Sudan University of Science and Technology

College of Graduate Studies

Immunohistochemical Detection of CD10 among Sudanese Patients with Renal Tumors

الكشف النسيجي الكيميائي المناعي لسي دي 10 لدى مرضى أورام الكلي السودانين

A Dissertation Submitted for Partial Fulfillment of the Requirement of the M.Sc. Degree in Medical Laboratory Sciences (Histopathology and Cytology)

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الاية

بسم الله الرحمن الرحيم

قال الله تعالى:

﴿آمَنَ الرَّسُولُ بِمَا أُنْزِلَ إِلَيْهِ مِنْ رَبِّهِ وَالْمُؤْمِنُونَ كُلٌّ آمَنَ بِاللَّهِ وَمَلائِكَتِهِ وَكُتُبِهِ وَرُسُلِهِ لا نُفَرِّقُ بَيْنَ أَحَدٍ مِنْ رُسُلِهِ وَقَالُوا سَمِعْنَا وَأَطَعْنَا غُفْرَانَكَ رَبَّنَا وَإِلَيْكَ الْمَصِيرُ(285) لا يُكَلِّفُ اللَّهُ نَفْساً إِلَّا وُسْعَهَا لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ رَبَّنَا لا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا رَبَّنَا وَلا تَحْمِلْ عَلَيْنَا إِصْراً كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا رَبَّنَا وَلا تُحَمِّلْنَا مَا لا طَاقَةَ لَنَا بِهِ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا أَنْتَ مَوْلانَا فَانْصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ (286)﴾

صدق الله العظيم

سورة البقرة الآيات(285-286)

Dedication

I dedicate this work to ……..

Who gave me the meaning of the life my parent….

My sweet sister (sroo) and brothers….

My colleagues, my teachers, my friends…

Every one whom I respect and appreciate….

Acknowledgement

Firstly I am grateful to Allah for give me the knowledge, strength, patience to complete this work.

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Abstract

This is retrospective case control study conducted in Omdurman teaching hospital during the period from January 2017 to Augustus 2017. The study aimed to detect the expression of CD10 in renal tumors using immunohistochemistry.

Atotal of 30 fixed paraffin blocks previously diagnosed as renal tumors, 10 (33.3%) samples were benign tumor and 20(66.7%) samples were malignant tumor were selected for this study. One section of 3 microns was cut from each block and stained by immunohistochemistry (avidin Biotin technique) for CD10 detection. The data obtained were analyzed using SPSS computer program version 15.

The patients ages ranged between 11-87 years with mean age of 49 years, most of them 23(76.7%) were above 50 years and the remaining 7(23.3%) were under 50 years.

Regarding gender patient revealed that 19(63.3%) patients were males and 11(36.7%) patients were females.

CD10 was positive in 20(66.7%) samples divided as follow; 15(50%) malignant and 5(16.7%) benign, while 10(33.3%) samples were negative 5(16.7)malignant (16.7)benign, with insignificant correlation between CD10 expression and renal tumors p-value (0.183).

The study concluded that there is no relation between CD10 expression and histological dignosis of renal tumors.

المستخلص

أجريت هذه الدراسة التحليلية الاسترجاعية المقترنة بحالات ضابطة في مستشفي امدرمان التعليمي خلال الفتره من يناير 2017 الي اغسطس ’ 2017 هدفت الدراسة للكشف عن افراز السي دي 10 في تشخيص النسيجي لاورام الكلي.

جمع ثلاثون قالب شمعي لهذه الدراسة تم تشخيصها مسبقا كأورام كلى منها10 عينات ورم حميد و 20عينة ورم خبيث .قطع من كل قالب مقطع واحد بسمك 3 مايكرون وصبغت بطريقة الكشف النسيجي الكميائي المناعي باستخدام تقنية الافيدين بايوتين .وحللت البيانات باستخدام برنامج الحزمة الأحصائية للعلوم الاجتماعية اصدار.15

تراوحت اعمار المرضي بين 87-11سنة بمتوسط عمر 49 سنة ومعظم اعمار المرضي كانت فوق 50 عاما (76.7%)23 والبقية تحت50 عاما .(23.3%)7 اما توزيع جنس المرضي فكان (63.3%)19ذكورا "و (36.7%)11 اناثا."

ظهر الافراز الايجابي ل سي دي 10 في(66.7%)20 عينة وزعت كالاتي (50%)15 عينة ورم خبيث و(16.7%)5 عينات ورم حميد و(33.3%)10عينة سلبية تم تقسيمها كالاتي (16.5)5ورم خبيث و (16.7)5ورم حميد مع عدم وجود علاقة بين ال سي دي 10 واورام الكلى) القيمه الاحتمالية تساوي.(0.183)

خلصت الدراسة لعدم وجود علاقة ذات دلالة احصائية بين افراز السي دي 10 والتشخيص النسيجي لاورام الكلى.

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List of abbreviations

CD	Cluster of Differentiation
DPX	Distyrene Plasticizer Xylene
DAB	3,3-Diaminobenzidene Tetrahydrochloride
RCC	Renal Cell Carcinoma
ID	Immune Diffusion
SPSS	Statistical package foe Social sciences
AST	Aspartate AminoTransferase
ALT	Alanine AminoTransferase
HRP	Horus Reddish Peroxides
FNA	Fine Needle Aspiration

Chapter One

Introduction

Chapter One

1.Introduction

Renal tumors is a disease in which kidney cell change and grow out of control forming amass called a renal cortical tumor, a tumor can be benign or malignant. A malignant tumor is cancerous, meaning it can grow and spread to other parts of the body but benign tumor can grow but will not spread (Curti, *et al*. 2014). Almost all renal tumor first appear in lining of tiny tubes in the kidney this tumor is called renal cell carcinoma, there are several types of kidney cancer include renal cell carcinoma, transitional cell carcinoma, sarcoma of the kidney and wilms tumor is most common in children (Elbart, 2013).

Common signs and symptoms of renal cancer are amass in abdomen and blood in the urine, also be no signs or symptoms in early stage of disease, however factors that increase the risk of kidney cancer include smoking, obesity , kidney stone , high blood pressure and faulty genes(Singer, *et al.* 2013).

Tests and procedures used to diagnose kidney cancer include: Blood and urine tests. Tests of blood and urine may give doctor clues about what's causing of signs and symptoms. Imaging tests allow the doctor to visualize a kidney tumor or abnormality. Imaging tests might include an ultrasound, a computerized tomography (CT) scan or magnetic resonance imaging (MRI). Removing a sample of kidney tissue (biopsy). In rare cases, doctor may recommend a procedure to remove a small sample of cells from a suspicious area of kidney. The sample is tested in a lab to look for signs of cancer (Ahmed, *et al.* 2013).

Treatment for renal tumor depend on the type and stage of disease , surgery is the most common treatment as kidney cancer does not often

respond to chemotherapy and radiotherapy. Other treatment options include biological therapies and immunotherapy (Pellicer, *et al.* 2007).

CD10 is cell surface enzyme with neutral metalloendopeptidase activity, is also known as common acute lymphocytic leukemia antigen.

CD10 are often expressed by clear cell renal cell carcinomas, and may they may be useful markers to suggest a renal origin of carcinoma, positive in 75% of cases (Ozolek, 2005).

CD10 is a membrane-bound zinc metalloproteinase, is one of the studied markers which originally was used for diagnosis and classification of malignant lymphomas and leukemia's (Mechtersheimer and Moller, 1989).

The diagnostic utility of CD10 in different in non-hematopoietic lesions including breast, renal, liver and uterus has been reported (Lau, *et al*.2002 , Moritani, *et al.*2002).

The usefulness of CD10 marker in discrimination of different benign and malignant renal tumor demonstrated in some reports (Yegen, *et al.2009*).

CD10expression is useful in the diagnosis of follicular carcinoma ,follicular variant of papillary thyroid carcinoma and renal tumor (Tomod, *et al.* 2005).

1.2 Objectives:

1.2.1 General objective:

To detect the expression of CD10 in renal tumors among Sudanese patients.

1.2.2 Specific objectives:

- To detect CD10 expression in renal tumors by using immuno-histochemistry
- To correlate between CD10 expression and histopathological diagnosis renal tumors.

Chapter Two

Literature Review

Chapter Two

2. Literature Review

2.1 Scientific background:

Kidney tumors are tumors or growth on or in kidney, this growth can be benign or malignant. Renal cancer that originates in the lining of the proximal convoluted tubule, a part of the very small tubes in the kidney that transport primary urine. Renal cell carcinoma is the most common type of kidney cancer in adults, responsible for approximately 90–95% of cases (Curti, *et al*. 2014). Initial treatment is most commonly either partial or complete removal of the affected kidneys (Rini, *et al.* 2008).

Where the cancer has not metastasized or burrowed deeper into the tissues of the kidney, the 5-year survival rate is 65–90%, but this is lowered considerably when the cancer has spread. The body is remarkably good at hiding the symptoms and as a result people with RCC often have advanced disease by the time it is discovered (Abbas, 2004).The initial symptoms of renal tumors often include: blood in the urine (occurring in 40% of affected persons at the time they first seek medical attention), flank pain (40%), amass in the abdomen or flank (25%), weight loss (33%), fever (20%), high blood pressure (20%), night sweats and generally feeling unwell (Curti, *etal* 2014). When renal tumor metastasizes, it most commonly spreads to the lymph nodes, lungs,liver, adrenal glands, brain or bones. Immunotherapy and targeted therapy have improved the outlook for metastatic RCC (Singer, *et al.* 2013).

2.2 Renal tumors:

Renal tumors may be discovered on medical imaging incidentally (i.e. an incidentaloma), or may be present in patient as an abdominal pain, abdominal mass or kidney cyst, hematuria or manifest first in a

paraneoplastic syndrome that seems unrelated the kidney (Gill, *et al.* 2010) .

A CT scan is the first choice modality for workup of solid masses in the kidneys. Nevertheless, hemorrhagic cysts can resemble renal cell carcinomas on CT, but they are easily distinguished with Doppler ultrasonography (Doppler US) (Kirstoffer, *et al.2015).*

2.2.1 Benign tumors:

A non cancerous tumors of renal is growth that does not spread to other part of the body, are not usually life-threatening. They are many type of benign tumors as: renal oncocytoma is thought to arise from the intercalated cells of collecting ducts of the kidney. It represent 5% to 15% of surgically resected renal neoplasms. Ultrastructurally, the eosinophilic cells have numerous mitochondria (Coburn, *et al.* 2007). Cystic nephromam lined by a simple epithelium with a hobnail morphology. Angiomyolipoma are tumors consisting of perivascular epithelioid cells. Metanephric adenoma cytology description is small, well differentiated epithelial tubules with bland nuclei and psammoma bodies. Renal medullary fibroma they consist of bland spindle-shaped or stellate-shaped cell in loose stroma , renal tubules may be entrapped (Crumley, 2013).

2.2.2 Malignant tumors:

2.2.2.1 Renal cell carcinoma:

Renal cell carcinoma is the most common type of adult kidney cancer, making up about 85% of diagnoses. This type of cancer develops in the proximal renal tubules that make up the kidney's filtration system. There are thousands of these tiny filtration units in each kidney (Elhassan, 2015).

2.2.2.2 Urothelial carcinoma

This is also called transitional cell carcinoma. It accounts for 10% to 15% of the kidney cancers diagnosed in adults. Urothelial carcinoma begins in the area of the kidney where urine collects before moving to the bladder, called the renal pelvis. This type of kidney cancer is treated like bladder cancer because both types of cancer start in the same cells (Singer, *et al.* 2013).

2.2.2.3 Sarcoma:

Sarcoma of the kidney is rare. This type of cancer develops in the soft tissue of the kidney; the thin layer of connective tissue surrounding the kidney or surrounding fat. Sarcoma of the kidney is usually treated with surgery. However, sarcoma commonly comes back in the kidney area or spreads to other parts of the body. More surgery or chemotherapy may be recommended after the first surgery (Coburn, *et al.* 2007).

2.2.2.4 Wilms tumor:

Wilms tumor is most common in children and is treated differently from kidney cancer in adults. This type of tumor is more likely to be successfully treated with radiation therapy and chemotherapy than the other types of kidney cancer when combined with surgery. This has resulted in a different approach to treatment (Kroeger, 2013).

2.2.2.5 Lymphoma:

Lymphoma can enlarge both kidneys and is associated with enlarged lymph nodes, in other parts of the body, including the neck, chest, and abdominal cavity. In rare cases, kidney lymphoma can appear as a lone tumor mass in the kidney and may include enlarged regional lymph nodes. (Robert, 2003).

2.3 Etiology of renal cancer:

2.3.1 Lifestyle:

The greatest risk factors for renal cancer are lifestyle-related; smoking, obesity and hypertension have been estimated to account for up to 50% of cases (Kroeger, 2013). Occupational exposure to some chemicals (Steven, *et al.* 2011; Lane and Brian, 2013; Metz, 2013). Another suspected risk factor use of non-steroidal anti-inflammatory drugs. Finally, studies have found that women who have had a hysterectomy are at more than double the risk of developing RCC than those who have not. Moderate alcohol consumption, on the other hand, has been shown to have a protective effect. (Kroeger, 2013).

2.3.2 Genetics:

Hereditary factors have a minor impact on individual susceptibility with immediate relatives of people with renal tumor having a two to fourfold increased risk of developing the condition. (Tarone, 2009) Other genetically linked conditions also increase the risk of renal tumor, including hereditary papillary renal carcinoma, hereditary leiomyomatosis, Birt–Hogg–Dub syndrome, hyperparathyroidism-jaw tumor syndrome, familial papillary thyroid carcinoma, von Hippel–Lindau disease and sickle cell disease (Baldewijns, *et al.* 2008).

2.4 Pathophysiology of renal tumor:

The tumor arises from the cells of the proximal renal tubular epithelium. It is considered an adenocarcinoma. There are two subtypes: sporadic (that is, non-hereditary) and hereditary. Both such subtypes are associated with mutations in the short-arm of chromosome 3, with the implicated genes being either tumor suppressor genes (VHL and TSC) and oncogenes (like c-Met) (Herbert, 2005).

2.5 Diagnosis of renal tumor:

The first steps taken to diagnose this condition are consideration of the signs and symptoms, and a medical history to evaluate any risk factors. Based on the symptoms presented, a range of biochemical tests may also be considered as part of the screening process to provide sufficient quantitative analysis of any differences in electrolytes, renal and liver function, and blood clotting times (Lane and Brian, 2013). Upon physical examination, palpation of the abdomen may reveal the presence of a mass or an organ enlargement. Although this disease lacks characterization in the early stages of tumor development, considerations based on diverse clinical manifestations, as well as resistance to radiation and chemotherapy are important. The main diagnostic tools for detecting renal cell carcinoma are ultrasound, computed tomography (CT) scanning and magnetic resonance imaging (MRI) of the kidneys (Elizabeth, 2008).

2.5.1 Laboratory tests:

Laboratory tests are generally conducted when the patient presents with signs and symptoms that may be characteristic of kidney impairment. They are not primarily used to diagnose kidney cancer, due to its asymptomatic nature and are generally found incidentally during tests for other illnesses such as gallbladder disease. In other words, these cancers are not detected usually because they do not cause pain or discomfort when they are discovered. Laboratory analysis can provide an assessment on the overall health of the patient and can provide information in determining the staging and degree of metastasis to other parts of the body before treatment is given (Lane and Brian, 2013).

2.5.1.1 Urine analysis:

The presence of blood in urine is a common presumptive sign of renal cell carcinoma. Alternatively, urinalysis can test for sugar, protein and

bacteria which can also serve as indicators for cancer. A complete blood cell count can also provide additional information regarding the severity and spreading of the cancer. Complete blood cell count provides a quantified measure of the different cells in the whole blood sample from the patient. Such cells examined for in this test include red blood cells, white blood cells and platelets. A common sign of renal cell carcinoma is anemia (Crumley, *etal.* 2013).

2.5.1.2 Blood chemistry:

Blood chemistry tests are conducted if renal cell carcinoma is suspected as cancer has the potential to elevate levels of particular chemicals in blood. For example, liver enzymes are found to be at abnormally high levels (Hatzaras, *etal.* 2012). The staging of the cancer can also be determined by abnormal elevated levels of calcium, which suggests that the cancer may have metastasized to the bones, also assess the overall function of the kidneys can allow the doctor to decide upon further radiological tests (Nuttaya and Muttarak, 201).

2.5.1.3 Radiology:

The characteristic appearance of renal tumor is a solid renal lesion which disturbs the renal contour. However, the advances of diagnostic modalities are able to incidentally diagnose a great proportion of patients with renal lesions that may appear to be small in size and of benign state (Margaret, *etal.* 2011). Deciding on the benign or malignant nature of the renal mass on the basis of its localized size is an issue as renal cell carcinoma may also be specific imaging features. The main imaging tests performed in order to identify renal cell carcinoma are pelvic and abdominal CT scans, ultrasound tests of the kidneys , MRI scans, intravenous pyelogram or renal angiography (Rubin, 1993).

2.5.1.4 Histopathology of renal tumors:

Histology of kidney cancer the diagnostic material from renal tumors, are needle biopsies or operation specimens (nephrectomy or resections). When report on an operation specimen detailed information must be included, such as tumor size, whether the os cystic, solid, necrotic or with bleeding, and relation to normal renal tissue as well as resection borders. When the kidney with surrounding fat and adrenal gland are removed, the pathologist must also describe the tumor relation to the capsule (Gerota's fascia), adrenal gland, renal tissue, and lymph nodes if present. It is important for the pathologist to study many microscopic sections in order to correctly classify the tumorand determine whether it is benign or malignant (Elhassan, 2015).

2.6 Immunohistochemistry:

Immunohistochemistry the process of selectively imaging antigens in cell of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues (Miller, 2014).

2.7 Tumor marker:

A tumor marker is a biomarker found in blood, urine, or body tissues that can be elevated by the presence of one or more types of cancer. There are many different tumor markers, each indicative of a particular disease process, and they are used in oncology to help detect the presence of cancer. An elevated level of a tumor marker can indicate cancer; however, there can also be other causes of the elevation (false positive values). Tumor markers can be produced directly by the tumor or by non-tumor cells as a response to the presence of a tumor. Although mammography, ultrasonography, computed tomography, magnetic resonance imaging scans, and tumor marker assays help in the staging and treatment of the cancer, they are usually not definitive diagnostic

tests. The diagnosis is mostly confirmed by biopsy (Kilpatrick and lind, 2013).

2.8 CD10:

CD10, a membrane-bound zinc metalloproteinase, is one of the studied markers which originally was used for diagnosis and classification of malignant lymphomas and leukemia's (Mechtersheimer and Moller, 1989). Recently the diagnostic utility of CD10 in different in non-hematopoietic lesions including renal, breast, liver and uterus has been reported (Luuawa, *et al*. 2002; lau, *et al*.2002; Moritani, *et al.* 2002; McCluggage, *et al.* 2003).

Study of Ortiz-Rey (2006), who report that CD10 positive expression with renal malignant tumor especial clear cell carcinoma 75% of cases.

Chapter Three

Materials and Methods

Chapter Three

Materials and Methods

3.1 Materials:

A archived tissue blocks obtained from renal tumor samples previously diagnosed as renal tumors.

3.2 Methods:

3.2.1 Study design:

This is analytical retrospective hospital based case control study, aimed to detect the expression of CD10 in renal tumor tissues among Sudanese patients.

3.2.2 Study setting:

The study was conducted at Omdurman teaching hospital, during the period from January 2017 to Augustus 2017.

3.2.3 Sample size:

Thirty formalin fixed paraffin wax embedded tissue was used in this study as followed: 10 samples form benign renal tumors and 20 samples from malignant renal tumors.

Date collection:

Age and sex patient data was collected from file.

3.2.5 Sample processing:

3.2.5.1 Sectioning:

One section (3µm) was cut from each block using Rotary microtome (Leica RM 2125).

Sections was then floated on a floating water bath adjusted to 45°C. Finally clean coated glass slides (thermos) in addition to ordinary slides

were used to pick up the floated section and slides were left in a 60℃ for 2 hours.

3.2.6.2 Immunohistochemistry staining:

The immunehistochemistry procedure were done as follows , sections were deparaffinied in xylene, slides were rehydrated through a graded series of alcohol and were placed in running water. Section were put in antigen retrieval by using PT link. After slides reached the room temperature, slides were washed in phosphate buffer saline, pH 7.6 for 2 minutes. After that a circle made around the sections by using Dako pen (Dako Denmark A/S). The sections were covered by endogenous peroxidase activity were blocked with 3% hydrogen peroxidase and methanol for 10 min, and then slides were washed in phosphate buffer saline for 3min, then slides were incubated with 100µl of primary antibodies (CD10) for 20 min at room temperature the primary antibody for CD10 were ready to use, and then were washed in phosphate buffer saline for 3 min, binding of antibodies were detected by incubated for 20 minutes with horus reddish peroxidase (ready to use), then sections were washed in three changes of PBS, followed by adding 3, 3 diaminobenzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. Slides were washed in running water 3 min and then section were counter stained with haematoxylin for 1 min, washed in distilled water and left to air dry for 5min. Finally slides were cleared in xylene and mounted with a cover glass used DPX mounting media. For each run of staining, positive and negative control slides were also prepared. The positive control slide was contain the antigen under investigation and the negative control slide were prepared from the same tissue block, but was incubated with PBS instead of the

primary antibody. Each slide was evaluated with investigator then the results were confirmed by consultant histopathologist.

3.2.7 statistical analysis:

The data were analyzed used version 15.0 SPSS computer program frequencies, means and chi square test were calculated.

3.2.8 Ethical consideration:

The study was performed after taker permission to use tissue blocks from archive samples from the Omdurman teaching hospital administration.

Chapter Four

Results

Chapter Four

4. Results

4. Results:

Thirty samples were collected from patient pruriently diagnosis as renal tumor, from Omdurman teaching hospital, the patients ages ranged between 11-87 years with mean age 49 years, most of them 23(76.7%) were more than 50 years and the remaining 7(23.3%) were less than 50 years as shown in table (4.1).

The patient sex revealed that 11(36.7%) patients were female and 19(63.3%) patients were male as shown in table (4.2).

In this study, 10 (33.3%) samples were benign 20 (66.7%) samples were malignant as shown in table (4.3).

The results of CD10 were positive in 20(66.7%), while 10(33.3%) of cases are negative as shown in table (4.4).

Positive expression of CD10 is common among renal tumor 50% samples are malignant, 16.6% are benign, negative expression of CD10 among study population was 10/30 samples, with insignificant correlation between CD10 and renal cell carcinoma (P. value 0.183), (table4.6).

Table (4.1): Distribution of age groups among study population:

Age groups (years)	Frequency	Percent
≥50	7	23.3
<50	23	76.7
Total	30	100%

Table (4.2): Distribution of sex among study population:

Sex	Frequency	Percent
Male	19	63.3%
Female	11	36.7%
Total	30	100%

Table (4.3): Distribution of histopathological diagnosis among study samples:

Histopathological Diagnosis	Frequency	Percent
Malignant	20	66.7%
Benign	10	33.3%
Total	30	100%

Table (4.4): Expression of CD10 among study samples:

expression CD10	Frequency	Percent
Positive	20	66.7%
Negative	10	33.3%
Total	30	100%

Table (4.5): Relation between CD10 expression and renal tumor among study samples:

Histopathological diagnosis	Expression of CD10		Total	P. value
	positive	Negative		
Malignant	15(50%)	5(16.6%)	20 (66.6%)	0.183
Benign	5(16.7)	5(16.7%)	10 (33.4%)	
Total	20(66.7%)	10 (33.3%)	30 (100%)	

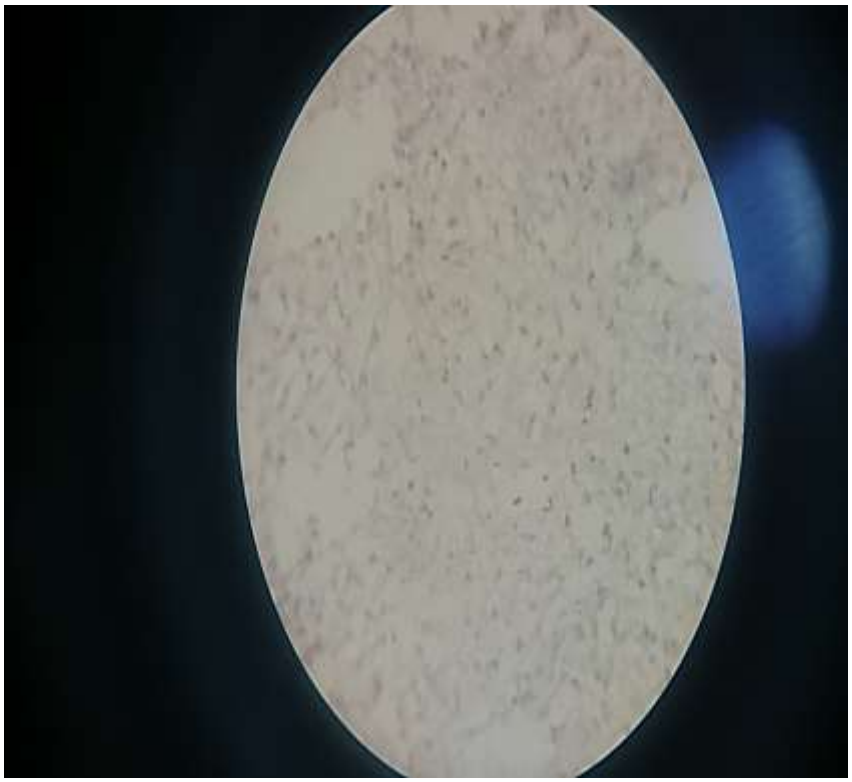

Fig.(4.1) Benign Tumor show negative expression of CD10

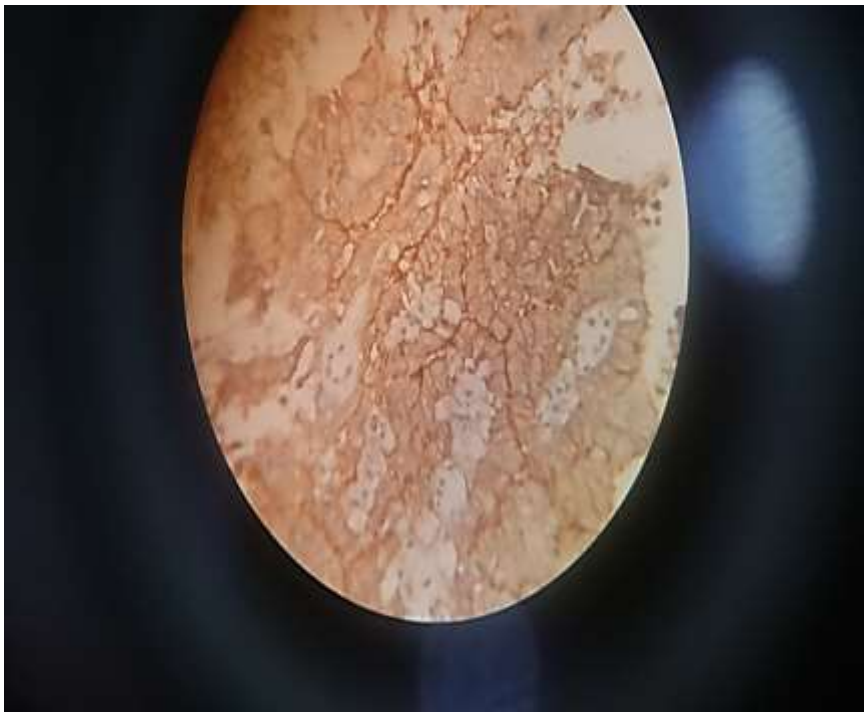

Fig (4.2) Malignant Tumor show positive expression of CD10

Chapter Five

Discussion

Chapter Five

5.Discussion

5.1 Discussion:

Thirty samples were collected from patient pruriently diagnosis as renal tumor, from Omdurman hospital, were used for immunohistochemical detection of CD10.

Regading the age of the study population the study revealed that most of patients were more than 50 years this result compatible with Bonifaz, *et al* (2014), they reported that number of patients was increased inagriculture or rural patients from 67-89 years more than other groups of age. Also Fahal, *etal* (2015) reported that 64% of renal tumors more than 57 years.

The study revealed that most of patients were men, with female: male ratio 1:2, the lower female: male ratio.this result agree with Bonifaz , *et al* (2014), they reported that renal tumors incidence and mortality with sex ratio 3:1 higher in male than in female. Also Fahal, *et al* (2015) reported that76% renal tumor cases is male.

The study found present positive of CD10 is common, with insignificant relation between CD10 and renal cell carcinoma (P. value 0.183), this result disagree with Avery, *et al* (2014) study which reported that CD10 is sensitive for renal tumors.

Chapter Six

Conclusion and Recommendation

Chapter Six
Conclusion and Recommendations

6.1 Conclusion:

On basis of this study we conclude the follow:

The age of the renal tumor patient in our study is commonly more than 50 years old.

The sex of the renal tumor in our study is commonly male .

There is no association between CD10 and histological diagnosis of renal tumors.

6.2 Recommendation:

On basis of this study we recommended the follow:

Further studies should be done with large sample size.

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Appendices

Appendices

Appendix (1)

Instrument and material:

Instrument:

Gloves.

Rotary microtome.

Microtome knife.

Coplin jars.

Oven.

Staining racks.

Water path.

Coated slides.

Dako pen.

Humidity chamber.

Cover glass.

Materials:

Ethyl alcohol (absolute, 90%, 70%, 50%).

Xylene.

Distell water.

Peroxidase blocker.

Primary antibody (CD10).

Secondary antibody (biotinylated secondary antibody).

3, 3 di amino benzidine tetra hydrochoride in substrate buffer.

DPX mounting media.

Phosphate (PH 7.4) component:

Solution A (0.2 M sodium di hydrogen orthophosphate, 3.12g disodium hydrogen orthophophate, 100 ml DW).

Solution B (2.1g citric acid, 100 ml DW) (72.7ml from solution A+22.8 ml from solution B).

Citrate buffer (PH 6.8) component:

Solution A (0.2 M sodium di hydrogen orthophosphate, 2.83 g disodium hydrogen orthophophate, 100 ml DW).

Solution B (2.1g citric acid, 100 ml DW) (72.7ml from solution A+22.8 ml from solution B).

Appendix (2)

ZYTOMED SYSTEMS

CE IVD

Mouse anti-CD10 (CALLA)

Cat. No.: BMS043 (16 ml Ready-to-use)

Instructions for use

Intended use
This antibody is designed for the specific localisation of CD10 in formalin-fixed, paraffin-embedded tissue sections. Anti-CD10 antibody is intended for in vitro diagnostic use.

Specifications

Specificity:	Human CD10
Immunogen:	Recombinant protein according to the external domain of human CD10
Clone:	56C6
Isotype:	Mouse IgG1
Species reactivity:	Human +, rat +, others not tested

Summary and Description
CD10 (CALLA, Common Acute Lymphoblastic Leukemia Antigen) is an integral Type II-membrane protein with a molecular weight of 100 kDa. CD10 was identified as a human membrane-associated neutral metalloendopeptidase. Synonyms are NEP, encephalokinase or neprilysin.
CD10 is present on precursor B-cells, a subset of precursor T-cells, mature granulocytes, approximately 75 % of B-ALL, a subset of T-ALL/T-LBL, and on all ALL-subtypes. Burkitt's lymphomas and myelomas stain positive for CD10, as well as some diffuse large-cell B-cell lymphomas and most follicular lymphomas. Few tumours of epithelial origin like carcinomas of the kidney, bladder, prostate, uterus and liver stain also positive.
MALT lymphomas and mantel cell lymphomas are negative for CD10.
CD10 is a frequently utilised marker for differential diagnosis of lymphomas but is also used for discrimination of e.g. hepatocellular carcinomas vs. liver metastases of other origins.

Reagent provided
Mouse monoclonal antibody in buffer with carrier protein and preservative for stabilisation in the following formats:
Ready-to-use 16 ml (Cat. No. BMS043)

Dilution of primary antibody
None

Storage and handling
The antibody should be stored at 2-8°C without further dilution.
If necessary, dilutions of the antibody should be done with a suitable antibody dilution buffer (e.g. ZUC025 from Zytomed Systems). The diluted antibody should be stored at 2-8°C after use. Stability of this working solution depends on various parameters and has to be confirmed by appropriate controls.
The antibody provided is suitable for use until the expiry date indicated on the label, if stored at 2-8°C. Do not use product after the expiry date. Positive and negative controls should be run simultaneously with all specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Zytomed Systems' technical support or your local distributor.

Precautions
Use through qualified personnel only. Wear protective clothing to avoid contact of reagents and specimens with eye, skin and mucous membranes. If reagents or specimens come in contact with sensitive area, wash with large amounts of water.
Microbial contamination of the reagent must be avoided, since otherwise non-specific staining may occur.
ProClin300 and sodium azide (NaN_3) are used for stabilisation. Reaction of sodium azide with lead or copper in drainage pipes can result in the formation of highly explosive metallic azides. Discard the antibody solution in a large volume of running water to avoid formation of deposits. A material safety data sheet (MSDS) for the pure substances is available upon request.