

بسم الله الرحمن الرحيم

**Sudan University of Science and Technology**

**College of Graduate Studies**

**Immunohistochemical Expression of E cadherin in Renal Tumors**

**الظهور المناعي النسيجي للواسمة الورمية E cadherin في أورام الكلى**

A dissertation submitted for partial fulfillment for requirements of M.Sc.

degree in Histopathology and Cytology

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## الآية

بسم الله الرحمن الرحيم

قال تعالى:

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العظيم

سورة البقرة الآية 32

## **Dedication**

***To .....***

*My father ..... who worked hardly for us*

***To .....***

*My mother ..... Who taught me*

*How I could be human*

***To .....***

*My wife ..... Who support me at any time*

***To .....***

*For whom he knows himself*

*I produce this humble effort*

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## Abstract

This is analytical retrospective case control study conducted at Ibn Seina Hospital and Sudan University of Science and Technology-College of Medical Laboratory science during the period from January to May 2016. The study aimed to detect E cadherin in renal tumors among Sudanese patients using immunohistochemical method. 72 paraffin blocks samples were collected from patients previously diagnosed as renal tumors (54 diagnosed as malignant renal tumors, 18 as benign renal tumors). Of whom 30 (41.6%) were male and 42 (58.4%) were female. Their ages ranged between 21 to 85 years with mean age 55 year. From each block one section was cut (3 $\mu$ m) by using rotary microtome to detect E cadherin in renal tumors.

Data was analyzed using SPSS version 20 computer programs; mean, frequency and chi square were calculated

E cadherin was positive in 43 (59.7%) samples, while 29 (40.3%) samples were negative. From positive results, 17 (23.6%) in benign renal tumors, while 26 (36.1%) in malignant renal tumors with significant differences between E cadherin expression and renal tumors ( $P = 0.001$ ). Positive result in malignant renal tumors distributed as follow, 11 (20.4%) in papillary RCC, 7 (13%) in non classified tumors, 6 (11.1%) in chromophobe RCC, and 2 (3.7%) in clear cell RCC with significant differences between E cadherin expression and subtypes of malignant renal tumors ( $P = 0.000$ ).

On the basis of these results the study concluded that, there is a relation between E cadherin expression and benign renal tumors. There is a relation between E cadherin expression and subtypes of malignant renal tumors, strongly expressed in chromophobe RCC and papillary RCC.

## المستخلص

أجريت هذه الدراسة التحليلية الإسترجاعية في مستشفى ابن سينا وجامعة السودان للعلوم والتكنولوجيا- كلية علوم المختبرات الطبية خلال الفترة بين يناير إلى مايو 2016. هدفت الدراسة إلى توضيح الواسمة الورمية E cadherin في أورام الكلى لدى المرضى السودانيين بإستخدام كيمياء ومناعة الأنسجة. تم جمع 72 قالب نسيجي لعينات من مرضى مشخصين مسبقا بأورام الكلى, منهم 54 عينة مشخصة سرطان كلى و18 عينة مشخصة أورام حميدة. تراوحت أعمار المرضى بين 21 إلى 85 سنة بمتوسط عمر 55 سنة. نسبة الذكور كانت 30 (41.6%) و42 (58.4%) كانت نسبة الإناث. تم قطع مقطع واحد ( $3\mu\text{m}$ ) من كل قالب بإستخدام المشرع الدوار للكشف عن E cadherin في أورام الكلى .

حللت البيانات بإستخدام برنامج الحزم الإحصائية للدراسات الإجتماعية (SPSS) وتم حساب الوسط الحسابي والتردد ومربع كاي. أعطى ال E cadherin نتيجة إيجابية في 43 (59.7%) عينة و نتيجة سلبية 29 (40.3%) عينة. من مجموع 43 عينة والتي أعطت نتيجة إيجابية كان منها 17 (23.6%) عينة في الأورام الحميدة و26 (36.1%) في الأورام الخبيثة مع وجود علاقة ذات دلالة إحصائية بين ظهور ال E cadherin وأورام الكلى ( $P= 0.001$ ). النتائج الإيجابية في الإورام الخبيثة كانت كالآتي 11 (20.4%) في السرطانة الحليمية و7 (13%) في الأورم الغير مصنفة و6 (11.1%) في النوع اللاصبوغي و2 (3.7%) في سرطان الخلية الكلوية من النوع الإعتيادي ذات الخلايا الصافية, مع وجود علاقة ذات دلالة إحصائية بين ظهور E cadherin وأنواع أورام الكلى الخبيثة ( $P= 0.000$ ) .

على أساس هذه النتائج, خلصت الدراسة إلى وجود علاقة بين ظهور ال E cadherin وأورام الكلى وكان ظهور ال E cadherin عالي في الأورام الحميدة, كما توجد علاقة بين ظهور ال E cadherin وأنواع أورام الكلى الخبيثة مع ظهور قوي لل E cadherin في النوع اللاصبوغي والسرطانة الحليمية.

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# Introduction

## 1.1 Introduction:

Renal cell carcinoma (RCC) is the most common type of kidney cancer worldwide, accounts for 2%–3% of all adult malignancies, representing the seventh most common cancer in men and the ninth most common cancer in women worldwide (Escudier *et al.*, 2014). Worldwide, there are 209 000 new cases and 102 000 deaths per year, whereas the incidence of all stages of RCC has increased over the past several years, contributing to a steadily increasing mortality rate per unit population (Znaor *et al.*, 2015). In Ibn siena Hospital there are about 45 case diagnosed as renal cancer in 2015. Cigarette smoking, obesity and hypertension are well established risk factors for renal cell cancer (Chow *et al.*, 2010).

The 2004 World Health Organization (WHO) classification benign renal neoplasms on the basis of histogenesis, and histopathology. Oncocytoma, papillary adenoma, metanephric neoplasms, angiomyolipoma, and cystic nephroma classified as benign renal tumors. Renal cell carcinoma (RCC), clear cell renal cell carcinoma (ccRCC), papillary renal cell carcinoma and chromophobe renal carcinoma classified as malignant tumors of the kidney (Prasad *et al.*, 2008).

Many renal masses remain asymptomatic and non palpable until the late stages of the disease. Currently, most RCCs are detected incidentally by the frequent use of imaging examinations for a variety of unrelated symptoms or diseases. Physical examination has only a limited role in diagnosing RCC. However, it is important for the clinical evaluation, especially findings such as a palpable abdominal mass. The most commonly assessed laboratory parameters are serum creatinine, and also use ultrasound (US) for diagnosis and computed tomography (CT). Magnetic Resonance Imaging (MRI) provide additional information regarding the renal mass (Ljungberg *et al.*, 2010).

Renal tumor biopsy is increasingly being used in diagnosis and is always indicated before ablative and systemic therapy without previous histopathology and in surveillance strategies to stratify follow-up and treatment (Ljungberg *et al.*, 2010).

Immunohistochemical techniques with a variety of markers have been applied more frequently in diagnostic pathology of renal neoplasms such as cytokeratin, vimentin, PAX2 and PAX8, CD10, E-cadherin and kidney-specific cadherin (Ljungberg *et al.*, 2010).

It is important to take time and think about treatment if kidney cancer is diagnosed, treatment options may include surgery, ablation and other local therapies, Active surveillance, radiation therapy, targeted therapy, immunotherapy (biologic therapy), and chemotherapy (Escudier *et al.*, 2014)

E cadherin is a calcium dependent protein crucial for cell-cell interactions and embryogenesis. It is normally expressed by renal tubular cells and many other cell types it is one of the most important molecules in cell-cell adhesion in epithelial tissues. It is localized on the surfaces of epithelial cells in regions of cell-cell contact known as adherents junctions, as a member of a large family of genes coding for calcium-dependent cell adhesion molecules (Slaus, 2003). Previous study found that in all cases, E cadherin expression was membranous marker in normal human kidney localized in distal tubules and collecting ducts in the examined sections, consistent, intense brown color in membrane, In all benign lesions, E cadherin immunoreactivity was noted in epithelial tissue staining intensity was comparable to that of normal renal tissue and also E cadherin expression in malignant renal tumor according to type and grade of tumor (Katagiri *et al.*, 1995).

## **1.2 Rationale:**

Renal cell carcinoma (RCC) is the most common type of kidney cancer worldwide, accounts for 2%–3% of all adult malignancies, representing the seventh most common cancer in men and the ninth most common cancer in women worldwide (Escudier *et al.*, 2014). This study was done to fill the gap in theory and practical focused on kidney cancer in Sudan. Expression of E cadherin in renal cells is very good can used as marker to prognostic and differentiate RCCs.

### **1.3 Objectives:**

#### **General objective:**

To study the expression of E cadherin in renal tumors using immunohistochemical methods.

#### **Specific objectives:**

1-To detect expression of E cadherin in subtypes of malignant renal tumors using immunohistochemical methods.

2-To correlate the expression of E cadherin with histological diagnosis.



## **2. Literature Review**

### **2.1. Anatomy and physiology of the kidney:**

The kidneys are a pair of bean shaped organs, each about the size of a fist. They are attached to the upper back wall of the abdomen. The lower rib cage protects the kidneys. Small glands called adrenal gland above each of the kidneys. Each kidney and adrenal gland is surrounded by fat and a thin, fibrous layer known as Grote's fascia. The main job of kidney filters the blood coming in from the renal arteries to remove excess water, salt, and waste products. These substances become urine. Urine leaves the kidneys through ureters, which connect to the bladder. The place where the ureter meets the kidney is called the renal pelvis. The urine is then stored in the bladder until you urinate (pee) (Barron, 2012).

The outer layer of kidney is called renal cortex and the inner layer is called the renal medulla. The renal medulla consist of 10-15 medullary pyramids (Barron, 2012).

Each kidney contains over one million functioning units called nephrons. Each nephron is composed of a glomerulus and tubule. The glomerulus acts to clean the blood free of cells and large proteins, producing an ultrafiltrate composed of the other smaller circulating elements. The ultrafiltrate enters the tubule, which is highly specialized at various segments, to produce the final urine by removing substances from the tubular fluid (reabsorption) or adding substances to the tubular fluid (secretion). Filtration, reabsorption, and secretion keep the organism in balance in terms of water, minerals, electrolytes, and hydrogen ion concentration and eliminate the toxic substances produced by the body (Preuss *et al.*, 1993).

### **2.2 Pathology of the kidney:**

#### **2.2.1 Chronic kidney disease:**

Chronic kidney disease (CKD) is defined as the presence of kidney damage, manifested by abnormal albumin excretion or decreased kidney function, quantified by measured or estimated glomerular filtration rate (GFR) that persists for more than three months. CKD is characterized by progressive scarring that ultimately affects all structures of the kidney (Thomas *et al.*, 2008). Possible mechanisms of progressive renal damages include systemic and glomerular hypertension, various cytokines and

growth factors, podocyte loss, dyslipidemia and proteinuria. CKD is associated with many diseases such as anemia, mineral and bone disorders, and cardiovascular risk such as, hypertension and diabetes (Thomas *et al.*, 2008).

### **2.2.2 Nephrotic syndrome:**

The nephrotic syndrome is one of the best known presentations of adult or pediatric kidney disease. The term describes the association of proteinuria with peripheral oedema, hypoalbuminaemia, and hypercholesterolaemia. Nephrotic syndrome is a relatively rare but important manifestation of kidney disease. It has serious complications and must be on the differential diagnosis for any patient presenting with new onset oedema. It can be caused by a wide range of primary (idiopathic) and secondary glomerular diseases and these is very important to cause renal failure (Hull and Goldsmith., 2008).

### **2.2.3 Benign tumor of the kidney:**

Renal neoplasms are classified into renal cell metanephric, mesenchymal, and mixed epithelial and mesenchymal tumors (Prasad *et al.*, 2008).

Oncocytoma is a benign renal cell neoplasm that accounts for approximately 5% of all adult primary renal epithelial neoplasms in surgical series. Oncocytoma, a subgroup of renal adenoma, is a benign neoplasm that arises from tubular epithelial cells of the kidney (Prasad *et al.*, 2008).

Papillary adenomas are the most common renal epithelial neoplasms. According to autopsy series, approximately 40% of patients older than 70 years harbor renal adenomas. Papillary adenomas have cytogenetic changes include loss of the Y chromosome and combined trisomy of chromosomes 7 and 17 (Prasad *et al.*, 2008).

Metanephric neoplasms define as a heterogeneous group of benign renal neoplasms that include metanephric adenoma (epithelial tumor), metanephric stromal tumor (stromal neoplasm), and metanephric adenofibroma (mixed epithelial and stromal neoplasm). These tumors are histogenetically related to Wilms' tumor and are postulated to represent the most hyperdifferentiated, benign end of the nephroblastoma spectrum (Prasad *et al.*, 2008).

Angiomyolipoma (AML) is the most common benign mesenchymal neoplasm, it is composed of variable proportions of blood vessels, smooth muscle, and adipose tissue. Renal AMLs consist of two different histological subtypes, classic triphasic and monotypic epithelioid. Epithelioid AMLs typically do not show macroscopic fat and appear as soft tissue masses and are thus impossible to differentiate from other solid renal masses (Prasad *et al.*, 2008).

#### **2.2.4 Malignant tumor of the kidney:**

Renal cell carcinoma is the most common tumor to influence the adult kidney, accounting for 80–90% of primary malignant renal neoplasms in adults. On gross pathology, tumors most often appear encapsulated. Tumors may be solid, cystic, or mixed, including or engulfing fat (Sircar *et al.*, 2013).

Histological subtypes according to the Heidelberg classification include clear cell (conventional) adenocarcinoma (80%), papillary (15%), chromophobe (5%), collecting duct (1%), and unclassified (4%) (Muglia and Prando, 2015).

Clear cell carcinoma displays large consistent cells with abundant clear cytoplasm rich in glycogen and lipid. Clear cell carcinoma is typically highly vascular.

Papillary tumors are subdivided into type I tumors, which occur sporadically and metastasize somewhat later, and type II, which are more likely inherited, may be multiple, and often present with a higher Fuhrman grade and poorer prognosis. Collecting duct tumors, which arise from the medullary collecting duct, often occur in younger patients and are associated with a poor overall prognosis (Sircar *et al.*, 2013).

Renal medullary carcinoma is a rare subtype, closely related to collecting duct carcinoma and having a poor prognosis, which occurs in young patients with sickle cell anemia or sickle trait (Ross *et al.*, 2012).

Chromophobe tumors and oncocytomas, both of which arise from collecting duct epithelium, may be confused on histologic examination but have differing immunohistochemical profiles. Chromophobe tumors have the best overall prognosis, and oncocytomas are benign (Ng *et al.*, 2008).

### **2.3 Epidemiology of renal cancer:**

The incidence of RCC varies internationally. The incidence of renal cancer in more developed regions (e.g.; Europe and North America) is more than twice that of less developed regions (e.g.; Africa and South America). In Europe, an estimated 16/100,000 individuals were diagnosed with a renal malignancy, whereas in Africa, an estimated 1/100,000 individuals were diagnosed with a renal malignancy in 2014.

The regional variation is likely due to a combination of a higher amount of incidental tumors discovered on diagnostic abdominal imaging (computerized tomography scan, ultrasound, and magnetic resonance imaging) and modifiable risk factors such as smoking, obesity, and hypertension (Terris *et al.*, 2016).

Worldwide estimated >320,000 new cases diagnosed in 2015. The estimated number of deaths globally is 140,000. During the past 10 years, the incidence of RCC has increased worldwide, yet death rates have decreased or remained stable in most countries under study. This could be explained by the fact that the size of renal tumors at diagnosis is decreasing with time in developed countries, such as the USA, which is characterized by better survival outcome (Terris *et al.*, 2016).

Symptoms of kidney cancer include, blood in urine, pain in side that doesn't go away, A lump or mass in side or abdomen, weight loss for no known reason, fever, feeling very tired (Escudier *et al.*, 2012).

### **2.4 Risk factor of the kidney cancer:**

**Cigarette smoking:** is most common a causal risk factor for renal cell cancer by the International Agency for Research on Cancer (IARC), although the risk associated with cigarette smoking is relatively modest. Compared to never smokers, risk increased about 50% in male and 20% in female smokers. A clear dose-response pattern of risk was observed with increasing amount of cigarettes smoked. Smoking cessation reduces the risk, but only among long-term quitters of ten or more years. Cigarette smoking is hypothesized to increase renal cell cancer risk through chronic tissue hypoxia due to carbon monoxide exposure and smoking-related conditions such as chronic obstructive pulmonary disease (Terris *et al.*, 2016).

**Excess body weight:** has been estimated to account for over 40% of renal cell cancers in the United States and over 30% in Europe. In prospective studies conducted worldwide, overweight and obese individuals at baseline were found to have elevated following risks of renal cell cancer in a dose-response manner, estimated to increase 24% for men and 34% for women for every 5 kg/m. The global rise in obesity likely has contributed to the upward RCC incidence trends, but does not explain the recent leveling of RCC in some countries. Several mechanisms have been hypothesized to influence renal cell cancer development in obese individuals (Sircar *et al.*, 2013).

**Hypertension:** Certain types of renal tumors and cancer treatment have been shown to cause hypertension. However, there is sufficient evidence to demonstrate that hypertension predisposes to renal cell cancer development. Most studies reported an association with a history of long-term hypertension, and cohort studies with blood pressure measurements taken at baseline generally reported a dose-response of increasing risks with rising levels of blood pressure. Users of diuretics and other anti-hypertensive medications also were associated with an elevated risk of renal cell cancer, but an independent effect from that of hypertension has not been established, renal cell cancer risk increased with further elevation of blood pressure and decreased with reduction in blood pressure over time, suggesting a tumor-promoting effect for hypertension and that effective control of blood pressure may reduce renal cell cancer risk (Washio *et al.*, 2014).

**A history of diabetes mellitus:** has been linked to renal cell cancer risk in several cohort studies, but its role independent of those of obesity and hypertension has not been demonstrated conclusively (Chow *et al.*, 2010).

## **2.5 Diagnosis of kidney cancer:**

### **2.5.1 Urine tests:**

The laboratory checks urine for blood and other signs of disease (Kuroda *et al.*, 2013).

### **2.5.2 Blood tests:**

The lab checks blood for several substances, such as creatinine. A high level of creatinine may mean the kidneys aren't doing their job (Kuroda *et al.*, 2013).

### **2.5.3 Radiological test:**

#### **2.5.3.1 Ultrasound:**

An ultrasound device uses sound waves that can't be heard by humans. The picture can show a kidney tumor (Escudier *et al.*, 2012).

#### **2.5.3.2 Computed Tomography (CT) scan:**

An x-ray machine linked to a computer takes a series of detailed pictures of abdomen. The CT scan can show cancer in the kidneys (Escudier *et al.*, 2012).

#### **2.5.3.3 Magnetic Resonance Imaging (MRI):**

A large machine with a strong magnet linked to a computer is used to make detailed pictures of kidney. Receive an injection of contrast material. MRI can show cancer in kidneys, lymph nodes, or other tissues in the abdomen (Escudier *et al.*, 2012).

#### **2.5.4 Biopsy:**

It is procedure in which the removal of tissue obtained to look for cancer cells. In some cases, biopsy done to diagnose kidney cancer, inserts a thin needle through your skin into the kidney to remove a small sample of tissue; use ultrasound or a CT scan to guide the needle, uses a microscope to check for cancer cells in the tissue (Escudier *et al.*, 2012).

#### **2.5.5 Immunohistochemical techniques:**

Variety of markers have been applied more frequently in diagnostic pathology of renal neoplasms, and in some situations, those techniques become indispensable, the immunohistochemical markers most commonly used for diagnosis of renal neoplasms (Rivera *et al.*, 2010).

### **2.6 Treatment of kidney cancer:**

**Surgery:** is the main treatment for most kidney cancers. Even people with highly developed kidney cancers are often helped by surgery.

**Radical nephrectomy:** In this operation, the whole kidney, the attached adrenal gland, and some nearby fatty tissue are removed.

**Radiation therapy:** Radiation therapy uses high-energy rays (such as X-rays) or particles to kill cancer cells or shrink tumors. It is sometimes used as the main treatment (Escudier *et al.*, 2014).

**Biologic therapy (immunotherapy):** The goal of biologic therapy is to boost the body's own immune system to help fight destroys cancer cells.

**Chemotherapy:** Chemotherapy (chemo) uses anti-cancer drugs that are given into a vein or by mouth (in pill form). These drugs enter blood and reach all areas of the body, which makes this treatment potentially useful for cancer that has spread (metastasized) to organs beyond the kidney (Escudier *et al.*, 2014).

## **2.7 E cadherin:**

E cadherin it is calcium dependent cell adhesion molecules and one of the most important molecules in cell-cell adhesion in epithelial tissues. It is localized on the surfaces of epithelial cells in regions of cell-cell contact known as adherent's junctions. The cadherin glycoproteins are expressed by a variety of tissues, mediating adhesion through homotypic binding such as E, N, K, P, R, OB. Classical cadherins, E and N cadherins being the best characterized play important roles in the formation of tissues during gastrulation, neurulation and organogenesis, can play a major role in malignant cell transformation, and especially in tumor development and proliferation. The suppression of E cadherin expression is regarded as one of the main molecular events responsible for dysfunction in cell-cell adhesion. Most tumors have abnormal cellular architecture, and loss of tissue integrity can lead to local invasion. Thus, loss of function of E cadherin tumor suppressor protein lead to increased invasiveness and metastasis of tumors, resulting in it being referred to as the suppressor of invasion gene. The human epithelial (E) cadherin gene CDH1 it is located in chromosome 16q22.1 (Shimazui *et al.*, 2000).

### **2.7.1 Role of E cadherin in normal cells:**

E cadherin expression is very early in epithelial tissue at the two cell stage epithelial differentiation and polarization occur early in ontogeny in the morula stage, when the embryo compacts and each cell polarizes along it's apicobasal axis to generate an epithelial-like phenotype. E cadherin plays an important role in the adhesion junction of epithelial cell, and early embryo's ability to compact. E cadherin is expressed in the membrane even before compaction of the morula occurs, is distributed in a non-polar

manner. The mechanism that renders E cadherin functional is unknown, but it does include phosphorylation of the protein (Katagiri *et al.*, 1995).

Epithelial tissue conversion is the most important exhibit of E-cadherin's function in development. Loss of epithelial adhesion and polarity causing mesenchymal cell morphology occurs during mesoderm formation. Heterozygous mutant animals were normal and fertile but abnormal in human (Jeanes *et al.*, 2008).

### **2.7.2 Role of E cadherin in malignant cells:**

Somatic mutations is very important to cause cancer in a number of different genes characterizes the process of tumorigenesis. Many genes involved in the process of tumorigenesis are components of one of a great many signal transduction pathways such as E cadherin and beta catenin. It is now apparent that epithelial malignancy can in certain aspects be explained by alterations in the adhesive properties of neoplastic cells, epithelial mesenchymal conversion is also observed in malignant tumors of epithelial origin. This process is similar to developmental events but with the important difference that it is uncontrolled. Malignant carcinoma cells are characterized in general by poor intercellular adhesion, loss of the differentiated epithelial morphology and increased cellular motility. Down regulation or a complete shutdown of E cadherin expression, mutation of the E cadherin gene, or other mechanisms that interfere with the integrity of the adherens junctions, are observed in carcinoma cells. In human tumors, the loss of E cadherin mediated cell adhesion correlates with the loss of the epithelial morphology and with the acquisition of metastatic potential by the carcinoma cells. Thus, a tumor invasion suppressor role has been assigned to E cadherin (Slaus, 2003).



### **3. Materials and methods**

#### **3.1 Study design:**

This is analytical retrospective hospital based case control study, aimed to detect the expression of E cadherin in renal tumors.

#### **3.2. Study area:**

The study was conducted at Ibn Seina Hospital and Sudan University of Science and Technology- College of Medical laboratory Science during the period from January to May 2016.

#### **3.3 Study population:**

Patients with renal neoplasm 72 paraffin blocks samples were collected previously diagnosed as renal tumors (54 diagnosed as malignant renal tumors, 18 as benign renal tumors).

#### **3.4 Sample collection:**

From each block one section was cut ( $3\mu$ ) by using rotary microtome to detect E cadherin in renal tumors.

#### **3.5 Sample processing:**

One section ( $3\mu\text{m}$ ) from formalin-fixed paraffin-embedded tumor was cut and mounted onto coated slides (Dako). Following deparaffinization in xylene, slides was rehydrated through a graded series of alcohol and placed in distilled water. Samples were steamed for antigen retrieval for E cadherin using PT link, slides was placed in the PT tank containing enough tris buffer (PH9.0) to cover the sections, then the machine was turn on and programmed as follow, 20 minutes to start heating from  $65^{\circ}\text{C}$  till it reach  $95^{\circ}\text{C}$  and then boiled at high temp ( $95^{\circ}\text{C}$ ) for 20 minutes then allow sections to cool to  $65^{\circ}\text{C}$ . Endogenous peroxidase activity was blocked with peroxidase blocking reagent (3% hydrogen peroxide and methanol) for 10 minutes.

#### **3.6 Sample technique:**

The sections were incubated with  $100\mu\text{l}$  of primary antibody (E cadherin)(Dako) for 20 minutes at room temperature in moisture chamber, and then were rinsed in phosphate buffer saline, after washed with PBS for 3minutes, binding of antibodies were detected by incubating for 20 minutes with dextran labeled polymer (Thermo –

ultra vision) . Finally, the sections were washed in three changes of PBS, followed by adding 3,3 diaminobenzidine tetra hydrochloride (DAB) as chromogen to produce. the characteristic brown stain for the visualization of the antibody/enzyme complex for 5 minutes. Slides were counter stained with haematoxylin (Mayer's) for one minute, then dehydrated, cleared and mounted in DPX.

### **3.7 Statistical analysis:**

Data was analyzed using SPSS version 20 computer programs; mean, frequency and chi square were calculated.

### **3.8 Ethical consideration:**

The study was performed after approval to use tissue blocks from the Ibn Seina Hospital, and also after approval from the ethics committee of Sudan University of Science and Technology.

### **3.9 Result interpretation:**

E cadherin strongly expression in benign renal tumors as membranous marker and also highly expressed in chromophobe RCC and papillary RCC but in ccRCC showed negative results.

## 4. Results:

The study involved 72 samples, previously diagnosed as renal tumors. Table(1) showed frequency of histopathological diagnosis, 54 (75%) malignant renal tumors distributed as follow 15 (20%) papillary RCC, 20 (27.8%) clear cell RCC, 13 (18.1%) non classified tumors, 6 (8.3%) chromophobe RCC and the remaining 18 (25%) benign renal tumors.

The study subjects age was ranged from 21 to 85 years, all subjects were grouped into two age groups. Figure (1) explain age groups, group one less than or equal to 60 years 43 (59.7%), while group two more than 60 years 29 (40.3%).

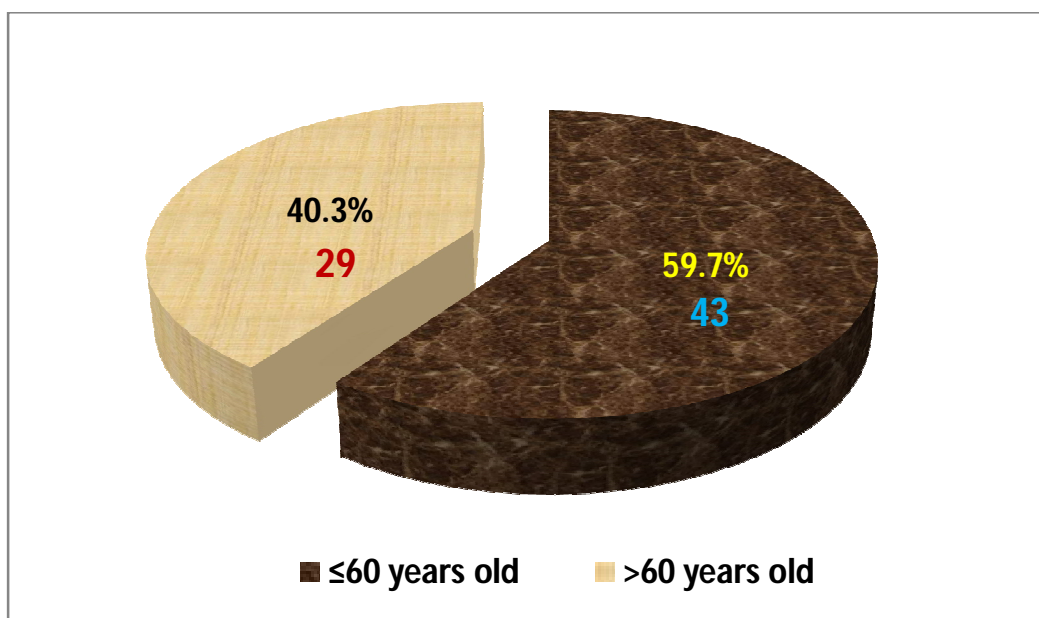
Figure (2) showed frequency of sex among study population, 30 (41.6%) were male, distributed as follow 6 (8.3%) in benign, 24 (33.3%) in malignant, while female were 42 (58.4%),distributed as follow 12 (16.7%) in benign, 30 (41.7%) in malignant.

Table (2) showed the compression of E cadherin expression in malignant and benign renal tumors, individual there was significant differences between E cadherin expression and renal tumors ( $P = 0.001$ )

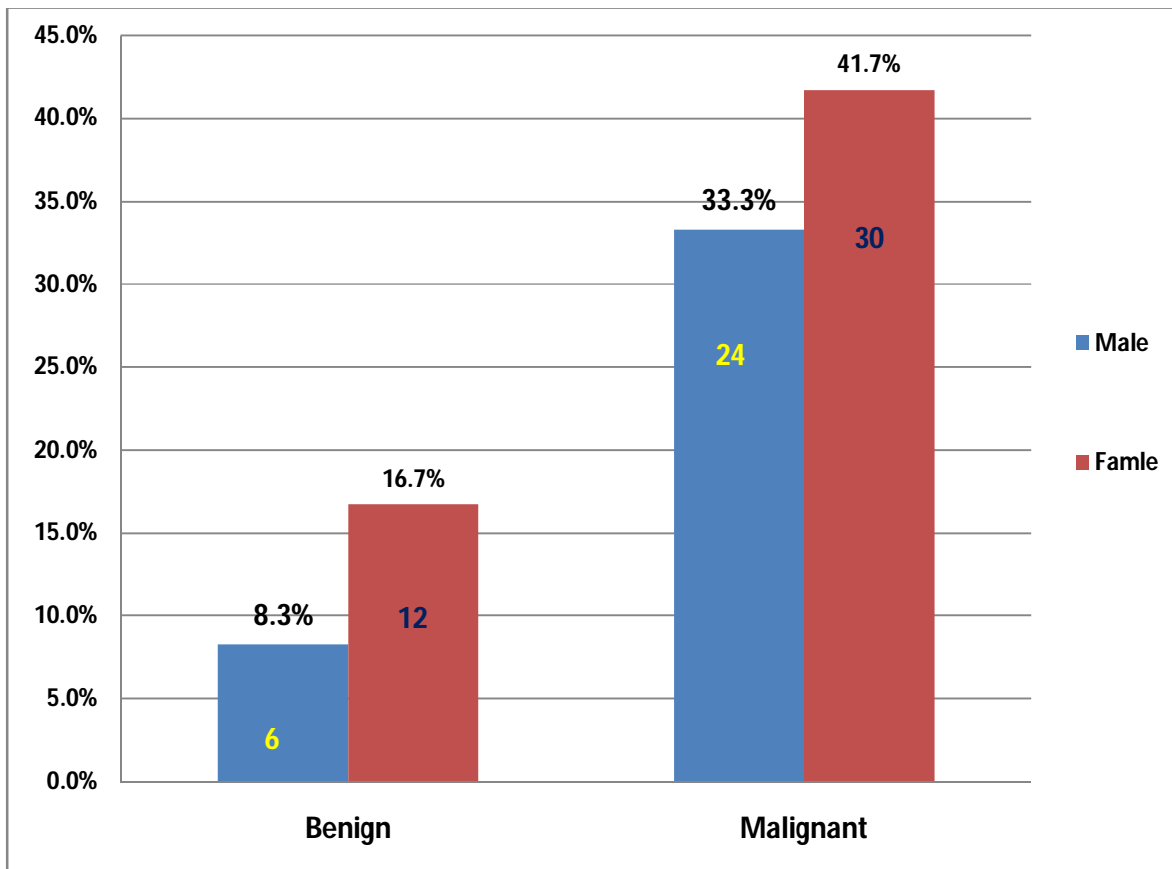
Table (3) showed the compression of E cadherin expression between subtypes of malignant renal tumor, there was significant differences between E cadherin expression and subtypes of malignant renal tumors ( $P = 0.000$ ).

**Table 4.1: Frequency of histopathological diagnosis**

<b>Histopathological Diagnosis</b>		<b>Frequency</b>	<b>Percentage</b>
<b>Benign</b>		18	25%
<b>Malignant</b>	Papillary RCC	15	20.8%
	Clear cell RCC	20	27.8%
	Non classified tumors	13	18.1%
	Chromophobe RCC	6	8.3%
<b>Total</b>		<b>72</b>	<b>%100</b>



**Figure 4.1: Age distribution among study population**



**Figure 4.2: Sex distribution among study population**

**Table 4.2: Relation between E cadherin expression and histopathological diagnosis**

Histopathological Diagnosis	Positive	Negative
Benign	17 23.6%	1 1.4%
Malignant	26 36.1%	28 38.9%
Total	43 59.7%	29 40.3%

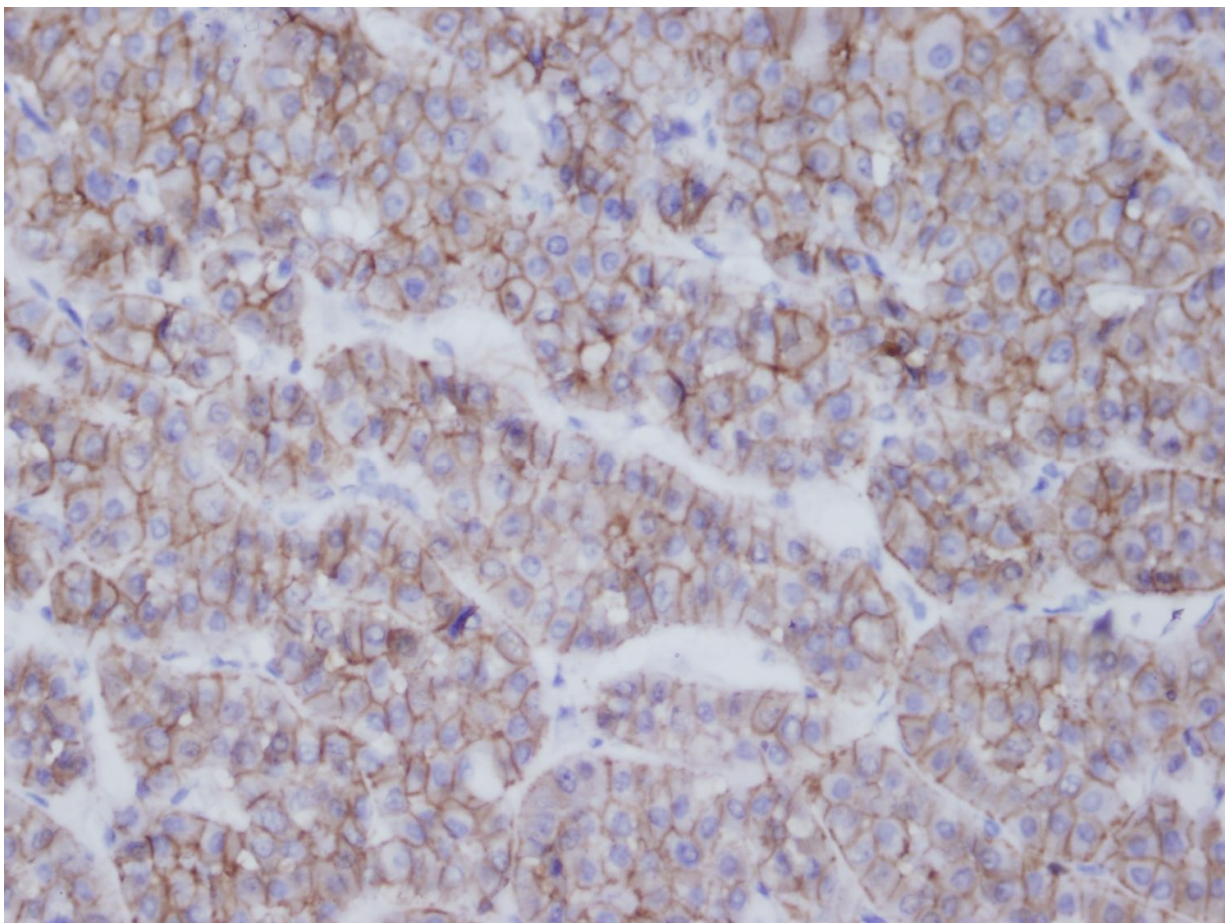
**$P = 0.001$**

**Table 4.3: Relation between E cadherin expression and subtypes of malignant renal tumors**

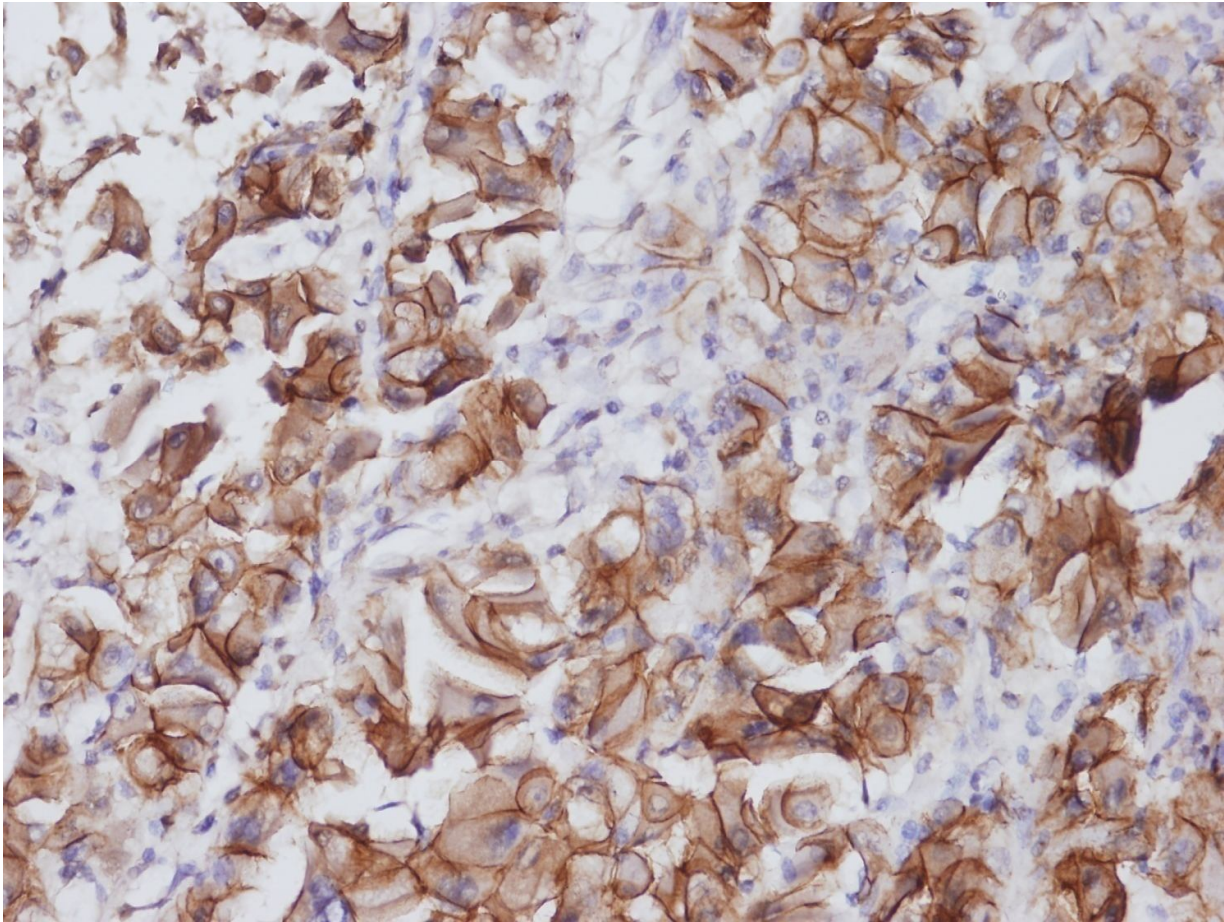
<b>Histopathological Diagnosis</b>		<b>Positive</b>	<b>Negative</b>
<b>Diagnosis</b>	<b>Papillary</b>	11 20.4%	4 7.4%
	<b>CCRCC</b>	2 3.7%	18 33.3%
	<b>Non classified tumors</b>	7 13.0%	6 11.1%
	<b>Chromophobe</b>	6 11.1%	0 0.0%
<b>Total</b>		<b>26</b> <b>48.1%</b>	<b>28</b> <b>51.9%</b>

$P = 0.000$



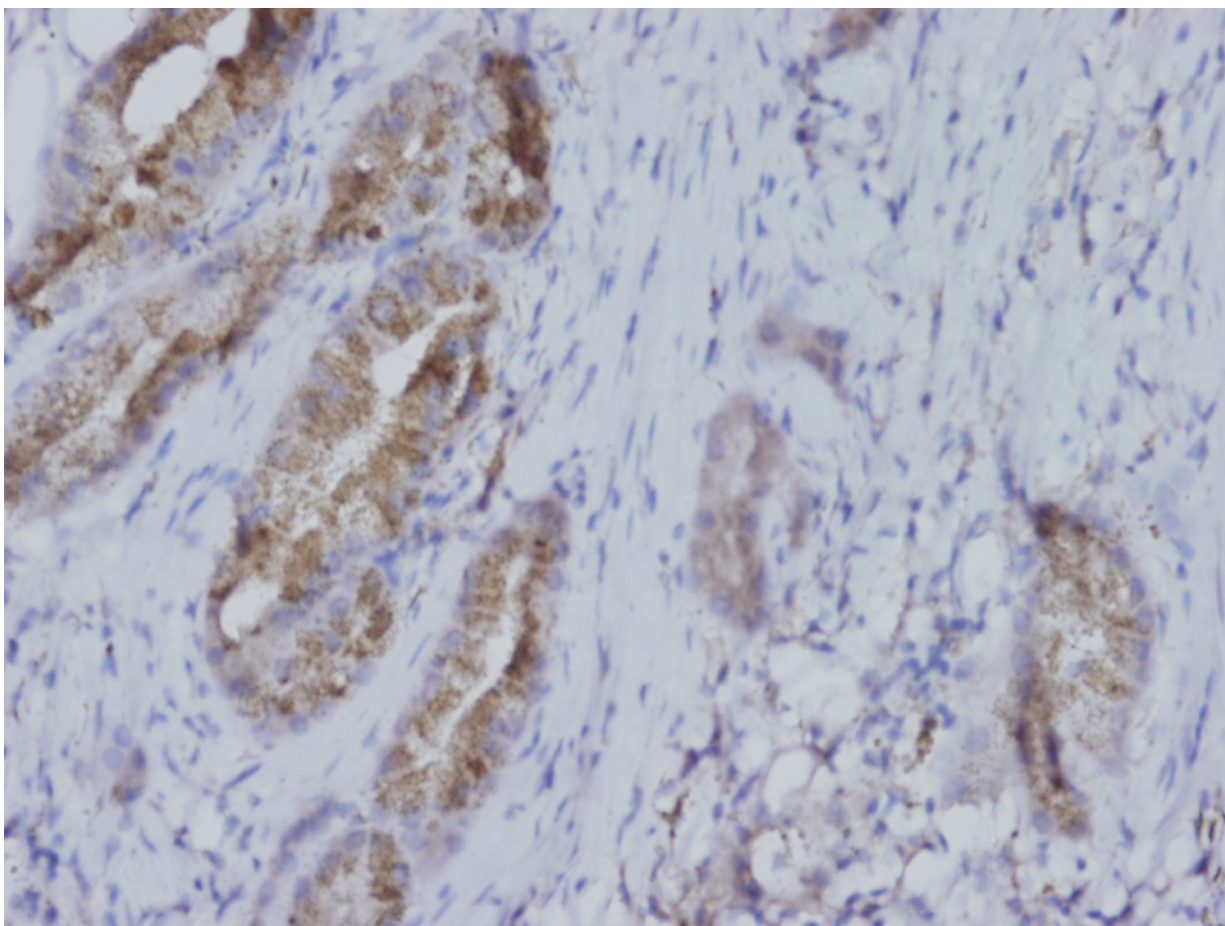


**Photograph (4.1): Benign renal tumors showing highly expression of E cadherin (40X)**

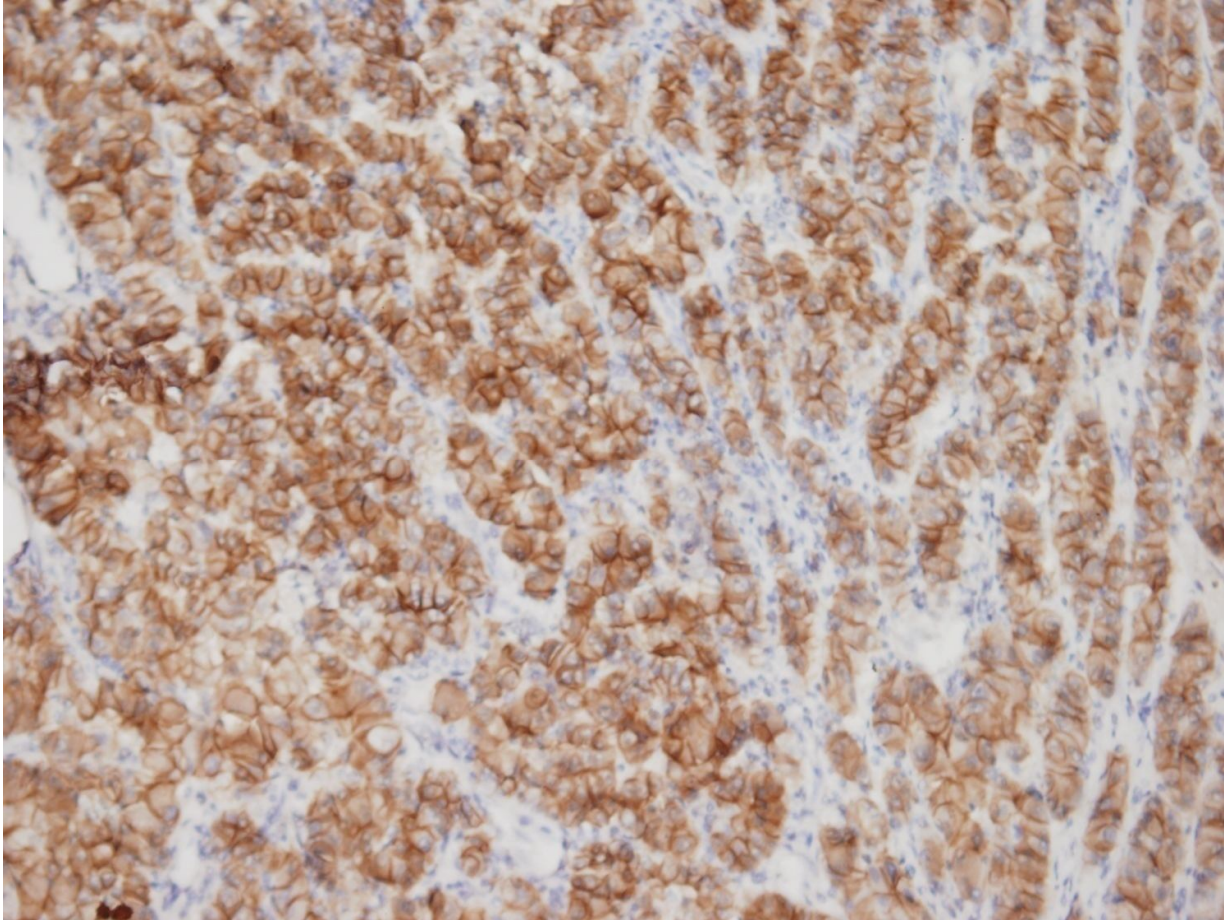


**Photograph (4.2):Chromophobe RCC showing highly expression of E cadherin (40X)**





**Photograph (4.3):Papillary RCC showing positive expression of E cadherin (40X)**



**Photograph (4.4): Non classified tumors showed positive expression of E cadherin (40X)**

## 5. Discussion

The present study found the majority of population approximately 60% of patient's age less than 60 years old, this result disagree with study of Terris 2016 who found the kidney cancer incidence is strongly related to age, being in older male and female aged 60 and over worldwide, small proportion of kidney cancer occur in children .

This study found that the common type of kidney cancer was clear cell carcinoma, this result disagree with study of Muglia and Prando 2015 who found the renal cell carcinoma is by far the most common type of kidney cancer. About 9 out of 10 kidney cancers are renal cell carcinomas worldwide. In this study, we demonstrated that E cadherin is specifically expressed in the benign and malignant renal tumors.

When we compared the expression of E cadherin in malignant and benign renal tumors individuals, there was significant difference between E cadherin expression in malignant and benign renal tumors. The positive result of E cadherin expression using immunohistochemical method were 43 (59.7%), and 29 (40.3%) were negative. Positive results distributed as follow, 17 (23.7%) in benign renal tumors while malignant renal tumor have 26 (36.2%) positive result, ( $P = 0.001$ ) this result explain that the expression of E cadherin occur in benign renal tumors and some malignant renal tumors such as chromophobe RCC and papillary RCC, this result supported by Langner and his colleagues found that the expression of E cadherin occur in RCC and chromophobe RCC (Langner *et al.*, 2003) and also agree with Katagiri and his colleagues they found that the expression of E cadherin occur in benign renal tumor and RCC (Katagiri *et al.*, 1995). And disagree with study of Shimazui and his colleagues they found that E cadherin negative in RCC and papillary RCC (Shimazui *et al.*, 2000).

When we compared the expression of E cadherin in subtypes of malignant renal tumors individuals, there was significant difference between E cadherin expression and subtypes of malignant renal tumors. The positive result of E cadherin expression using immunohistochemical method between subtypes of malignant renal tumor distributed as follow, 11 (20.4%) in papillary RCC, 7 (13%) in RCC, 6 (11.1%) in chromophobe RCC, and 2 (3.7%) in clear cell RCC ( $P = 0.000$ ), these results explain

that the expression of E cadherin occur in chromophobe RCC and some RCC and papillary RCC but in clear cell RCC showed negative result and also explain that E cadherin is a good differential marker in subtypes of RCC. This result supported by Zhou and his colleagues found that E cadherin expression in chromophobe RCC and good differential marker, used to differentiate between chromophobe and clear cell RCC (Zhou *et al.*, 2005), and also agree with Jin and his colleagues they found that strong expression of E cadherin occur in Chromophobe RCC (Jin *et al.*, 1995). And disagree with study of Steven and his colleagues they found that E cadherin are typically negative in RCC and papillary RCC (Steven *et al.*, 2012).

## **6. Conclusion and Recommendations:**

### **6.1 Conclusion:**

**On the basis of these results the study concluded that:**

- There is a significant relation between E cadherin expression and benign renal tumors.
- There is a significant relation between E cadherin expression and subtypes of malignant renal tumors.

### **6.2 Recommendations:**

**On the basis of this study we recommended:**

- More studies could be done about kidney cancer because the researches are very limited in Sudan and lack of data published.
- E cadherin is a good deferential and diagnostic marker for malignant renal tumors as membranous marker. Further studies could be done of E cadherin with another adhesion molecule such as beta catenin with large sample size.

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## **APPENDIX (1)**

### **1-Instrument and materials:**

#### **1-Instrument:**


- Rotary microtome
- Oven
- Coplinjare
- Staining racks
- Stainless microtome blade
- Dako coated slides
- PT link
- Cover glass
- Water bath
- Dako pen
- Moisture chamber
- Work station
- Pipettors

#### **2. Materials:**

- Xylene
- Ethyle alcohol
- Mayer's haematoxylene
- Distilled water
- Citrate buffer
- Peroxidase blocker
- Anti E cadherin antibodies (primary antibody)
- Dextran polymer conjugated secondary antibodies and HRP
- 3,3diaminobenzidinetetrahydrochloride in substrate buffer
- DPX mounting media

## APPENDIX (2)

P02080 001



CE

**FLEX**  
**Monoclonal Mouse**  
**Anti-Human**  
**E-Cadherin**  
**Clone NCH-38**  
**Ready-to-Use**  
**(Dako Omnis)**

**English**  
**Code GA059**

**Intended use** For in vitro diagnostic use.

**Synonyms for antigen** E-CD, uvomorulin, L-CAM, Arc-1, or cell-CAM 120/180 (2-4)

**Summary and explanation** E-cadherin is a 120 kDa transmembrane cell adhesion molecule. The gene has been localized on chromosome 16q22.1. In its extracellular domain, E-cadherin is involved in cell-cell adhesion through calcium-regulated homophilic interactions, whereas in its intracellular domain, E-cadherin connects to the actin cytoskeleton via catenins. E-cadherin has a significant function in intercellular adhesion of epithelial cells, the establishment of epithelial polarization, glandular differentiation, and stratification. It is localized mainly in the adherens junctions and concentrates the urokinase plasminogen activator and the epidermal growth factor receptor to cell contact sites (5, 6). Down-regulation of E-cadherin expression has been observed in a number of carcinomas and is usually associated with advanced stage and progression (5-8).  
Refer to Dako's *General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures.

**Reagent provided** Ready-to-use monoclonal mouse antibody provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L sodium azide.  
Clone: NCH-38. Isotype: IgG1, kappa.

**Immunogen** E-cadherin (uvomorulin) (9).

**Specificity** Anti-E-cadherin, NCH-38 recognizes the 120 kDa mature form and 82 kDa fragment of E-cadherin in Western blots of A431 cells lysates (9).

**Precautions**

1. For professional users.
2. This product contains sodium azide (NaN<sub>3</sub>), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
3. As with any product derived from biological sources, proper handling procedures should be used.
4. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
5. Unused solution should be disposed of according to local, State and Federal regulations.

**Storage** Store at 2-8 °C. During storage the cap should be closed. Do not use after expiration date stamped on vial. Onboard stability is 80 hours. Remaining on-board stability is tracked by the Dako Omnis software. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

#### Quick guide

Step		Comments
Fixation/embedding	Formalin-fixed, paraffin-embedded	Onboard deparaffinization
Pre-treatment	EnVision™ FLEX, High pH (Code GV804)	30 min HIER
Antibody	Ready-to-use	25 min incubation
Negative Control	FLEX Negative Control, Mouse (Code GA750)	25 min incubation
Visualization	EnVision™ FLEX (Code GV800) + EnVision™ FLEX+ Mouse LINKER (Code GV821)	Block: 3 min; Link: 10 min; Polymer: 20 min; Chromogen: 5 min
Counterstain	Hematoxylin (Code GC808)	3 min incubation
Control Tissue	Colon and liver	Cytoplasmic/membranous staining
Slides	FLEX IHC Microscope Slides (Code K8020)	Recommended for greater adherence of tissue sections to glass slides
Mounting	Non-aqueous, permanent mounting required	After staining, the sections must be dehydrated, cleared and mounted using permanent mounting medium
Instrumentation	Dako Omnis	Reagents are provided in instrument-specific vials

\*The user must always read the package insert for detailed instructions of the staining procedure and handling of the product.

#### Specimen preparation

**Paraffin sections:** The antibody can be used for labeling formalin-fixed, paraffin-embedded tissue sections. Tissue specimens should be cut into sections of 4 µm.

**Pre-treatment:** Pre-treatment of formalin-fixed, paraffin-embedded tissue sections with heat-induced epitope retrieval (HIER) is required. Pretreating tissues with HIER using diluted EnVision™ FLEX Target Retrieval Solution, High pH (50x) (Dako Omnis), Code GV804 is recommended. Deparaffinization, rehydration and target retrieval are performed onboard Dako Omnis. Please refer to Dako Omnis Basic User Guide.

The tissue sections should not dry out during the pre-treatment process or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of FLEX IHC Microscope Slides, Code K8020 is recommended.

#### Staining procedure

**Program:** The staining steps and incubation times are pre-programmed into the Dako Omnis software. Please refer to the Dako Omnis Basic User Guide for detailed instructions on loading slides and reagents. If the protocols are not available in the Dako Omnis system, please contact Dako Technical Services. All incubation steps are performed at 32 °C onboard Dako Omnis.

**Visualization:** The recommended visualization system is EnVision™ FLEX, High pH (Dako Omnis), Code GV800 in combination with EnVision™ FLEX+ Mouse LINKER (Dako Omnis), Code GV821. The visualization is performed onboard Dako Omnis.

**Counterstaining:** The recommended counterstain is Hematoxylin (Dako Omnis), Code GC808. The counterstaining is performed onboard Dako Omnis.

**Mounting:** After staining onboard Dako Omnis the sections must be dehydrated, cleared and mounted using permanent mounting medium.

**Controls:** Positive and negative controls should be run simultaneously using the same protocol as the patient specimens. The positive control tissue should include colon and liver the cells/structures should display reaction patterns as described for this tissue in the "Performance characteristics" section. The recommended negative control reagent is FLEX Universal Negative Control, Mouse (Dako Omnis), Code GA750.

The cellular staining pattern is cytoplasmic and membranous.

#### Staining interpretation Performance characteristics

**Normal tissues:** In colon, the epithelial cells show a strong staining reaction. In liver, the hepatocytes and ductal cells show a moderate to strong reaction.

#### Summary of Normal Tissue Reactivity

Tissue Type (# tested)	Positive Tissue Elements	Tissue Type (# tested)	Positive Tissue Elements
Adrenal (3)	3/3 epithelium (50-80%), membrane and cytoplasm	Pancreas (3)	3/3 epithelial cells (100%), membrane and cytoplasm
Bone marrow (1)	0/1	Parathyroid (3)	3/3 epithelium (100%), membrane and cytoplasm
Breast (3)	3/3 epithelial cells (100%), membrane and cytoplasm	Pituitary (3)	0/3
Cerebellum (3)	0/3	Prostate (3)	3/3 epithelium (100%), membrane and cytoplasm
Cerebrum (3)	0/3	Salivary gland (3)	3/3 epithelium (100%), membrane and cytoplasm
Cervix (1)	1/1 epithelium (100%), membrane and cytoplasm	Skin (3)	3/3 epithelium (100%), membrane and cytoplasm
Colon (3)	3/3 epithelial cells (100%), membrane and cytoplasm	Small intestine (3)	3/3 epithelium (100%), membrane and cytoplasm
Esophagus (3)	3/3 epithelium (100%), membrane and cytoplasm	Spleen (3)	3/3 small vessel endothelium (50%), membrane and cytoplasm
Kidney (3)	3/3 tubular epithelium (80%), membrane and cytoplasm	Stomach (3)	3/3 epithelium (100%), membrane and cytoplasm



Liver (3)	3/3 hepatocytes, membrane and cytoplasm 3/3 bile ducts, membrane and cytoplasm	Testis (3)	0/0
Lung (3)	3/3 alveolar bronchial epithelium cells (100%), membrane and cytoplasm	Thyroid (3)	3/3 epithelium (100%), membrane and cytoplasm
Muscle, cardiac (3)	0/3	Thymus (3)	Hassall's corpuscles - cortico reticular (100%), membrane and cytoplasm
Muscle, skeletal (3)	0/3	Tonsil (3)	3/3 epithelium (100%), membrane and cytoplasm
Nerve, peripheral (2)	2/2 nerve (60%), cytoplasm	Uterus (3)	3/3 epithelium (100%) membrane and cytoplasm
Ovary (3)	2/3 epithelium in primary follicles (<1-100%), membrane and cytoplasm		

Abnormal tissues: The antibody labeled 203/204 invasive ductal breast carcinoma, 5/49 invasive lobular breast carcinoma, 3/10 pleomorphic lobular carcinoma; 4/4 cases of tubulolobular breast carcinomas; and 5/ 9 invasive breast carcinomas, with uncertain classification between lobular and ductal type (1).