بسم الله الرحمن الرحيم

Shendi University

College of Higher Studies

Immunohistochemical Expressions of HER2\new among Sudanese Patients with Renal Cell Carcinomasin Khartoum States

A thesis submitted for the partial fulfilment of the requirements of M.Sc. degree in Medical Laboratory Sciences (Histopathology and Cytology)

By

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بسم الله الرحمن الرحيم

﴿قَالَ تَعَالَىٰ: أَعُوذُ بِٱللَّهِ مِنَ ٱلشَّيۡطَٰنِ ٱلرَّجِيمِ ﴾ ۞ إِنَّ ٱللَّهَ لَا يَسۡتَحۡيِۦٓ أَن يَضۡرِبَ مَثَلٗا مَّا بَعُوضَةٗ فَمَا فَوۡقَهَاۚ فَأَمَّا ٱلَّذِينَ ءَامَنُواْ فَيَعۡلَمُونَ أَنَّهُ ٱلۡحَقُّ مِن رَّبِّهِمۡۖ وَأَمَّا ٱلَّذِينَ كَفَرُواْ فَيَقُولُونَ مَاذَآ أَرَادَ ٱللَّهُ بِهَٰذَا مَثَلٗاۘ يُضِلُّ بِهِۦ كَثِيرٗا وَيَهۡدِي بِهِۦ كَثِيرٗاۚ وَمَا يُضِلُّ بِهِۦٓ إِلَّا ٱلۡفَٰسِقِينَ ﴿٢٦﴾ ﴾ البقرة: ٢٦

صدق الله العظيم

Dedication

To the one who taught me how to be available member in community?

Dear MY ……

Friend and dear colleagues

Finally to all of them here and there I dedicate to fruit of exertion with sincerity of love.

Nidal

Acknowledgment

All the thanks to Allah for the gift of life, health and faith and for the strength and desire that he gave me to continue this way, and look always for the best.

I am grateful to my supervisor Dr. Ibrahim Bakheit Yousif Elemam for his help, patience, care, invaluable support and for providing me with the materials that I need to carry out this study.

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List of contents

Chapter One

Chapter Two

Chapter Three

Chapter Four

Chapter Five

List of tables

List of figures

List of abbreviations

HER2……..…….human epidermal growth factor receptor 2

RCC……...…..……..Renal Cell Carcinoma

ccRCC…….....clear cell renal cell carcinoma
pRCC…….....Papillary renal cell carcinoma

chRCC……...……..Chromophobe renal cell carcinoma

IHC…….……..……...Immunohistochemistry

ISH .....…...In situ hybridization

PCR……..….Polymerase chine reaction

Abstract

BackgroundRenal cell carcinoma (RCC) is a kidney 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. RCC is the most common type of kidney cancer in adults, responsible for approximately 90–95% of cases.HER2 is a member of the epidermal growth factor receptor (EGFR) family with intrinsic protein tyrosine kinase activity and its increased activity is the assumed mechanism underlying cell transformation.

Materials and methods;this was observational case study conducted in Khartoum state-Sudanduring period from September 2017 to May 2018.An 80 paraffin-embedded tissue blocks with ovarian tumors were included in this study;were subjected to detection of HER2/neu antigenusingimmunohistochemistry (IHC) technique.

Result;HER2/neu antigen was detected in 2 cases (2.5%) with no statistically significant different, the P value was 0.222.There was relation between type of malignant tumortumor and mraker expression P.value was0.000.There are relation between gender and marker expression P.value was0.000.

Conclusions;There was no relationship between tumors type and HER2/neu expression.There was relationship between type of malignant tumor, gender and HER2/neu expression.

ملخص البحث

الخلفية : سرطان الخلايا الكلوية هو سرطان في الكلى ينشأ في بطانة الأنابيب الملتوية القريبة وهي جزء من الأنابيب الصغيرة جدًا في الكلية التي تنقل البول الأساسي . ار سي سي والنوع الأكثرشيوعًا من سرطان الكلى لدى البالغين، وهومسؤول عن حوالي 90-95٪ من الحالات هير2 نيو هو عضو في عائلة مستقبلات عامل نموالبشرة مع نشاط كيناز البروتين التيروزيني الداخلي وزيادة نشاطه هوالآلية المفترضة التي تقوم عليها تحول الخلية .

الطرق والمواد: اجريت هذه الدراسة المتعلقة بالمشاهدة والتي شملت مجموعة الحالة بولاية الخرطوم في الفترة من سبتمبر 2017 الي مايو 2018.اشتملت الدراسة على 80 عينية نسيجية منا لكلى في شمع البرافين جميع العينات شخصت على انها اورام كلوية.اخضعت هذه العينات للكشف عن انتجين(هير 2) عن طريق تقنية الصبغ المناعي.

النتائج : ظهر انتجين (هير 2) عينتان والتي تمثل نسبة 2.5% مع عدم وجود دلالة ذات قيمة احصائية وكانت القيمة الاحتمالية 0.222. ايضا اتضح انه هناك علاقة بين نوع الورم الخبيث وظهور الانتجين (هير2) وكانت القيمة الاحتمالية0.000.ايضا اتضح انه ليست هناك علاقة بين الجنس وظهور الانتجين) وكانت القيمة الاحتمالية 0.000.

الاستنتاج : لا توجد علاقة بين ظهور الانتجين (هير2) ونوع الورم توجد علاقة بين نوع الورم الخبيث والجنس وظهور الانتجين.

Chapter one

1. Introduction:

1.1. Background:

Renal cell carcinoma (RCC) is a kidney 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. RCC is the most common type of kidney cancer in adults, responsible for approximately 90–95% of cases (Kush Sachdeva; 2019). The five-year survival rate is 65–90% but this is lowered considerably when the cancer has spread (Brian Rini et al., 2008).

Histological diagnosis Renal cell carcinomas comprise a broad spectrum of histopathological entities described in 2004 4classifications and modified by ISUP Vancouver Classification (Eble et al 2004). There are three main RCC types: clear cell (ccRCC), papillary (pRCC - type I and II) and chromophobe (chRCC). RCC type classification has been confirmed by cytogenetic and genetic analyses (Srigley JR et al 2013).The renal tumors are discussed in Section of Histological diagnosis includes, besides RCC type, evaluation of nuclear grade, sarcomatoid features, vascular invasion, tumour necrosis, and invasion of the collecting system and prerenal fat. Fuhrman nuclear grade has been the most widely accepted grading system (Yang XJ et al; 2005). At the ISUP conference, a simplified, nuclear grading system, based only on size and shape of nucleoli, has been proposed which will replace the Fuhrman grading system (.Hemamali et al; 2014). The identification of tissue-based RCC biomarkers that can improve early tumor detection and predict patient prognosis and response to therapies is warranted. Despite long-standing efforts toward biomarker discovery and validation, no tissue biomarker is currently used in the clinical management of patients with kidney cancer. Many variables are involved in the various phases of RCC tissue biomarker analysis and the lack of standardization in many of the procedures involved in the

biomarker development process represents a major limitation for the advancement of the field. Herein, we review current issues in tissue-based biomarker research in kidney cancer, with particular emphasis on immunohistochemical assays.

The expression of candidate markers identified by proteomic, genomic, and gene expression profiling studies needs to be validated on tumor tissue by molecular pathology techniques such as immunohistochemical analysis. Immunohistochemical analysis allows one to not only detect the presence or absence of the antigen but also to localize it within cellular and subcellular compartments and to provide potential quantitative data. This is particularly important in kidney cancer, where tumors are characterized by substantial heterogeneity, not only among the different tumor subtypes but also within the same lesion. Immunohistochemical evaluation of cancer-specific marker expression in tissue microarrays (TMAs). Doctors know that kidney cancer begins when some kidney cells acquire mutations in their DNA. The mutations tell the cells to grow and divide rapidly. The accumulating abnormal cells form a tumor that can extend beyond the kidney. Some cells can break off and spread (metastasize) to distant parts of the body. Kidney cancer is a heterogeneous entity that not only comprises histologically and genetically different subtypes but also shows a significant heterogeneity within the same lesion. Detection and quantification of tissue biomarkers within different areas from the same lesion may be extremely important to clarify the molecular mechanisms underlying this phenotypic heterogeneity. To achieve this goal, it is crucial to use high-quality tissues, in which both morphology and biomarker integrity are guaranteed. Unfortunately, tissue acquisition, processing, and storage procedures have been shown to affect the quality of the specimen collected.

1.2. Justification:

Renal cell carcinoma (RCC) is one of the most common types of malignant tumor of the human urinary system. To date, the benefit of the HER2, is a member of the epidermal growth factor receptor (EGFR) family with intrinsic protein tyrosine kinase activity and its increased activity is the assumed mechanism underlying cell transformation. HER2 combines with the other EGFRs to form heterogeneous dimers and is involved in signal transduction, cell proliferation, development, differentiation, migration and tumor formation. Previous studies have reported that HER2-positive status was an independent predictor of poor prognosis in multivariate analysis. Herceptin, which is targeted against the HER2 cell-surface receptor, has been successfully used for the treatment of breast cancer. At present, there are conflicting reports concerning HER2 expression in RCC due to different laboratory conditions. Since it has been found that HER2 is expressed by the normal adult kidney, the presence of this oncoprotein in the normal kidney may affect the possibility of using HER2-targeted therapy for the treatment of RCCs overexpressing HER2.

1.3. Objectives:

1.3.1. General objective:

To study of HER2\new immunoexpression among Sudanese patient withrenal cell carcinoma

1.3.2. Specific objectives:

- To correlate the present of HER2\new in renal cell carcinoma among Sudanese patient.
- To correlate the gender with HER2\new in renal cell carcinoma among Sudanese patient.

Chapter tow

2. Literature review:

2.1 Epidemiology of renal cell carcinoma

2.1.1 Incidence and prevalence

Renal cell cancer (RCC) is the 9th most common cancer worldwide and approximately 337 860 new cases were diagnosed in 2012 (Jonasch, Gao, Rathmell 2014). The incidence varies geographically, being highest in developed countries (Jonasch, Gao, Rathmell 2014). During the last ten years the incidence of RCC has risen slightly, 1.7% in men and 0.6% in woman in Finland (Nordcan a). There were 534 new cases among men (incidence 10.3/100 000) and 401 among women (incidence 6.3/100 000) in Finland in 2013 (Nordcan b; Nordcan c). RCC was 9th in men (3.3% of all tumors) and 12th in women (2.6% of all tumors) cancer disease in Finland in 2013 (Nordcan d; Nordcan e). At the beginning of the year 2014 the prevalences were 3807 in men and 3359 in women in Finland (Nordcan f; Nordcan g). The five-year proportional survival were 63% in men and 63% in woman among patients with RCC diagnosed between 2005-2012 (Cancer.fi).

2.1.2 Etiology

Cigarette smoking is an established risk factor for RCC (Ljungberg et al. 2011). The relative risk is low but ever-smokers have a higher risk of RCC compared to never-smokers (HR 1.38, CI 95%= 1.27-1.50) and heavier smoking increases the risk even more (Hunt et al. 2005). Ten years after quitting smoking the risk of RCC seems to be reduced to the similar level than in non-smokers (Hunt et al. 2005; Parker et al. 2003). High BMI (body mass index) has been established in several studies as a risk factor for RCC (Renehan et al. 2008). Every 5 kg/m2 increase in BMI elevates the risk 1.24 in men and 1.34 in women (Renehan et al. 2008). On the other hand, RCC patients with normal or excess body mass at the time of

diagnosis had better survival compared to patients with low BMI (Sunela, Kataja, Kellokumpu-Lehtinen 2013). Two large prospective studies have shown elevated blood pressure or use of antihypertensive drugs to be associated with the risk of RCC (Chow et al. 2000; Weikert et al. 2008). Use of acetaminophen and non aspirin non-steroidal anti-inflammatory have also associated with risk of RCC (Choueiri, Je, Cho 2014). Patients with end-stage renal disease (ESRD) or on long-term hemodialysis have a risk of acquired renal cystic disease (ARCD). ARCD patients have been shown to have a three to six times risk of RCC (Ljungberg et al. 2011). Diabetes mellitus (DM) type 2 increases the risk of many cancers but has not been demonstrated an association with RCC (Zucchetto et al. 2007). The incidence of RCC varies substantially in worldwide and this is suggested to be caused by lifestyle, especially diet (Ljungberg et al. 2011). Both men's and women's daily intakes of fat and protein areassociated with RCC but the type of fat and its linkage to the condition has yielded conflicting result in the literature (Ljungberg et al. 2011). Vegetable and fruit consumption has been shown to reduce the risk of RCC (Lee et al. 2009). Occupational exposure to different chemical substances may involve a potential risk of carcinogenesis. Exposure to trichloroethylene (TCE), lead, glass fibers, wool fibers, brick dust, blast-furnace or working coke-oven, iron or steel industry have shown associations with an increased incidence of RCC (Boffetta et al. 2011; Moore et al. 2010; Sim et al. 2009).

2.2 Diagnostic of renal cell carcinoma

The first possible renal cell tumor was reported by Daniel Sennert in 1613, but the first unequivocal case report of RCC published by Miril in 1810 (Bhatt and Finelli 2014). Nowadays radiological examinations such as abdominal ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI) are performed

increasingly. Over 50% of RCC cases are currently detected incidentally and the proportion of small tumors has increased significantly (Escudier et al. 2014). The classical clinical triad of RCC, flank pain, palpable abdominal mass and gross hematuria, has decreased due to increased radiological imaging (Sunela et al. 2014), but is still present in some patients. Metastatic RCC may cause paraneoplastic syndromes such as unexplained fever, erythrocytosis or wasting syndromes, and in addition bone pain or lung nodules may occur (Escudier et al. 2014). Laboratory tests such as serum creatinine, haemoglobin, leucocyte and platelet counts, lactate dehydrogenase (LD) and serum-corrected calcium should be made, if RCC is suspected (Escudier et al. 2014). Some of these laboratory tests correlate with survival and are used for risk assessment (Escudier et al. 2014). According recommendations, initial radiological examination for diagnosing RCC is US and CT is performed for assessment of local invasiveness, lymph node involvement or other metastases. MRI may be used when intravenous contrast medium is contraindicated. It is recognized to be superior to CT in detecting local advancements and venous involvements of tumor thrombus (Escudier et al. 2014). Abdominal and chest CT or MRI should be done for accurate staging of RCC. Bone scan or brain CT are not recommended unless the patient has clinical symptoms. PET-CT could be useful in detecting extra-renal metastasis rather than renal lesion (Wang et al. 2012).

2.3 Classification of renal cell carcinomas

2.3.1 Histopathology and grading

The first classification of renal tumors was published as early as 1826 by Koenig (Bhatt and Finelli 2014). In the past decade it has been realized that all RCCs are not related and it is no longer reasonable to place these tumor types in the same

category (Jonasch, Gao, Rathmell 2014). RCC is not a single disease, but rather many cancers occurring in the kidney. In 1997 the Heidelberg classification of RCC was issued by the International Union against Cancer (UICC) and the American Joint Committee on Cancer (AJCC). This classifies RCC into five groups according to histological features and genetic alterations, as described below (Kovacs et al. 1997). In the International Society of Urological Pathology (ISUP) 2012 Concensus Conference additional recommendations were made (Delahunt et al. 2013). According to it, morphotypes of RCC have prognostic significance, e.g. papillary types 1 or 2 and tumor necrosis are of prognostic significance, and in addition a chromophobe RCC should not be graded. In our study, the histology of RCCs was classified according to the Heidelberg classification and the different types of RCC are described below (Kovacs et al. 1997). Classification is based on morphological characteristics. Genetic alterations are linked to morphological features, but not normally available in clinical practice.

2.3.1.1. Clear cell renal carcinoma (ccRCC)

Is most frequent subtype of RCC, accounting 75% of cases. Tumor cells have a predominantly clear cytoplasm. Cells grow in solid, trabecular, tubular and cystic patterns, and additionally focal papillary growth may be seen. These tumors evince a highly specific deletion of chromosome 3p and mutation of the VHL gene is typical. In addition, deletion of chromosome arms 6q, 8p, 9p and 14q and duplication of chromosome band 5q22 may be typically observed.

2.3.1.2.Papillary renal cell carcinoma (pRCC)

Is the second most common type of RCC, compromising 10% of cases in surgical series.pRCC cells may be small with scanty cytoplasm, but may also have abundant cytoplasm with basophilic, eosinophilic or pale staining incidence. A

papillary growth pattern predominates, though solid and tubulopapillary architecture have also been seen. Characteristic alternations are a trisomy of 3q, 7, 8, 12, 16, 17 and 20 and a loss of the Y chromosome.

2.3.1.3. Chromophobe renal cell carcinoma (chRCC)

Comprises 5% of all RCCs. Cells have pale and eosinophilic granular cytoplasm and cytoplasmic microvesicles can be seen in the electronic microscope. Genetically, loss of heterozygosity at chromosome 1, 2, 6, 10, 13, 17 and 21 and hypoploid DNA content are characteristic of this variant.

2.3.1.4 Collecting duct carcinoma

Accounts for approximately one per cent of RCCs. It has characteristically an atypical epithelium, sometimes with hobnail appearance and irregular channel lines. The channels are located in an inflamed stroma and focal mucin can be seen. Genetic abnormalities have not observed in the collecting duct type of RCC.

2.3.1.5.Unclassified renal cell carcinoma

Constitutes a diagnostic category which other RCC type criteria do not readily identify. To this category belong 4-5% of RCCs.

2.3.2. RCC grading:

The histological differentiation of RCC is classified according to the Fuhrman system to four categories (grades 1-4) (Fuhrman, Lasky, Limas 1982). The classification is based on tumor cell nuclear size, irregularity and prominence (Delahunt et al. 2013),

2.3.3. TNM classification and staging

The TNM classification (TNM) by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) is the most commonly used staging system for RCC. It contains three components: T (tumor) describes the size of the primary tumor and the extent of invasion; N (Node) the status of metastasis in regional lymph nodes and M (metastasis) indicates the status of metastasis or absence of it. The first TNM system for RCC dates from 1974 (Eggener 2010) and the 10th edition was last revised in 2010 (Sobin LH, Gorpodarowich MK, Wittekind CH 2009). The tumors in our study are classified according to TNM 2002 Classification of Malignant tumor (Sobin LH 2002), shown in Table 2. The anatomic stage (I-IV) of RCC is obtained from TNM information and it is used for RCC patient prognosis as described in Table 3. RCC patients with stage I disease have a fiveyear disease-specific survival of about 80-95% and patients with stage II have some 80 % (Elmore et al. 2003). If stage I-II RCC patients have invasions of the urinary collecting system, the five-year survival is decreases to 60% compared with over 90% without invasion (Verhoest et al. 2009). Patients with stage III disease have a five-year cancer-specific survival (CSS) of around 60%. Before the use of new targeted agents, RCC patients with stage IV disease had a five-year CSS of only 10% with a median overall survival (OS) of 10-15 months (Jonasch, Gao, Rathmell 2014). However, median OS has extended beyond two years since targeted agents have been available for the treatment of RCC (Jonasch, Gao, Rathmell 2014).

2.4 Treatment of renal cell carcinoma

2.4.1 Surgical treatment

The first recorded nephrectomy was performed in 1862, in fact accidentally as in the surgery Wolcott assumed the mass be hepatic (Bhatt and Finelli 2014). Gustav Simon performed the first planned successful nephrectomy in Heidelberg in 1869 (Bhatt and Finelli 2014). At present surgery continues to play an important role in RCC treatment, and it is the only effective treatment for localized RCC. Nephrectomy can be performed with either radical or partial techniques (Escudier et al. 2014).

2.4.2 Cryotherapy

For patients with a small (<3cm) cortical tumor, especially those with high surgical risk, solitary kidney, compromised renal function, hereditary RCC or multiple bilateral tumor radiofrequency or cryoablative treatments could be considered (Escudier et al. 2014). Cryoablation treatment causes direct cell damage by an argon gas based system and was first described in humans for renal cell cancer in 1995 (Bhatt and Finelli 2014; Uchida et al. 1995). These treatments have low recurrence rates and excellent cancerspecific survival (CSS) (Psutka et al. 2013).

2.5 Prognostic factors

At present, evaluation of RCC patients' prognosis is based on clinical factors such as tumor size and biological factors such as laboratory parameters and their combinations (Leibovich et al. 2003; Ljungberg et al. 2010; Patard et al. 2004). Several molecular markers have also been studied, but none has achieved status as

an independent prognostic factor and they are therefore not recommended in routine practice (Ljungberg et al. 2010).

2.5.1 Stage, grade and histology

The most important and powerful prognostic factor continues to be clinical classification (Stage I-IV) according to the TNM system in RCC (Delahunt et al. 2002; Fergany, Hafez, Novick 2000; Mejean, Oudard, Thiounn 2003; Zisman et al. 2002). Tumor stage represents the size and spreading of a tumor. Fuhrman and associates present a grading system for cell histological differentiation according to nuclear features and presence of nuclear pleomorphism, used since 1982 (Fuhrman, Lasky, Limas 1982). The system is widely accepted as an independent prognostic factor (Ljungberg et al. 2010; Mejean, Oudard, Thiounn 2003), although several problems have been identified. For example, nuclear prominence is more subjective and nuclear pleomorphism is poorly defined (Cheville et al. 2003). The prognostic value of this grading system for pRCC and chRCC has been debated, but it has been shown to constitute an independent prognostic factor for ccRCC in many studies (Cheville et al. 2003; Kallio et al. 2004; Klatte et al. 2010).

2.6.HER2/new

HER2, or ErbB-2, is a member of the epidermal growth factor receptor (EGFR) family with intrinsic protein tyrosine kinase activity and its increased activity is the assumed mechanism underlying cell transformation (KlapperLN,etal;1999).HER2 combines with the other EGFRs to form heterogeneous dimers and is involved in signal transduction, cell proliferation, development, differentiation, migration and tumor formation (Reese DM, Slamon DJ;1997).

2.7. HER2/new and renal cell carcinoma

Previous studies have reported that HER2-positive status was an independent predictor of poor prognosis in multivariate analysis.Herceptin, which is targeted against the HER2 cell-surface receptor, has been successfully used for the treatment of breast cancer. At present, there are conflicting reports concerning HER2 expression in RCC due to different laboratory conditions, case groups or the ethnicity of patients. In the present study, we evaluated the HER2 status of 42 RCC tumor and normal tissue specimens using immunohistochemistry (IHC) and 6 specimens using western blotting. Unlike the overexpression observed in breast cancer, IHC showed that HER2 is commonly expressed in normal renal, rather than RCC tissues. Since it has been found that HER2 is expressed by the normal adult kidney, the presence of this oncoprotein in the normal kidney may affect the possibility of using HER2-targeted therapy for the treatment of RCCs overexpressing HER2. The present study represents the rationale of the analysis(Revillion F, et al;1998)

2.8.Immunohistochemistry

Immunohistochemistry (IHC) is a technique for identifying cellular or tissue constituents (antigens) by means of antigen-antibody interactions, the site of antibody binding being identified either by direct labeling of the antibody or by use a secondary labeling method. The recent introduction of prognostic and predictive markers in IHC has made a tremendous impact on patient treatment and management. Immunohistochemistry is widely employed in establishing diagnosis, predicting prognosis and response to therapy and in the study of disease pathogenesis. (Bancroft and Gamble, 2008).

Chapter three

3. Materials and methods:

3.1. Study design:

This was observational case control study.

3.2. Study duration:

This study was carried in Sept 2017 – May 2018 .

3.3. Study area:

Samples involved in this study were collected from Alrahma Medical Centre in Khartoum State-Sudan.

3.4. Study population:

Populations subjected in this study were paraffin tissue blocks already with renal cell carcinoma.

3.5. Inclusion and exclusion criteria:

All samples are from Sudanese people from different states in Sudan. Age and race were not taken as exclusion criteria in this study.

3.6. Study sample:

Samples used in this study were renal cell carcinomas.

3.7. Sample size:

Sample size was 40 samples with renal cell carcinoma as case group and 40 control group (Normal Renal Cell).

3.8. Sampling technique:

Convenient sampling technique was used to collect samples.

3.9. Data collection tools and variables:

Master sheets were used to record all patients and sample data; age, presenting symptom, type of RCC. Master sheets were also used to record all IHC results.

3.10. Sample processing:

One section from each block measures four micrometers was cut using Leica microtome (Leica Microsystems, Nussioch Gmbll, model: RM 2125RT, ser NO. 8843/04-2005-China) and then stained in H&E to confirm diagnosis of each block. Then other one sections was cut from each recruited block using the same microtome, (measuring four microns) then floated in 70% ethanol and water bath (Electrothermal ser NO.18861434-China) at $40c^0$, consecutively. Each floated section was mounted on positive charge immune slide (Thermo Scientific- Italy) to detect immune expression of HERnew gene in each sample. All slides contained sections were dried in dry oven (WTC binder 7200 TUTTLINGEN, B28, NO.88485-USA) at $60c^0$ for 30 minutes.

3.11. Methods of detection:

Paraffin wax sections were detected using immunohistochemistry techniques. For Ab-3 (Clone K1H8) mouse monoclonal antibody biomarker was used to detect presence of HERnew gene and the antibody used was specific to our gene and does not cross react with other gene. The used biomarkers come from Thermo Scientific (Italy).

3.11.1. Hematoxylin and Eosin:

the sections were taken to water (dewaxed in xylene 4 min and rehydrated in ethyl alcohol 100% 4 min, 90% 2 min, 70% 2 min, and water 2 min) , then the nucleus was stained by Harris hematoxylin for 5 min, washed in running tap water until become blue, after that differentiated in 1% acid alcohol (1% acetic acid in 70% ethyl alcohol) for 5-10 sec. washed in running tap water for several minutes 7-10 (bluing), and the cytoplasm was stained in 1% eosin Y for 5 min, Washed in running tap water for 1–5minutes, finally Dehydrated through alcohols, cleared, and mounted in mixture of distyrene a plasticizer and xylene (DPX).

3.11.2. Immunohistochemistry:

Sections for IHC technique were stained and diagnosed in histopathology lab at alrhma lab. Paraffin sections from the samples were deparaffinized in two changes of xylene for 10 minutes in each change, then rehydrated in descending changes of ethanol as follows; sections were placed in two changes of absolute ethanol for 5 minutes in each change and then were placed in 90% ethanol for 3 minutes, and then placed in 70% ethanol for 2 minutes, then washed in distilled water for 2 minutes and washed two times in buffer. After rehydration antigens were retrieved in preheated water bath at 95oc in plastic coplinjar contained 1ml Target Retrieval. After antigen retrieval, slides were washed in Phosphate Buffer Saline (PBS) of pH 7.4 for 3-5 minutes, then endogenous peroxidase activity was blocked in hydrogen peroxide block for 10-15 minutes, then slides were washed in PBS for 3 minutes, then all slides were drained for a few seconds and wiped around the sections with tissue paper and encircled round the tissue using cytomation pen, then specific primary antibody to her2/new was applied to each section for 30 minutes, then slides were washed in PBS 3 minutes, then the second layer antibody biotinylated goat-anti-mouse/rabbit immunoglobulins was placed on each section for 30 minutes at room temperature, the slides then were washed in PBS for 10 minutes, the third and final antibody layer, Streptomyces Avidin Biotin Complex-Horse Radish Peroxidase (StABC/HRP) was placed on each section for 30 minutes at room temperature, the slides then were washed in PBS for 10 minutes. After that 1 drop from 3.3 diaminobenzidine tetrahydrochloride (DAB) Plus Chromogen added to 1 ml of DAB plus substrate, mixed and applied to tissue for 10 minutes, then all slides were rinsed in running tap water (RTW) for 5 minutes, counter stained in Mayer's Hematoxylin for 1 minute, blued in RTW for 10 minutes, dehydrated in absolute ethanol, cleared in xylene and mounted in DPX.

3.12. Control:

For each run of staining, positive and negative control slides were also prepared. The positive control slides contain the antigen under investigation and the negative control slides were prepared from the same tissue block, but Incubated with PBS instead of the primary antibody.

3.13. Statistical analysis:

Statistical analysis was carried out with the statically software package standard (SPSS) version16. The association of Her2/New protein expression with the urinary bladder transitional cell carcinoma was assessed by Pearson chi-square test. Statistical significance was reached if a $p < 0.05$ was obtained.

Chapter four

4. The Result

Figure 4.1: Frequency of diagnosis among study population

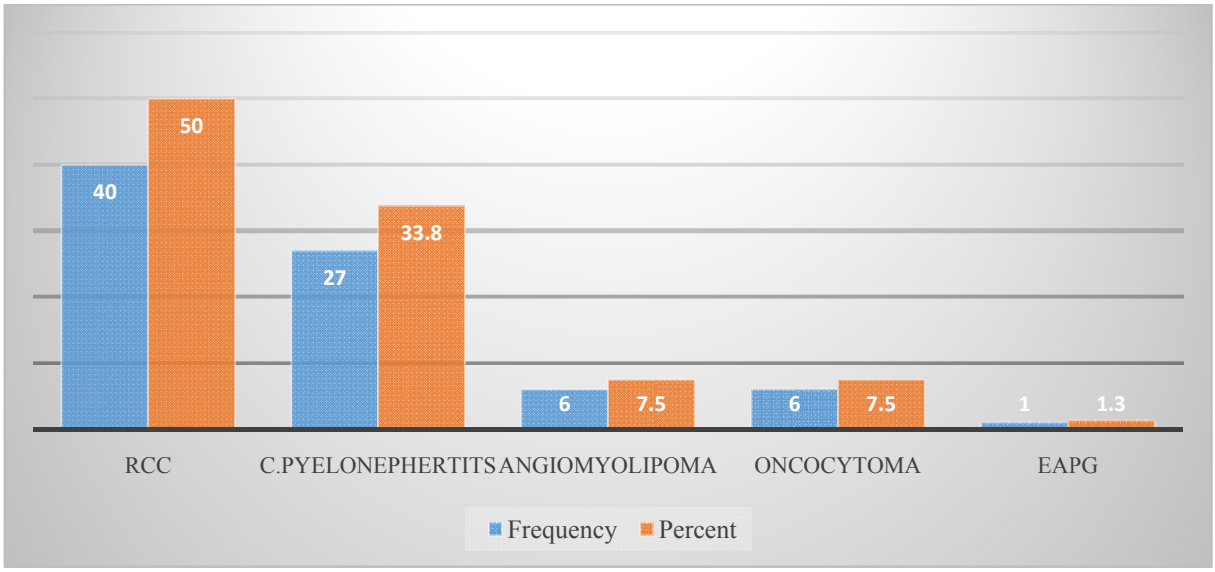
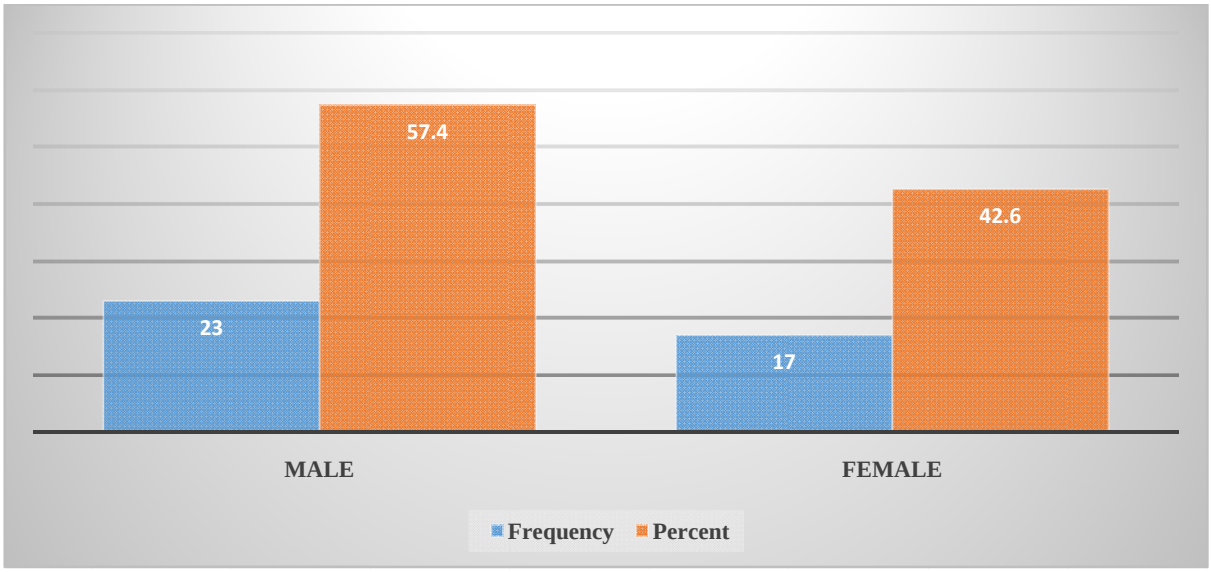


Figure.4.2: Frequency of gender among case group



Figure;4.3: Frequency of age group among case group

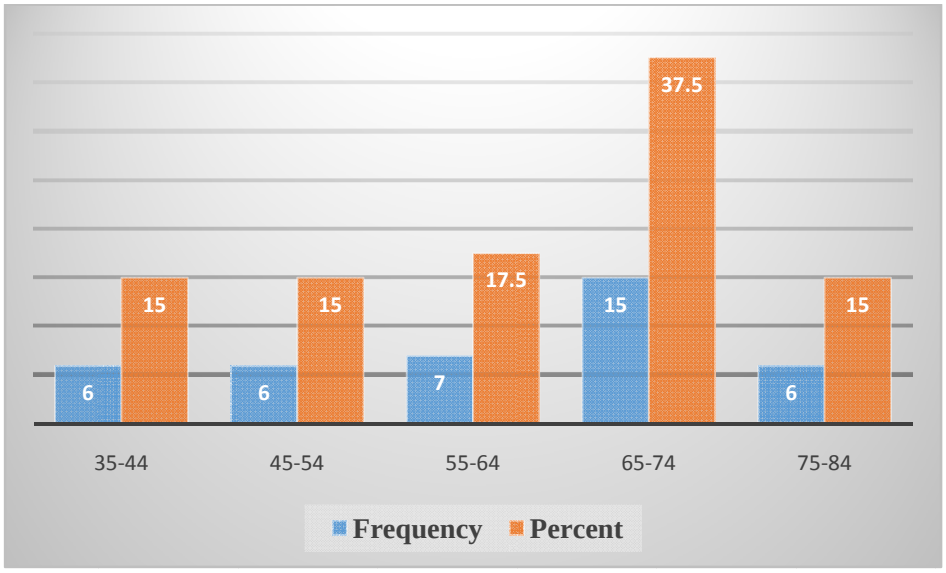


Figure: 4.5: Frequency of marker expression among study population

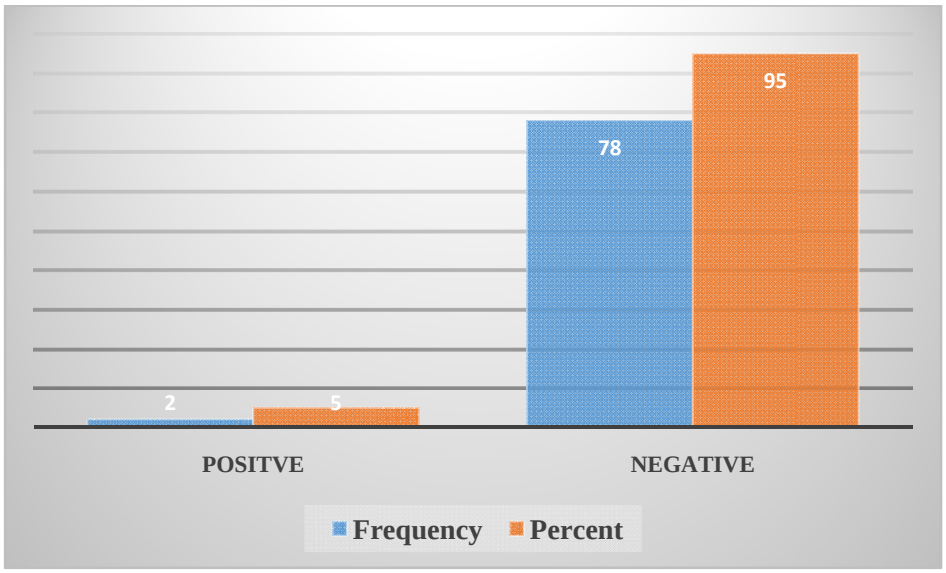


Table 4:1: Comparison between type of tumor and marker expression

Variables	Expression		Total	P.value
	Positive	Negative		
Malignant	2	38	40	0.222
Benign	0	40	40	
Total	2	78	80	

Table: 4.2: Correlation between type of malignant tumor and marker expression

Variables	Expression		Total	P.value
	Positive	Negative		
Clear cell type	1	22	23	0.000
Papillary type	1	13	13	
TCC	0	3	3	
Total	2	38	40	

Chapter Five

5. Discussion

This was a retrospective study and all information on patients was collected from patient records. The survival data were obtained from the Alrahma Medical Center.This study involved all patients, whose RCC was diagnosed.

Regarding association between type of tumor and marker expression our study summarized that; there are no relationship (P.value 0.222) this result was agree with study conduct byHUILI WANG, et al in China on 2012 which concluded that there was a negative correlation between the HER2 expression in normal tissue and that of RCC (P=0.007, r=−0.410).Also our result agreement with study conduct by Latif Z et al in U k on 2002 which concluded thatER2 amplification is absent and protein over-expression uncommon in RCC.

Regarding association between type of malignant tumor and marker expression our study summarized that; there are relationship (P.value 0.000)this result was disagree with study conduct byS. Spasova et al inBulgaria on 2015 which concluded thatThere was no significant association between HER2 overexpression and tumor stage, grade and the type of tumor this disagreement may be due variation in sample size and The genetic makeup of the community.

Regarding association between gender and marker expression our study finding there are relationship between gender and marker expression (P.value 0.000)regarding this result there was no publish data in the same point.

Regarding association between age of patients and marker expression our study finding there are no relationship between age of patients and marker expression (P.vlue (0.407).Also there was no publish data in the same point.

5.2. Conclusion

No relation between Her 2 New Expression & Renal Tumor

5.3. Recommendations

On the base of the obtained results we recommended that;

- ❖ Further study should be conducted in renal tumors using larger sample size.
- ❖ For future study, advance techniques like polymerase chain reaction (PCR) and In situ hybridization (ISH) should be conducted to detect the exact role of Her 2 in renal tumor.

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