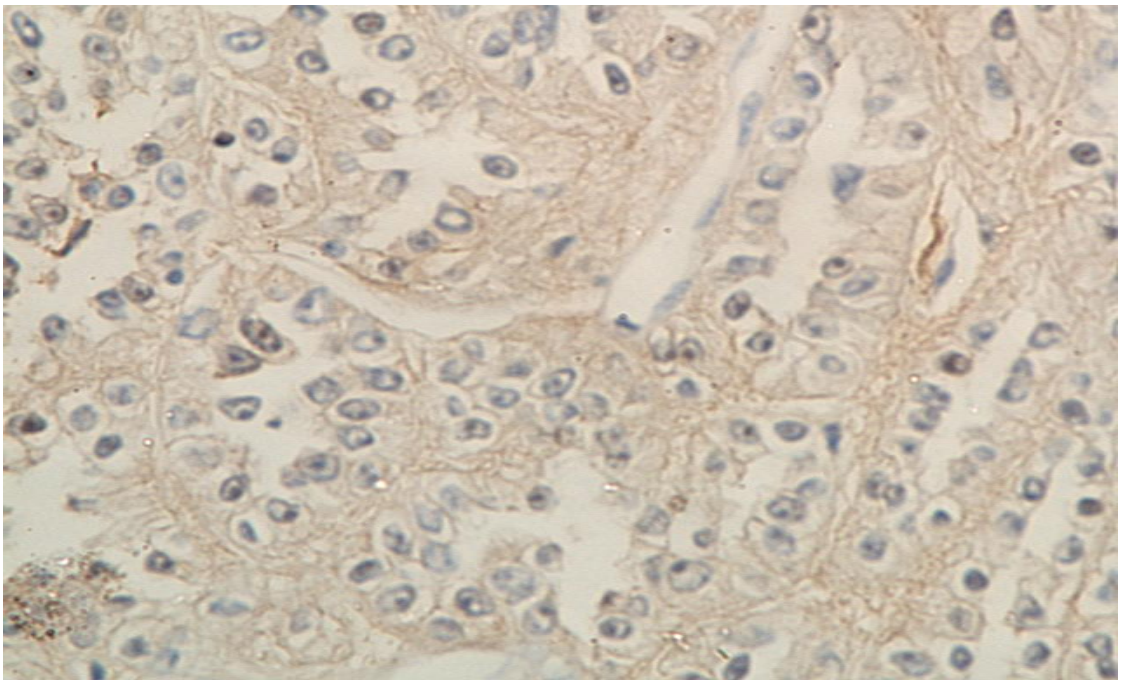


Fig. 4-7 Diffuse weak cytoplasmic Survivin expression in RCC (X40)(Dept of Pathol. Benghazi University, Benghazi, Libya)

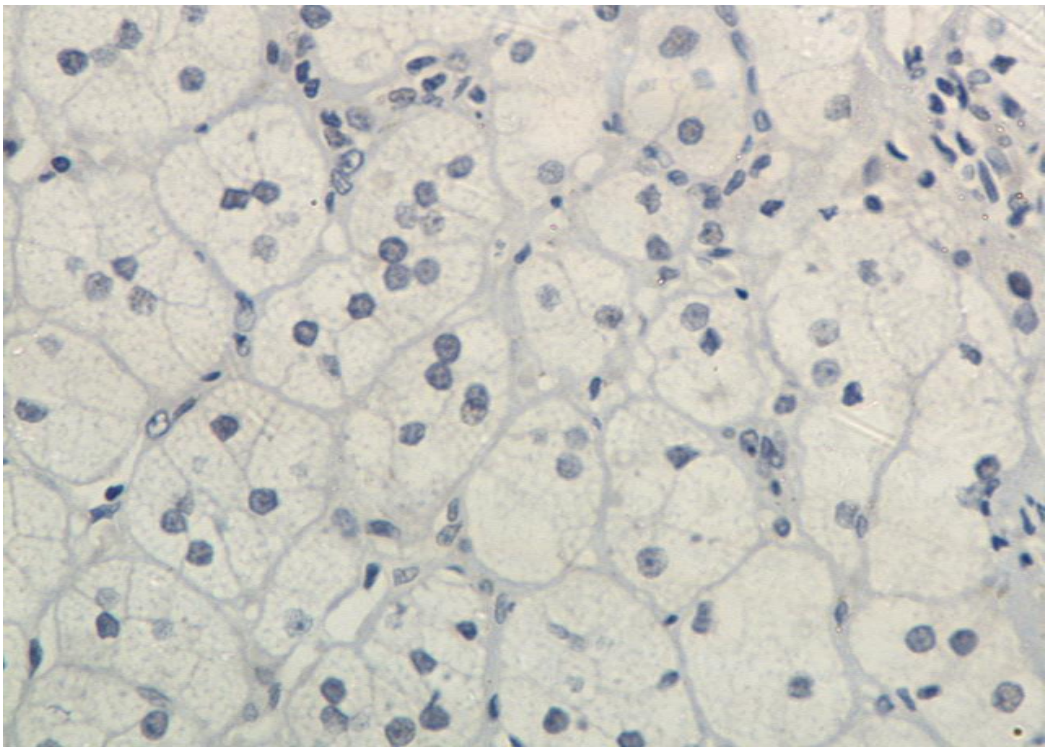


Fig. 4-8 Negative Survivin expression in RCC (X40) (Dept of Pathol. Benghazi University, Benghazi, Libya)

Correlation of Survivin expression with the clinicopathlogical features

The distribution of Survivin expression in tumor samples in relation to clinicopathological characteristics is presented in table. 4-1and 4-2.

Using different cut- off points (mean, median, and 2- tier score (0 vs 1,2,3 and 0,1 vs 2,3) and 4-tier score (0,1,2, and 3). The present study revealed that a significant correlation between Survivin expression and venous invasion (tumor thrombus). Patients who had tumors with high Survivin expression were more likely to have tumor thrombus ($p<0.042$), larger tumor size (>5cm) ($P<0.051$), advanced tumor stage ($P<0.033$). Survivin expression associated significantly with primary tumor classification (pT1, pT1 vs pT2, $p<0.013$).

Survivin expression showed a borderline association ($p<0.068$) with tumor location in that tumor arising in right kidney expresses survivin more than tumors arising in left kidney.

On the other hand, early tumor recurrence (relapse), gender, age, distant metastasis, lymph node involvement, perinephric and capsular invasion as well as tumor grade and status at end point, response to treatment had no significant relationship with the expression of Survivin.

Table. 4-1 Correlation between Survivin expression and clinico-pathological features of RCC at cut-off point (0 vs 1,2,3).

Features	*Number of cases (%)*	*Survivin expression*		*p-value*
		Negative(O)	*Positive(1,2,3)*	
Gender				***0.267***
Male	***23(62%)***	***7(30%)***	***16(70%)***	
Female	***14 (38%)***	***2(14%)***	***12(86%)***	
Age group (years)				***0.917***
≤ 55	***20(54%)***	***5(25%)***	***15(75%)***	
> 55	***17(46%)***	***4(24%)***	***13(76%)***	
Lymph node involvement[1]				***1.000***
Yes	***3(17%)***	***1(33%)***	***2(67%)***	
No	***15(83%)***	***5(33%)***	***10(67%)***	
Distant metastasis				***0.484***
Yes	***7(24%)***	***1(14%)***	***6(86%)***	
No	***22(76%)***	***6(27%)***	***16(73%)***	
Tumor Stage				***0.833***
I	***15(43%)***	***5(33%)***	***10(67%)***	
II	***11(31%)***	***2(18%)***	***9(82%)***	
III	***4(12%)***	***1(25%)***	***3(75%)***	
IV	***5(14%)***	***1(20%)***	***4(80%)***	
Tumor grade[2]				***0.734***
G1	***2(6%)***	***0(0%)***	***2(100%)***	
G2	***26(72%)***	***6(23%)***	***20(77%)***	
G3	***8(22%)***	***2 (25%)***	***6(75%)***	
Tumor location				***0.068***
RT. Kidney	***18(49%)***	***2(11%)***	***16(89%)***	
LT. Kidney	***19(51%)***	***7(37%)***	***12(63%)***	
Primary tumor size				***0.051***
≤5cm	***9(24%)***	***4 (44%)***	***5(56%)***	
>5cm	***28 (76%)***	***9(32%)***	***19(68%)***	
Tumor Thrombus				***0.771***
Yes	***7(19%)***	***2(29%)***	***5(71%)***	
NO	***30 (81%)***	***7(23%)***	***23(77%)***	
Primary tumorclassification				***0.864***
pT1	***14(38%)***	***4(29%)***	***10(71%)***	
pT2	***15(40%)***	***3(20%)***	***12(80%)***	
pT3	***8(22%)***	***2(25%)***	***6(75%)***	
Recurrence				***0.832***
No	***26(70%)***	***18(69%)***	***8(31%)***	
Yes	***11(30%)***	***8(73%)***	***3(27%)***	

1 There were no data about the number of the lymph node in some cases.

2 In G4,there was only one case, which was sarcomatoid RCC. It was excluded.

Table. 4-2 Correlation between Survivin expression and clinico-pathological features of RCC at median as cut-off point.

Features	*Number of cases (%)*	*Survivin Expression*		*p-value*
		<Median(0.20)	*>Median(0.20)*	
Gender				***0.623***
Male	***23(62%)***	***15(65%)***	***8(35%)***	
Female	***14(38%)***	***8(57%)***	***6(43%)***	
Age group (years)				***0.699***
≤55	***20(54%)***	***13(65%)***	***7(35%)***	
> 55	***17(46%)***	***10(59%)***	***7(41%)***	
LN involvement[1]				***0.18***
Yes	***3(17%)***	***1(33%)***	***2(67%)***	
No	***15(83%)***	***11(73%)***	***4(27%)***	
Distant metastasis				***0.452***
Yes	***7(24%)***	***3(43%)***	***4(57%)***	
No	***22(76%)***	***13(59%)***	***9(41%)***	
Tumcor Stage				***0.033***
I	***15(43%)***	***8(53%)***	***7(47%)***	
II	***11(31%)***	***11(100%)***	***0(0%)***	
III	***4(12%)***	***1(25%)***	***3(75%)***	
IV	***5(14%)***	***1(20%)***	***4(80%)***	
Tumor grade[2]				***0.429***
G1	***2(6%)***	***2(100%)***	***0 (0%)***	
G2	***26(72%)***	***16(62%)***	***10(38%)***	
G3	***8(22%)***	***4(50%)***	***4(50%)***	
Tumor location				***0.898***
RT. Kidney	***18(49%)***	***11(61%)***	***7(39%)***	
LT. Kidney	***19(51%)***	***12(63%)***	***7(37%)***	
Primary tumor size				***0.079***
≤5cm	***9(24%)***	***2(22%)***	***7(78%)***	
>5cm	***28(76%)***	***18(64%)***	***10(36%)***	
Tumor thrombus				***0.042***
Yes	***7(19%)***	***2(29%)***	***5(71%)***	
No	***30(81%)***	***21(70%)***	***9 (30%)***	
Primary tumor classification				***0.013***
pT1	***14(38%)***	***8(57%)***	***6(43%)***	
pT2	***15(40%)***	***13(87%)***	***2(13%)***	
pT3	***8(22%)***	***2(25%)***	***6(75%)***	
Recurrence				***0.534***
No	***26(70%)***	***17(65%)***	***9(35%)***	
Yes	***11(30%)***	***6(55%)***	***5(45%)***	

1 There were no data about the number of the lymph node in some cases.

2 In G4, there was only one case, which was sarcomatoid RCC. It was excluded.

Survival analysis

In the Kaplan–Meier survival analysis (At median as cut-off point) there was a borderline (P=0.08, log rank) difference in disease-free survival between patients who have Survivin expression above median and those with Survivin expression below median (Fig. 4-15). Interestingly, at 2-years follow- up, 85 % of the patients with tumors expressing Survivin below median showed longer disease free survival in comparison with only 57% of patients with tumors expressing Survivin above median.

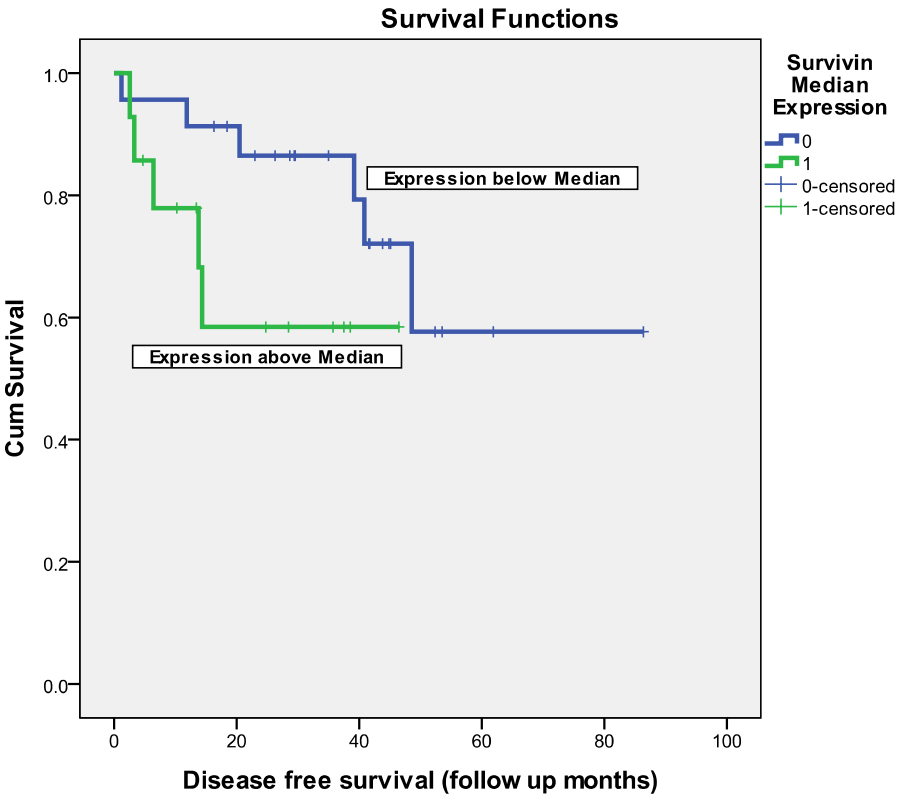


Fig. 4-9 Survivin expression (< median & > median) as determinant of disease-free survival in univariate (Kaplan–Meier) analysis.

In current study the survival analysis (at 0,1 vs. 2,3 as cut-off point), there was a difference (p=0.167, log rank) in betw disease-free survival een patient with survivin positive tumor and those with negative expression. At 5-years follow up 89% of patients with no survivin showed longer compa disease-free survival red with 38% of patient with survivin expression.

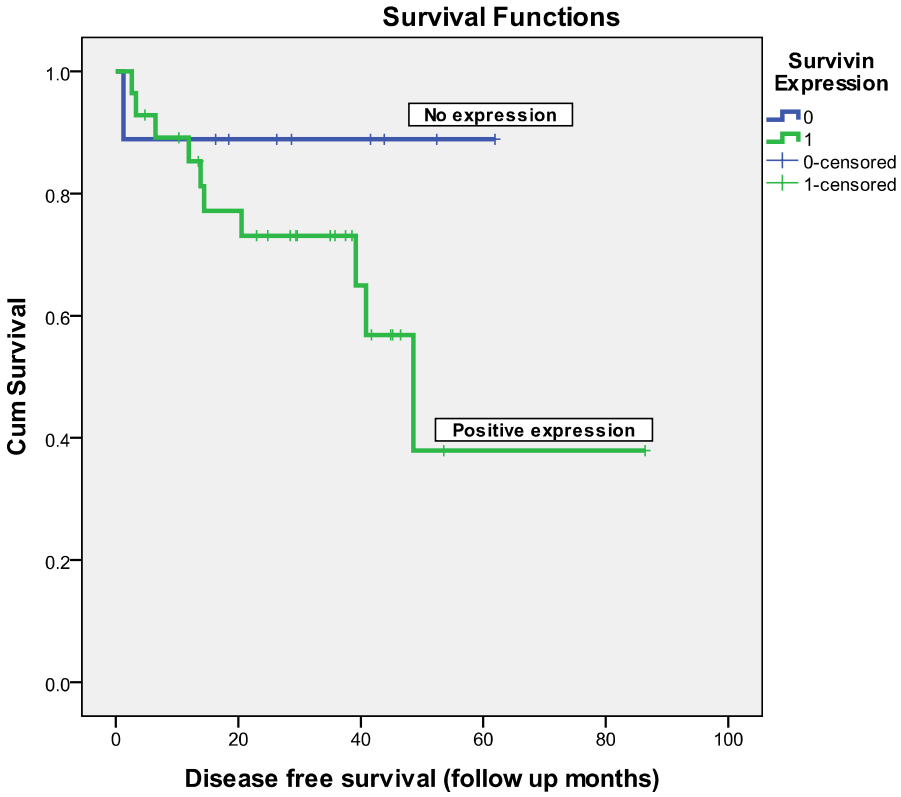


Fig. 4-10 Survivin expression (negative\positive) as determinant of disease-free survival (DFS) in Kaplan-Meier analysis of RCC patient.

Kaplan-Meier disease free survival and other clinicopathological data

We also performed these survival analyses in relation to clinicopathological data. Kaplan-Meier survival analysis showed difference (p=0.0001, log rank) in disease-free survival between patients without venous invasion compared with those with venous invasion. At 3-year follow up 90% of patients without venous invasion showed longer disease-free survival compared with 25% patients with venous invasion.

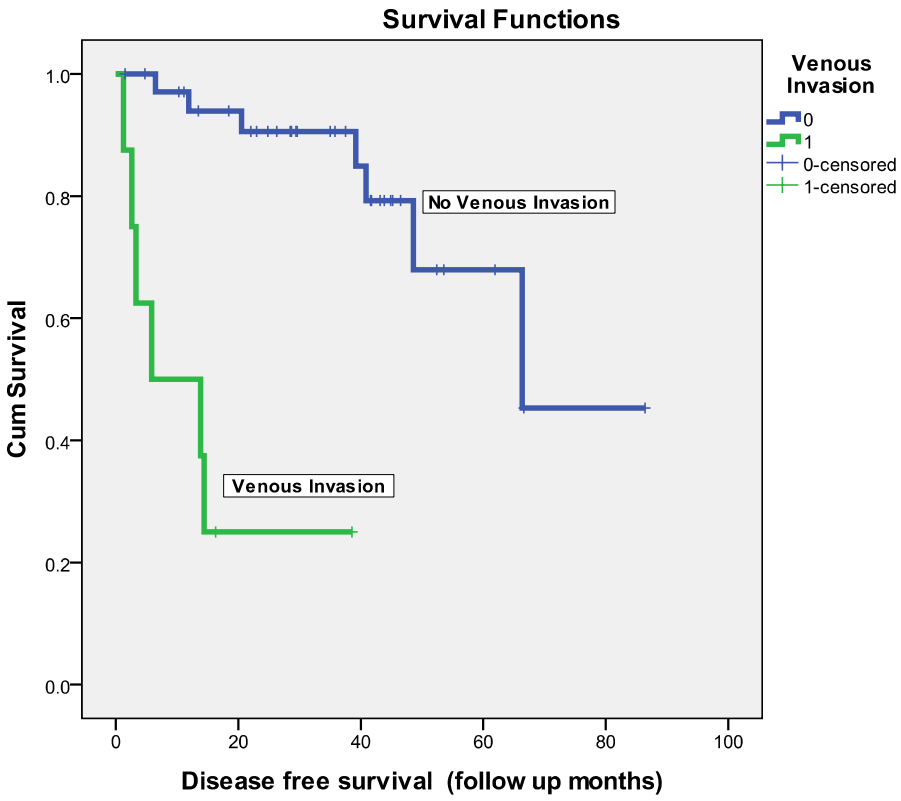


Fig. 4-11 Disease-free survival predicted by tumor venous invasion

Asimilar trend was noticed for garde (p=.038, log rank), G1 tumors showed longer as disease-free survival compared with G2 and G3. At 3-year follow up 100% patients with G1, showed longer disease-free survival comparaed with 82% for G2, and 42% for G3.

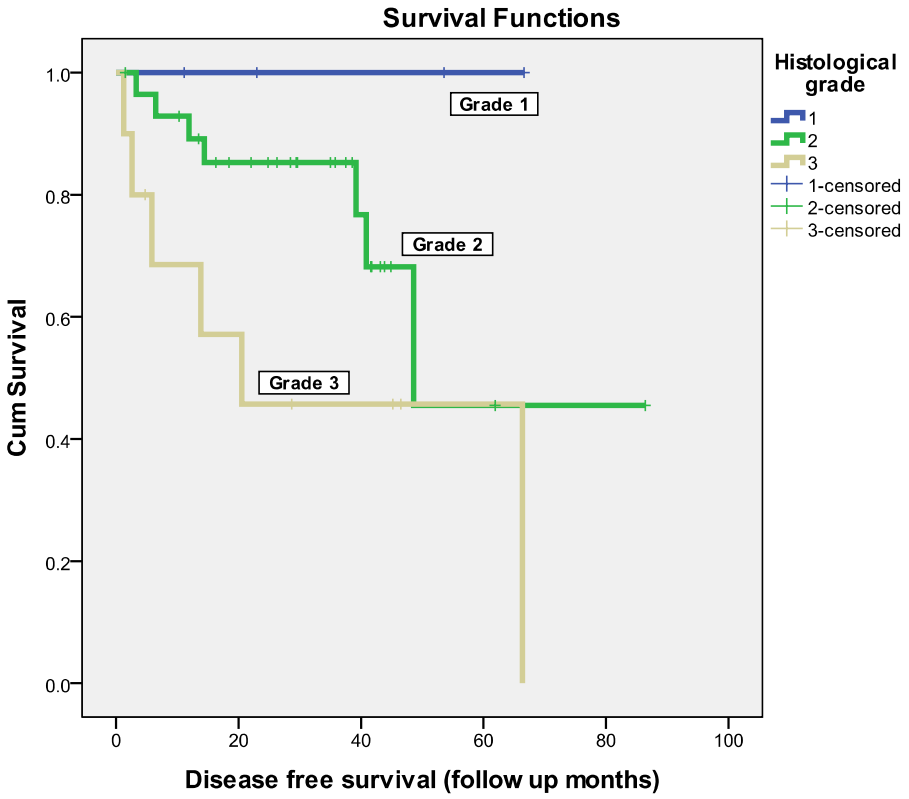


Fig. 4-12 Disease-free survival predicted by histological grades

Similarly, a siginificant (p=0.0001, log rank) difference in disease-free survival and primary tumor status, patients with T1 have longer disease-free survival compared with T2, T3. At 3-years follow up 100% patients with T1 showed longer disease-free survival compared with 80% for T2, and 38% for T3.

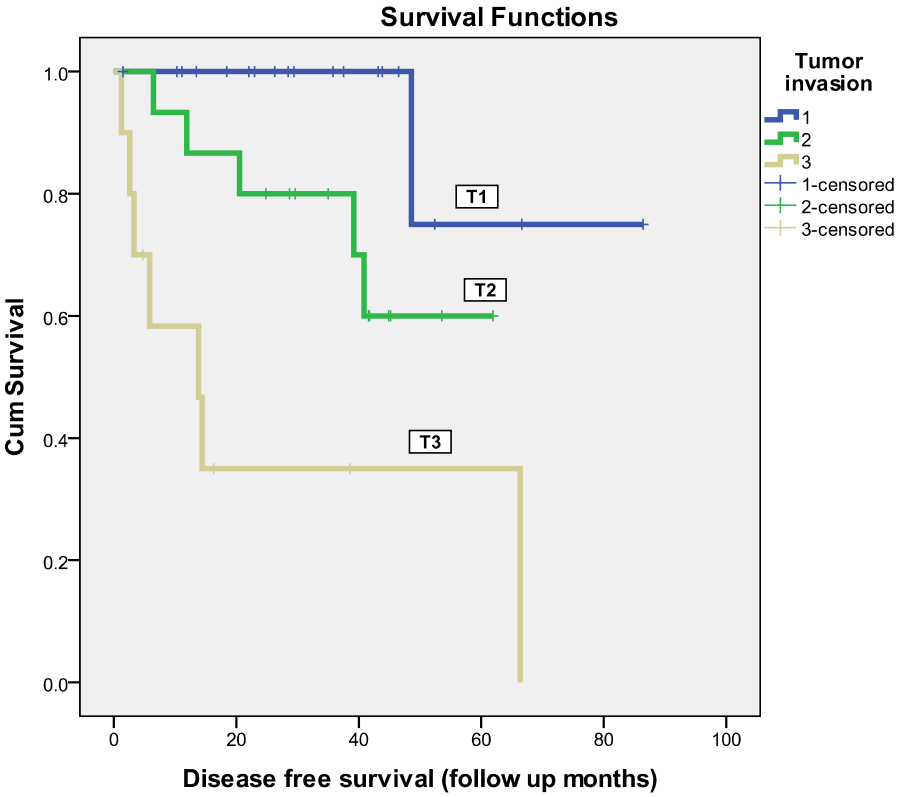


Fig. 4-13 Disease-free survival predicted by primary tumor status

There was a siginificant (p=0.0001, log rank) difference in disease-free survival and lymph node involvement, patients without lymph node involvement have longer disease-free survival compared with those with lymph node involvement.

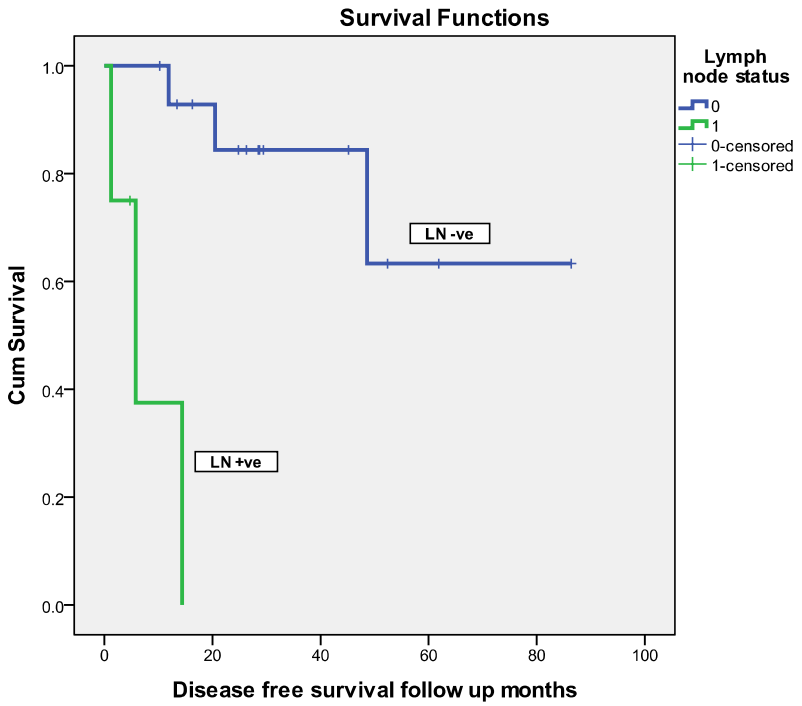


Fig. 4-14 Disease-free survival predicted by lymph node involvement

Moreover, there was significant difference (p=0.0001, log rank) in disease-free survival and metastasis, patients without metastasis have longer disease-free survival as compared with those with metastasis.

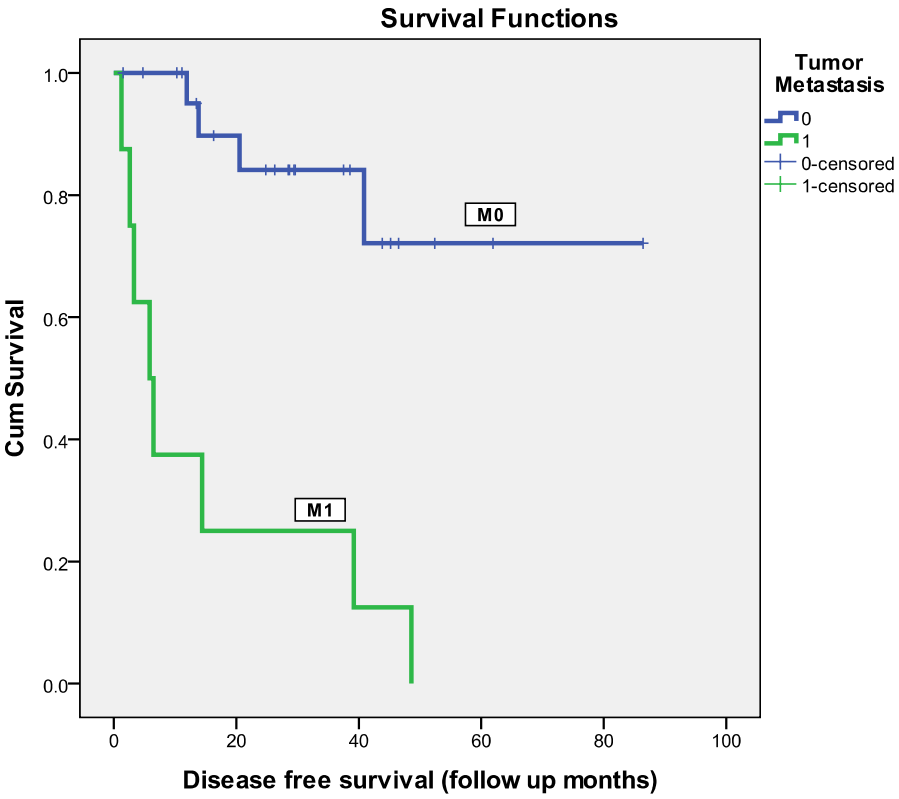


Fig. 4-15 Disease-free survival predicted by metastasis

There was significant difference (p=0.0001, log rank) in disease-free survival and stage, when we divided our cases into low stage and high stage. Patients with stage I and stage II have longer disease-free survival as compared with those with stage III and IV. At 5-years follow up 80% patients with low stage showed longer disease-free survival compared 20% for high stage.

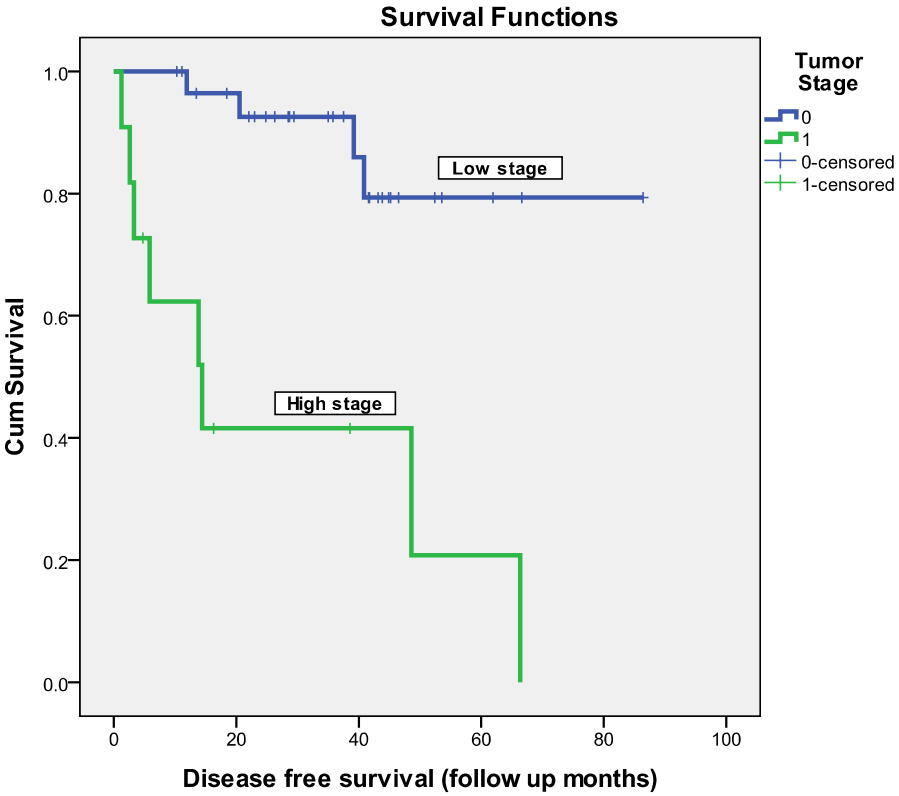


Fig. 4-16 Clinical stage as determinant of Disease-free survival in Kaplan-Meier analysis of RCC patients.

Kaplan-Meier disease specific survival and other clinicopathological data

In the current study, there was significant difference (p= 0.027, log rank) in disease specific survival and tumor size, patients with tumor size (≤5cm) have longer disease specific survival compared with those with tumor size (>5cm). At 5-years follow up 100% patients with tumor size (≤5cm) showed longer disease specific survival compared 45% for patients with tumor size (>5cm).

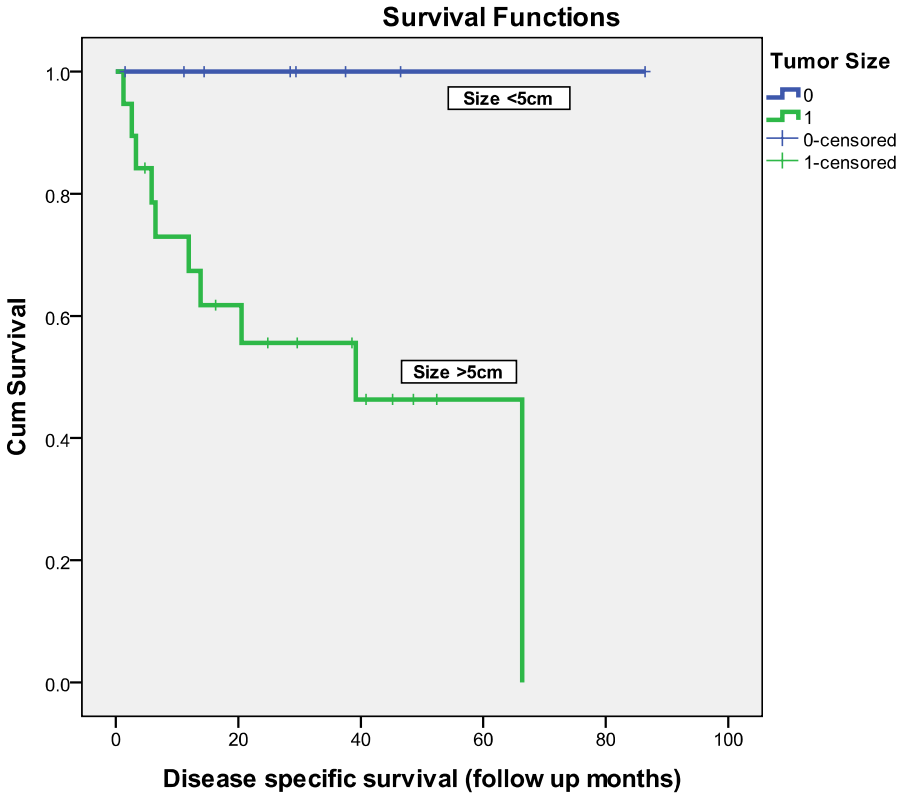


Fig. 4-17 Disease specific survival predicted by tumor size

There was significant difference (p= 0.021, log rank) in disease specific survival and Primary tumor status, patients with T1 have longer disease specific survival compared with T2, T3, at 3-years follow up 100% patients with T1 showed longer disease specific survival compared 62% for patients with T2, and 43% for patients with T3.

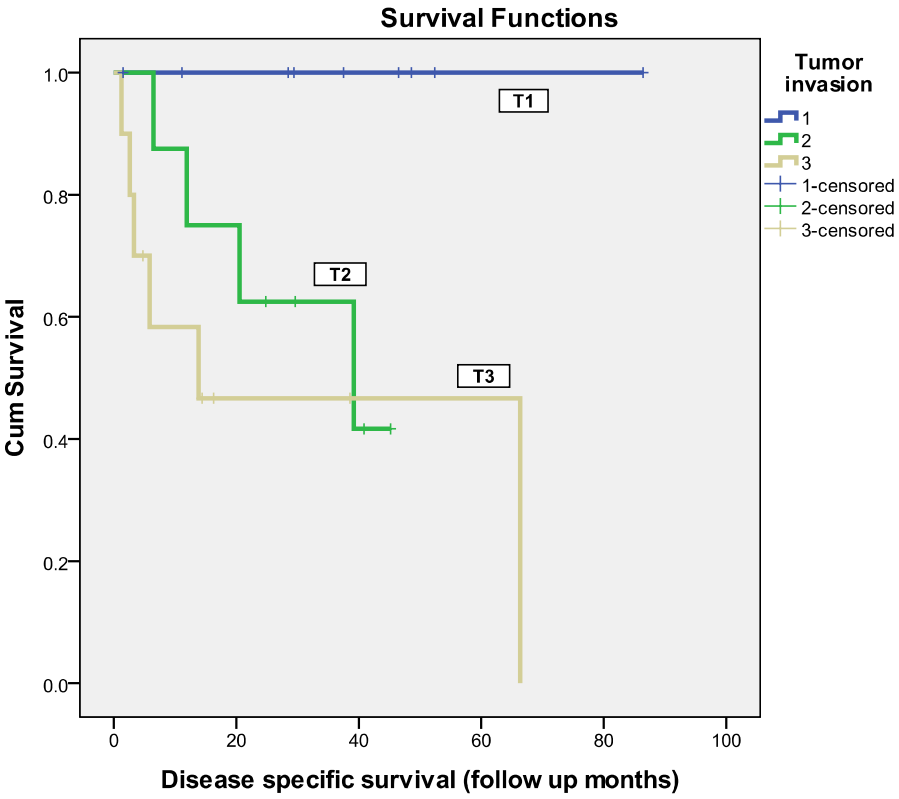


Fig. 4-18 Disease specific survival predicted by Primary tumor status

A similar trend was noticed for metastasis (p=0.002, log rank). Patients without metastasis have longer disease specific survival compared with those with metastasis.

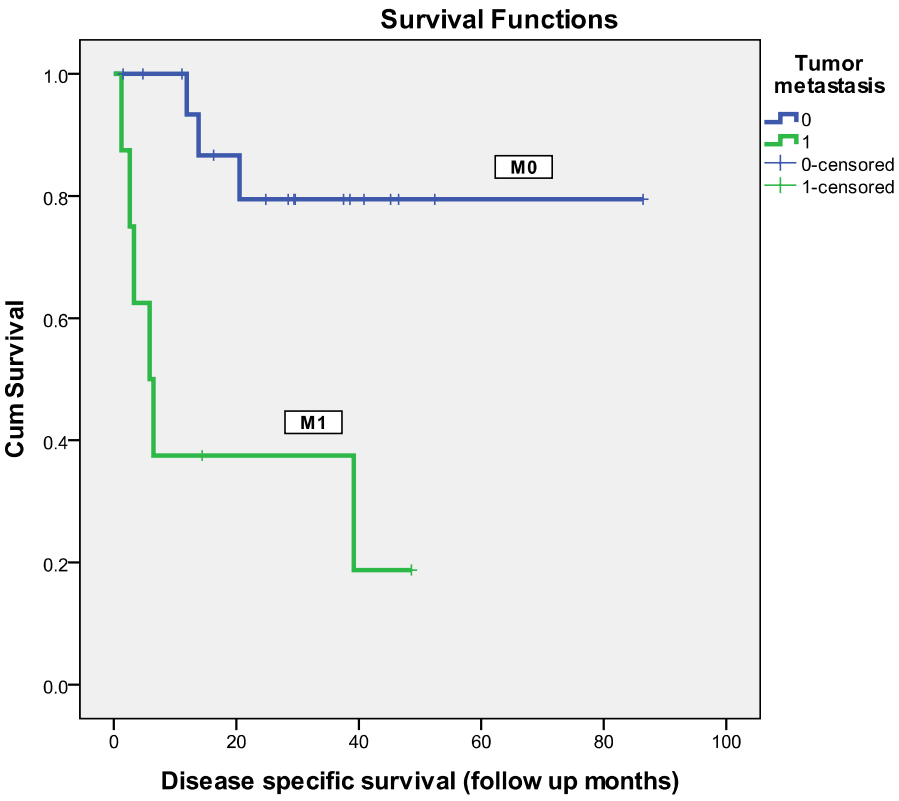


Fig.4-19 Disease specific survival predicted by metastasis

There was significant difference (p= 0.058, log rank) in disease specific survival and stage, patients with low stage have longer disease specific survival as compared with those with high stage. At 5-years follow up 70% patients with low stage showed longer disease specific survival compared with 50% for high stage.

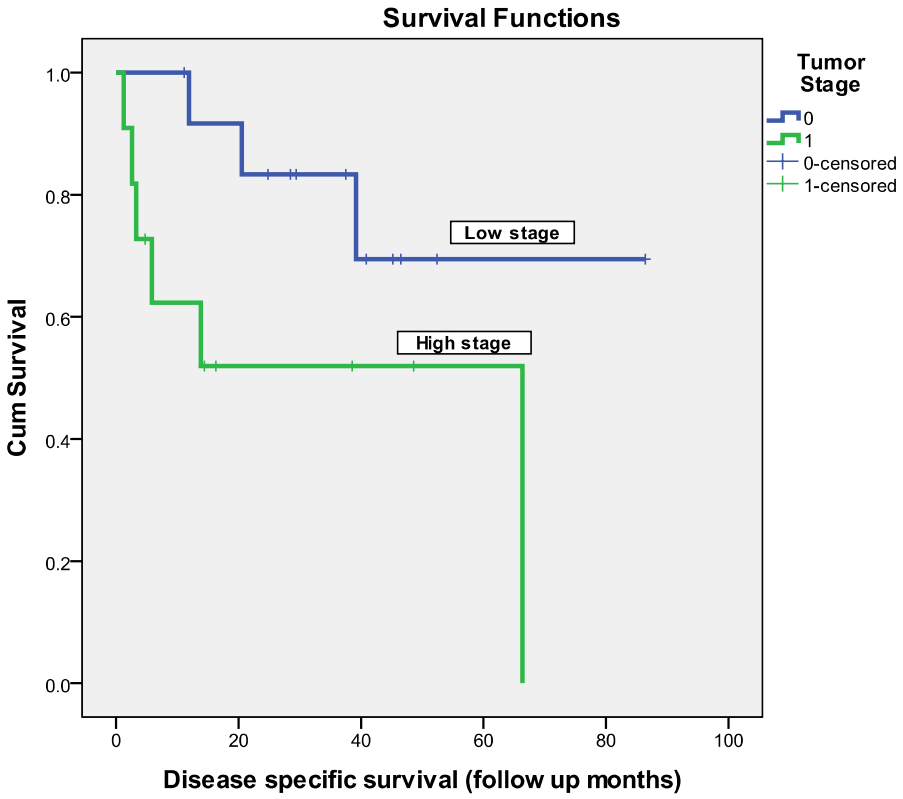


Fig. 4-20 Disease specific survival predicted tumor stage

Moreover, there was statistically significant (p=0.0001, log rank) in disease specific survival for recurrence. Patients without recurrence showed longer disease specific survival compared with patients with recurrence.

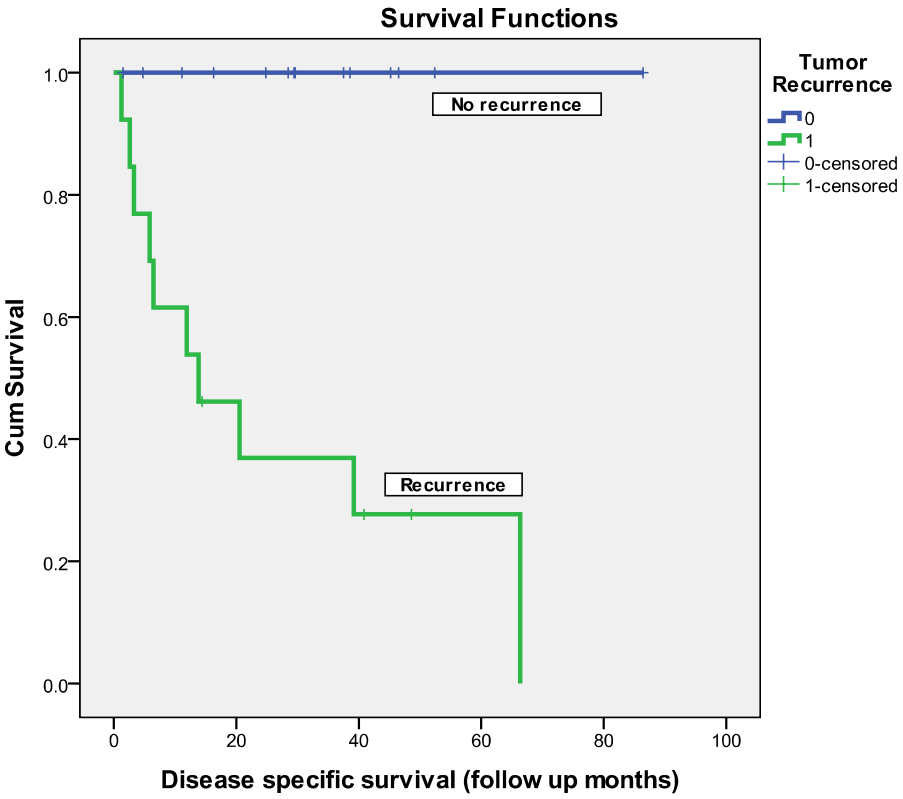


Fig. 4-21 Disease specific survival predicted recurrence

Finally there was statistically significant (p=0.0001, log rank) in disease specific survival for response to treatment, patients with objective response to treatment showed longer disease specific survival compared with patients no response to treatment. At 5-years follow up 100% patients with objective response to treatment showed longer disease specific survival compared with 25% for those with no response to treatment.

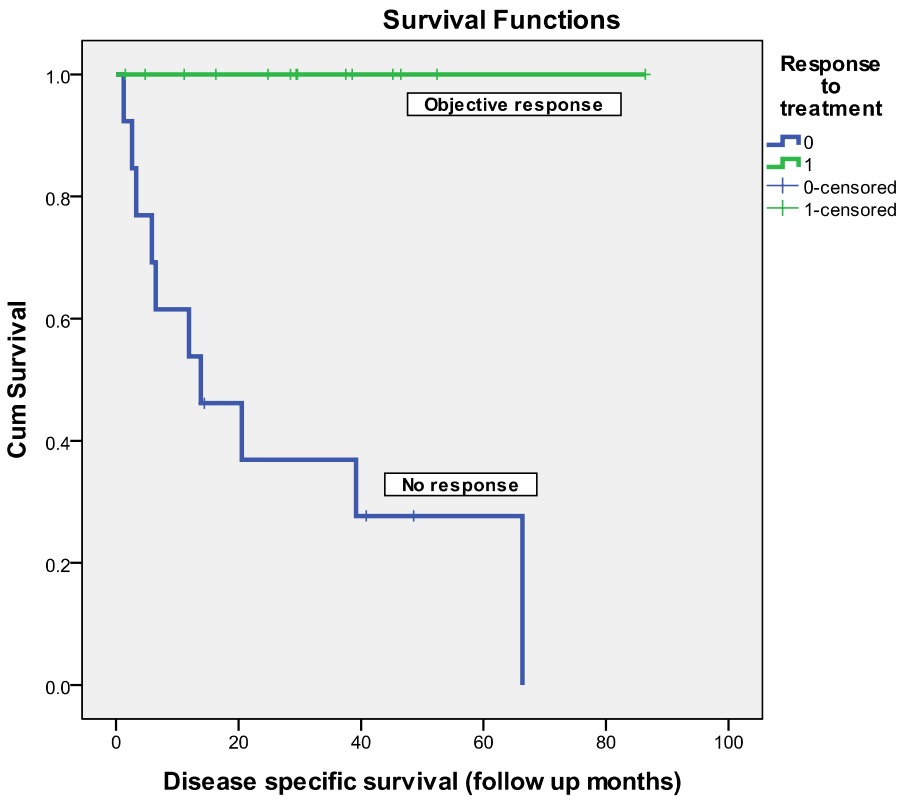


Fig. 4-22 Disease specific survival predicted treatment

Chapter V

Discussion

Discussion

Cancer of kidney in Libya, (including cancers of renal pelvis) comprises 2% of all cancer patients. The incidence is remarkably less as compared to US and European rates (Abuageila et al., 2006). In the present we found that the mean age of occurrence is 55.6 years which is a decade earlier than other study conducted by Siemer et al., 2006 and Thompson et al., (2008), which showed that mean age of occurrence is 64 year, more over, cancer research UK (2009) showed that 74% of cases above 60 years.

In the present study, incidence of RCC was more common in males (62%) than females which constituted 38% of all cases collected from 2003-2012 in Eastern Libya and this is comparable with other studies. Cancer research UK during 2009, there were 61% in men and 39% in women, giving a male: female ratio of 16:10.

The ccRCC is the most common histological subtype which constitutes 76% of all cases, while pRCC 19%, and chRCC is 2% and this finding is comparable with other study by Sean (2012), which also showed that the ccRCC is the commonest followed by Prcc then chRCC respectively.

In the present study, tumor size at the time of diagnosis was > 5 cm in diameter in 76% of cases, and 24 % were ≤ 5 cm in diameter. That means small tumor size does not rule out malignant tumor and this fact is matching other previous studies by Bosniak et al., (1995) and Schlomer et al, (2006). Most of patients in the present study were free from metastasis at the time of diagnosis (59.5%), while only 7 (18.9%) have evidence of metastasis. This reflects the improvement in investigation facility and ability of USS and CT scan for diagnosis of renal mass even if the patient is investigated for other reason, and this fact is matching previous study (Nguyen et al., 2006).

In the current study K-M survival analysis of clinico-pathological data, there was significant correlation between outcome of patients with RCC and venous invasion, histological grades, T classification, lymph node involvement, metastasis, tumor stage (p=0.0001, p=0.038, p=0.0001, p=0.0001, p=0.0001, p=0.0001, respectively). Patients without venous invasion, low histological grade, low T classification, without lymph

node involvement, without metastasis, and those low tumor stage showed longer disease-free survival. There was also significant correlation between disease specific survival and tumor size, T classification, metastasis, tumor stage, recurrence, and response to treatment, ($p=0.027$, $p=0.021$, $p=0.002$, $p=0.058$, $p=0.0001$, $p=0.0001$, respectively). Patients with small tumor size, low T classification, without metastasis, low tumor stage, without recurrence, and those with objective response to treatment showed longer disease specific survival. And this fact is matching previous study conducted by Siddiqui et al., (2007), which found that among patients with T3a disease, the size of the primary tumor remains a prognostic factor (ten-year survival rates of 77, 54, and 46 % for tumors <4, 4 to 7, and >7 cm, respectively. Other similar study conducted by Tsui et al., (2000), the five-year survival rate based upon tumor grade was 89, 65, and 46 % for tumors of histologic grade 1, 2, and 3 to 4, respectively. Also tumor stage, according to TNM staging, was found significantly correlated with patient outcome (Verhoest et al., 2009; Atkins, 2011) states that patients with stage I (T1N0) RCC have a five-year survival rate over 90 % in most contemporary series and this fact is also agreed with our study.

Renal tumorigenesis is a complex and a multistep process determined by environmental and genetic factors. Thus, it's essential to identify novel molecular markers underlying the development of RCC and predicting its prognosis, which will help us to explore additional prognostic factors to identify RCC patients at high risk of tumor progression and develop more effective therapeutic strategies (Altieri et al., 2003). Apoptotic cell death is a physiologic mechanism of cellular death that occurs during metamorphosis, embryogenesis, hormone-induced organ involution, and neoplasia (Tatabe et al., 1996). Inhibition of apoptosis has been implicated in carcinogenesis, tumor progression, and resistance of tumor cells to chemotherapy (Rudin et al., 1997).

Survivin, a member of the family of inhibitors of apoptosis proteins (IAPs), is a bifunctional protein that suppresses apoptosis and regulates cell division (Altieri et al., 2003). Survivin seems to aid tumor progression and survival by a different mechanism i.e., inhibition of apoptosis (Verhagen et al., 2001). Survivin is expressed during foetal development but not within adult tissues *(*Adida et al., 1998*).* Aberrant expression of this protein, however, has been reported in most adult human

malignancies (Ambrosini et al., 1997). Survivin is thought to promote tumor progression by rendering cells refractory to apoptosis (Okada et al., 2004). Recently, it has been reported that Survivin is linked with poor cancer-specific survival when expressed at high levels in ccRCC (Parker et al., 2006).

Survivin also promotes and stabilizes mitotic microtubules necessary for cell development and proliferation (Uren et al., 2000; Li et al., 2005). Survivin is a structurally unique member of the inhibitor of apoptosis protein family that suppresses apoptosis and regulates cell division (Reed 2001; Altieri 2008). However, over-expression of Survivin was frequently observed in different types of cancer, including RCC. Lei et al., (2010), have demonstrated that the expression of Survivin was elevated both in RCC cell lines and in tumor tissues. Over-expression of Survivin was frequently observed in a variety of human malignancies, such as colorectal cancer (Chen et al., 2004), lung cancer (Falleni et al., 2003), hepatocelluar carcinoma (Fields et al., 2004), pancreatic cancer (Liu et al., 2011), and osteosarcoma (Wang et al., 2006).

Over-expression of Survivin is correlated with poor prognosis of these cancers. It has been demonstrated that sufficient expression of Survivin messenger RNA and protein were detected in RCC cell lines but not in normal human kidney epithelial cell line (Lei et al, 2010). Elevated expression of Survivin was also observed in RCC tissues compared with adjacent normal tissues (Baytekin et al., 2009; Lei et al., 2010).

Survivin and with its distribution among multiple sub-cellular pools that are independently regulated, differentially modified at a post-translational level, and with unique immuno-reaction properties (Fortugno et al., 2002). Survivin absence in mithocondrial fractions of normal tissue, suggests that its localization to mithocondria may be preferentially, or exclusively, associated with oncogenic transformation**.** (Dohi et al., 2004). On the other hand, cytoplasmic pool is representative of its active anti-apoptotic function and is associated with parameters of poor prognosis in most human cancers, including carcinomas of the oral cavity, lung, breast, colon, stomach, oesophagus and pancreas (Lo Muzio et al., 2003; Sohn et al., 2006). The current study shows that the intracellular localization of Survivin in tumor cells was predominantly

nuclear and only weakly cytoplasmic. In a previous study conducted by Zamparese et al., (2008), intracellular localization of Survivin in tumor cells was found to be predominantly and diffusely cytoplasmic and only weakly and focally nuclear.

The present study revealed that a significant correlation between Survivin expression and venous invasion (tumor thrombus), patients who had tumors with high Survivin expression were more likely to have tumor thrombus ($p<0.042$), larger tumor size (>5cm) ($P<0.051$), advanced tumor stage ($P<0.033$). Survivin Expression associated significantly with primary tumor classification (pT1, pT2 VS pT3; $P< 0.013$). Survivin expression showed a borderline association ($p< 0.068$) with tumor location in that tumor arising in right kidney expresses Survivin more than tumors arising in left kidney, shorter disease-free survival of RCC patients.

The same observations were demonstrated by Parker et al., (2006), who reported that high Survivin expression in RCC was associated significantly with tumor thrombus, larger tumor size, advanced tumor stage, higher grade, tumor necrosis, lymph node involvement and distance metastases compared with patients who had tumors with low Survivin expression. Fields et al., (2004), also showed that Survivin expression correlates with poor prognostic parameters (high nuclear and histologic grade, microvascular invasion), increased proliferation (mitotic count, MIB-1), local recurrence, and shorter disease-free survival of hepatocelluar carcinoma patients. Survivin expression was significantly associated with poorly differentiated, advanced stages and more aggressive ccRCCs ($p<0.05$). Patients with low Survivin expression had statistically significant better survival rates (Zamparese et al., 2008). The present study revealed that a significant correlation between Survivin expression with advanced tumor stage, and this is mentioned in previous studies which states that, for the outcome of RCC patients, over-expression of Survivin was significantly associated with advanced tumor stage, tumor grade and lymph node metastasis (Baytekin et al., 2009; Okamura et al., 2009).

In the current study high level of Survivin expression did not correlate with clinicopathological factors including age, sex, and distant metastasis of RCC patients. Lei et al., (2010), reported that high level of Survivin expression was significantly correlated with tumor pathological stage, grade, and lymph node metastasis, but not

with other clinicopathological factors including age, sex, tumor size, histology, and distant metastasis of RCC patients.

In current study Survivin expression did not correlate with the emergence of early recurrence (relapse), although its expression seems to be associated with the stage of tumors. Seok-Soo Byun et al., (2007), reported that the results of previous study suggest that Survivin-mediated inhibition of apoptosis is associated with progression and recurrence of RCC. Thus, Survivin is a useful independent prognostic marker for this condition.

The present study, showed that there was difference in disease-free survival between patients who have positive Survivin expression and those with negative Survivin expression. At 5-years follow up 89% of patients with no Survivin showed longer DFS compared with 38% of patient with Survivin expression. Also in present study at 2- years follow- up 85 % of the patients with Survivin expression below median showed longer disease free survival in comparison with only 57% of those survivin above median, Zamparese et al., (2008); Kosari et al., (2005), reported RCC patients with high survivin levels had a significantly shorter overall survival time than those with low levels. The 5-year cancer-specific survival rate was 43.0% for patients with high Survivin expression and 87.2% for patients with low Survivin expression. Survivin expression is an independent predictor of ccRCC progression and death from RCC. Thus, survivin has the potential to offer additional prognostic information and to provide a novel target for the development of new adjuvant therapies (Parker et al., 2006). Increased Survivin expression is an unfavorable prognostic marker associated with decreased overall survival in many malignancies, including renal, colon, and breast cancer (Altieri 2003; Krambeck et al., 2007; Zamparese et al., 2008).

Our data obtained from a 37 series of RCC of Libyan patients, suggest that Survivin expression in RCC may identify patients at risk of a more aggressive disease and a worse prognosis. Further investigations, on a larger and more heterogeneous population, should be carried out to validate and extend our results.

Chapter VI

Summary and conclusions

Summary and conclusions

- RCC accounts for 2% to 3% of all malignant diseases in adults.
- Cancer of kidney in Libya, (including cancers of renal pelvis) comprises 2% of all cancer patients, being more common in males than females with ratio of 2.3:1.9.
- Among 37 patients involved in the current study 62 % were males and 38 females. The mean age at diagnosis 55.6 years.
- ccRCC is commonest was histological subtype in Eastern Libyan patients followed by pRCC and chRCC respectively.
- Most of Libyan patients have no vascular invasion at time of diagnosis, and at low TNM stage (75% stage I & stage II), also 59% were free from metastasis.
- There was a statistically significant correlation between Survivin expression and venous invasion (tumor thrombus), larger tumor size, advanced tumor stage. Also associated significantly with primary tumor classification (pT1, pT2 VS pT3; $P< 0.013$) showed a borderline association with tumor location .
- Survivin expression in RCC may identify patients at risk of a more aggressive disease and a worse prognosis. These findings underline the importance of de-regulation of apoptosis as a critical pathogenetic component of tumor progression and identify Survivin as a potential novel marker of aggressive cancer of the kidney. Although these associations appeared to be statistically significant in our study, these initial findings should be independently verified by other large independent population-base studies.

Recommendations

General

- Establishment of electronic archive to facilitate collection of data for the future studies.
- Establishment of the National Cancer Registry of Libya that will give a complete figure about cancer biology in Libyan population.
- Establishment of National Guidelines in histopathology reporting.
- There should be good communication between the members of the oncology, pathology, surgery, and radiology teams with agreed terminology, regular meetings and clinical discussions.

Specific

- In Libyan population, Survivin is usually overexpressed in RCC cells and high Survivin protein expression might be an independent prognostic factor for RCC.
- Therefore, tumor-specific down regulation of Survivin gene may become a novel therapeutic strategy to RCC patients and open new horizon of therapy, including molecular targeted therapy and radiotherapy**.**
- Further investigations, on a larger and more heterogeneous population, should be carried out to validate and extend our results, taking also into account the association of the various survivin isoforms with the RCC patient's outcome and examining the prognostic significance of their different topographical intracellular distribution.

Chapter VI

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إستخدام الصبغات المناعية لإكتشاف البروتينات المانعه من موت الخلايا المبرمج في سرطان الكلى ودراسة علاقتها ببعض العوامل المتعلقة بالمرضى ومدى علاقتها بمستقبل المرضى بسرطان الكلى

دراسة للحصول على درجة الماجستير في علم الامراض

للطبيبة: هيام محمودالنهوي

بكالوريس طب و جراحه

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بنغازي – ليبيا

(2013)

الملخص العربى

سرطان الكلي

الكلى هن عضوين في الجسم تُشبه بذر الفاصولياء في شكلها وتقع الكلى في الجدار الخلفي لجوف البطن، وتحديداً من جانبي العمود الفقري تحت الحجاب الحاجز. ان الضلعين الأخيرين من القفص الصدري يحميان الجانب الخلفي لكل كلية.

تصل الكلى الى ال 8 الي 12 سم في الطول, 5 الي 7 سم في العرض, 2 الي 3 سم في السمك.

أنواع سرطان الكلى

غالباً ما يتم تصنيف سرطان الكلى وفقاً للخلايا التي ينشأ منها. من المُتفق عليه أن سرطان الكلى يُصنف لنوعين أساسيين:

1. **سرطان الخلايا الكلوية**: ويُشكل 80-85% من أورام الكلى.
2. **سرطان الخلايا الانتقالية**: ويُشكل ما يُقارب 10% من أورام الكلى

توجد أنواع أخرى من أورام الكلى والتي قد تكون خبيثة، أو حميده

يشكل سرطان الخلايا الكلوية 3% من كل أنواع السرطان المشخص في الولايات المتحدة الأمريكية سنة 2010 . السرطان يصيب كل الأعراق وهو شائع حول العالم ولكن أكثرها شيوعا في شمال أوروبا ودول أمريكا الشمالية. عالميا,هناك حوالي 270,000 حالة من سرطان الخلايا الكلوية تم تشخيصها سنة 2008, و 116,000 حالة وفاة من السرطان. في دراسة أجريت سابقا في شرق ليبيا (المستيري 2004) وجد ان سرطان الكلي و الجهاز البولي يشكلان حوالي 2% من مجموع الأورام المشخصة في سجل بنغازي للسرطان سنة 2004 منهم 16 ذكور و 7 إناث بمعدل 2.07\100000 للذكور و معدل .091\100000 للإناث. سرطان الخلايا الكلوية يحدث غالبا بين العقدين السادس و الثامن من العمر, بمتوسط عمري يبلغ 64 عاما, وهو نادر في عمر اقل من 40 عاما.

مسببات حدوث سرطان الخلايا الكلوية

1. التدخين
2. ارتفاع ضغط الدم
3. السكري
4. التعرض لمواد سامة في العمل خاصة الكادميوم والأسبست.
5. الوزن الزائد والسمنة.
6. أمراض الكلى: مثل تكيس الكلى.
7. ديال الكلى: يُسمى أيضاً بغسيل الكلى. المرضى اللذين يمرون بعلاج بديال الكلى مُعرضون للاصابة بسرطان الخلايا الكلوية أكثر من غيرهم، لذا من المهم مُتابعة هؤلاء المرضى مرة في السنة على الأقل.

8. العوامل الوراثية: رغم أن أغلب حالات سرطان الخلايا الكلوية تُعتبر مُكتسبة، الا أن عوامل وراثية مُعينة قد تلعب دوراً في بعض حالات سرطان الخلايا الكلوية، وقد تكون هذه الحالات وراثية. وجود سرطان الخلايا الكلوية لدى أحد أفراد العائلة يزيد من احتمال الاصابة بسرطان الخلايا الكلوية.
9. أمراض وراثية: بعض من حالات سرطان الخلايا الكلوية تكون بسبب أمراض وراثية وأهمها داء فون هيبل ليندلو.

قد توجد عوامل أخرى تزيد من خطورة الإصابة بسرطان الخلايا الكلوية مثل العلاج الكيميائي. إلا أن الأمر لم يُثبت نهائياً.

أعراض وعلامات سرطان الخلايا الكلوية

قد يكون سرطان الخلايا الكلوية عديم الأعراض، وذلك في أغلب الحالات، مما يجعل الأعراض تظهر في المراحل المتقدمة من سرطان الخلايا الكلوية. أهم الأعراض التي قد تظهر هي: البيلة الدموية: أي ظهور الدم في البول, ألم في الخاصرة, الشعور بكتلة في البطن أو الخاصرة, فقدان الوزن, الحرارة المُرتفعة.

الا أن أغلب حالات سرطان الخلايا الكلوية يكون عديم الأعراض ويتم تشخيصه خلال الاختبارات التصويرية والتي تُجرى لأسباب أخرى للمريض.

مُضاعفات سرطان الخلايا الكلوية

يؤدي سرطان الخلايا الكلوية إلى عدة مضاعفات، أهمها: دوالي الخصية, ارتفاع نسبة الكالسيوم في الدم, كثرة الحمر: أي ارتفاع عدد كريات الدم الحمراء، مما يؤدي لاحمرار الجلد وأعراض أخرى, انسداد الوريد الأجوف السفلي.

قد ينتشر سرطان الخلايا الكلوية لأعضاء أخرى في الجسم كالعقد اللمفاوية والعظام والرئتين ويؤدي لأعراض تبعاً للعضو المُصاب. الا أن سرطان الخلايا الكلوية يُعد من أنواع السرطان البطيئة النمو والتي لا تنتشر بسرعة.

سيرڤيڤن(Survivin)

سيرڤيڤن(Survivin), احد البروتينات الكابحة لموت الخلايا المبرمج, يلعب دور هام جدا في إحداث و استمرارية نمو السرطان كما انه يجعل من السرطان المقاوم للأشعة العلاجية. سيرڤيڤن يعبر عنه بشكل كبير في الخلايا السرطانية ولا يوجد في الخلايا الطبيعية في الإنسان. ويعبر عته بشكل خاص في سرطان الرئة والقولون والثدي والكلي. وهو يلعب دور هام في ظهور الورم السرطاني من خلال خاصيته ككابح لموت الخلايا المبرمج.

الشفرة الوراثية الخاصة بالسرڤيڤين ممكن إن تكون قاصرة في خلايا سرطان الكلي , بمعني إن تثبيط سيرڤيڤن يثبط نمو الورم وكذلك يزيد من حساسية الورم للعلاج الإشعاعي. ولهذا فان سيرڤيڤن يمكن اعتباره كعامل مهم في تحديد النتيجة العلاجية للمربض. وتبني إستراتيجية علاجية تتضمن تثبيط سيرڤيڤن و العلاج بالإشعاع .

كما ان الدراسات الحديثة أثبتت ان هناك علاقة هامة بين زيادة التمثيل الخلوي لسيرفيفن وحجم الورم و مرتبة الورم وانتشاره في الأوعية الدموية وكذلك مرحلة المرض. ولقد حاولت في هذه الدراسة اثبات هذه العلاقة كما هو مبين في أهداف الأطروحة.

ملخص البحث

هدف هذه الدراسة البحثية هي تسليط الضوء على عوامل التنبؤ في سرطان الكلى بواسطة الكشف عن البروتينات المانعة من موت الخلايا المبرمج ومنها السيرفيڤن وتقييم دورها في حالات مرضى سرطان الكلى فى شرق ليبيا وتقييم علاقة السيرفيڤن بعوامل التنبؤ التقليدية وأثرها على حياة المريض.

المرضى وطرق الدراسة

تم اخذ 37 مريض من مرضى سرطان الكلى في شرق ليبيا من واقع سجلات قسم علم الإمراض بجامعة بنغازي ومن ثم متابعة هذه الحالات في قسم الأورام بمركز بنغازي الطبي وقد تم صبغ هذه العينات بمضاد السيرفيڤن واختبار هذه العينات إحصائيا.

النتائج

لا توجد علاقة بين السيرفيڤن وجنس وعمر المريض ومدى انتشار السرطان الى الاعضاء الاخرى, والى العقد الليمفاوية وكذلك الغشاء المحيط بالكلى. و لكن وجدت علاقة بين مستوي السرفيڤن مع حجم الورم والمراحل المتقدمة من الورم وهذا يدل على فائدة هذا البروتين فى سرطان الكلى وعلاقته بمستقبل المرضى.