Sudan University of Science and Technology

College of Graduate Studies

Immunohistochemical Detection of CK5/6 in Bladder Tumors among Sudanese Patients

الكشف النسيجى الكيميائى المناعى عن السايتوكيرتين5\6 فى اورام المثانه عند المرضى السودانيين

A dissertation submitted in partial fulfillment for the requirements of M.Sc Degree in Medical Laboratory Science (Histopathology and Cytology)

By:

Linda Omer Altayb Elhag

B.Sc in Medical Laboratory Science (Histopathology and Cytology)

(University of Science and Technology 2010)

Supervisor:

Dr. Abu Elgasim Abass Awad Elkareem

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الآيـة

بسم الله الرحمن الرحيم

قال تعالي :

لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا ۚ لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ ۗ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا ۚ رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إِصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا ۚ رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ ۖ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا ۚ أَنْتَ مَوْلَانَا فَانْصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ ﴿٢٨٦﴾

صدق الله العظيم

سورة البقرة الآية 286

Dedication

To my mother

To my husband

To my sister

To my son

To my family

To all my teachers

To all my colleagues and friends

With love and respect.

Acknowledgment

Im gratefull to Allah for the care, insight,peaceful and pity in my life. I would like to express my profound thanks to my supervisor, Dr. Abu Elgasim Abass, for his patience, guidance, unlimited assistance, encouragement and sustained interest throughout the course of this work.

I wish to extend my warmest thanks to the staff of the histopathology and cytology department, college of medical laboratory science, Sudan university of science and technology for their continuous support and encouragement.

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Abstract

This is an analytical retrospective case control hospital based study conducted in Ibn Sena hospital in Khartoum state, during the period from April to August 2016. The study aimed to detect CK5/6 expression in bladder tumors using immunohistochemistry.

Forty paraffin embedded blocks previously diagnosed as bladder tumors were collected. Samples include 30(75%) malignant tumors, (including transitional cell carcinoma 28(70%), adenocarcinoma 2(5%) samples) and 10(25%) bengin samples.

The patient's age ranged between 49 and 70 years with mean age of 59 years, most patients were more than 55 years representing 22(55 %) and the remaining 18(45%) patients were less than 55 years.

One section of 3micrometer thickness was cut from each paraffin block by rotary microtome and stained by immunohistochemical method (indirect streptoavidin-biotin immunoperoxidase technique) for detection of CK5/6. Data collected from patients files and results obtained were analyzed using SPSS computer program.

Immunohistochemical expression of CK5/6 was revealed positive result in 20/30 samples and negative result in 10/30 samples in malignant tumors, while all benign tumors gave positive result for CK5/6 (10/10), with significant statistical association between CK5/6 expression and histopathology diagnosis (P =0.035).

This study concludes that CK5/6 expression is associated with bengin forms of bladder tumors.

الخلاصة

أجريت هذه الدراسة التحليلية التراجعية المستشفويه الحالة و الحالة الضابطة فى مستشفى إبن سيناء فى ولاية الخرطوم خلال الفتره من ابريل الى اغسطس 2016 . هدفت الدراسة للكشف عن سايتوكراتين 6/5 فى اورام المثانه باستخدام كيمياء الانسجه المناعية.

جمعت أربعون عينة مطمورة بشمع البارفين من عينات مرضى تم تشخيصهم مسبقا بأورام المثانه. تتكون العينات من 30 (75%) عينة لآورام خبيثة , تضمنت سرطان الخلايا الانتقالية 28(70%) عينة, سرطان الخلايا الغديه 2(5%)عينة و 10(25%)عينات لآورام حميدة.

تراوحت اعمار المرضى بين 49- 70 سنة ومتوسط العمر 59 سنة ,أغلب المرضى 22(55%) كانت أعمارهم اكثر من 55 سنة وبقية المرضى 18(45%) كانت اعمارهم اقل من 55 سنة.

تم قطع مقطع واحد من كل عينة بسمك 3µm بواسطة جهاز المشراح الدوار. تم صبغ العينات بواسطة كيمياء الانسجة المناعية (طريقة تقنية البيوتين المناعى غير المباشره) للكشف عن سايتوكيرتين5\6. تم جمع البيانات من ملفات المرضى .تم أستخدام برنامج الحزمة الاحصائية للعلوم الاجتماعية SPSS لتحليل البيانات.

أظهرت الدراسة عن التعبير المناعى للواسمة سايتوكيرتين5\6 انها موجبة الظهور فى 30/20 وسالبة الظهور فى 30/10 عينة من عينات الاورام الخبيثة بينما كل عينات الأورام الحميدة اظهرتنتائج موجبه ل CK5/6 مع وجود علاقة احصائية بين CK5/6 ونوع الورم (القيمةالاحتماليه=0.035) .

خلصت الدراسة الى ان افراز السايتوكيرتين5\6 يكون دائما اكثر فى الاورام الحميدة.

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CHAPTER ONE

INTRODUCTION

Chapter One
Introduction

1.1 Introduction:

Bladder cancer is a disease in which malignant cells grow in the tissues of the bladder. When bladder cancer is found at an early stage, it is treatable. Many bladder cancers are found at this stage. However, bladder cancer often returns, even when it is found at an early stage (Bridget and Julie,2014).

Bladder cancer (BC) is the 11th most commonly diagnosed cancer in the world. Incidence rate (per 100,000 person-years) is 8.9 for men and 2.2 for women. Worldwide, BC is the 14th leading cause of cancer deaths, age-standardised mortality rate (per100.000 person-years) was 3.3 for men versus 0.9 for women in 2008. BC incidence and mortality rates vary across the countries due to differences in risk factors (Marko, *et al.* 2015).

Risk factors are age, cigarette smoking, excessive use of certain pain medications, treatment with alkylating agent chemotherapy drugs, family history of bladder cancer, exposure to hair dye, urologic conditions such as urinary tract infections and urinary stasis, infection with schistosoma, dietary factors-a diet low in fruits and vegetables, exposure to arsenic in drinking water (Adami, *et al.* 2002).

Bladder cancer is examined by physical examination and medical history, cystoscopy, bladder biopsy**,** urine cytology, FISH andimaging tests(Bridget and Julie,2014).

Bladder cancer is treated by surgery, immunotherapy, chemotherapy and radiation therapy (Adami, *et al.* 2002).

The role of cytokeratin5/6 (CK5/6) in diagnosis of bladder cancer : Cytokeratin is an intermediate filament protein, reflects the epithelial cell type, state of tissue

growth, differentiation, functional status and is used in various carcinomas. The diagnosis of carcinoma *in situ* in bladder specimens is of great benefit because it has prognostic and

therapeutic value. Morphology alone may not be sufficient in the differentiation of reactive urothelialatypia (RUA), urothelial dysplasia (UD) and carcinoma *in situ* (CIS). Ck 5/6 is promising to be reliable diagnostic markers of UD and CIS in conjunction with morphological changes (Hayam, *et al*. 2014).

1.2 Objectives:

General objective:

To detect Ck5/6 expression in bladder tumors among Sudanese patients.

Specific objective:

To correlate the Ck5/6 exepression with histopathological diagnosis**.**

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CHAPTER TWO

LITREATURE REVIEW

Chapter two

Literature Review

2.1 Scientific back ground:

Urinary bladder cancer is the 4th most frequent cancer diagnosed in men and the 9th most frequent cancer diagnosed in women.When first diagnosed with urinary bladder cancer,most people's cancers are confined to the bladder (74%) rather than it being an advanced stage of urinary bladder cancer. The incidence of urinary bladder cancer rises steeply after age 50 in both men and women. Surgery is the main treatment for urinary bladder cancer(Adami, *etal*. 2002).

2.2 Structure of the bladder:

The bladder is a hollow muscular organin the lower abdomen. It stores urine, the liquid waste made by the kidneys. Bladder is part of the urinary tract. Urine passes from each kidney into the bladder through a long tube called a ureter. Urine leaves the bladder through a shorter tube called the urethra. The wall of the bladder has four main layers, the inner layer is called the lining. This lining is made up of cells called urothelialor transitionalcells. This layer is called the urotheliumor transitional epithelium. Under the urothelium is a thin layer of connective tissue, blood vessels, and nerves, which is called the lamina propria. Next is a thick layer of muscle called the muscularis propria. Outside of this muscle,a layer of fatty connective tissue sets apart the bladder from other nearby organs. As the cancer grows or spreads into the other layers in the bladder, it becomes more progressive (Bridget and Julie,2014).

2.3Disorder of the bladder:

2.3.1 Transitional cell (urothelial) carcinoma:

This is the most common form of bladder cancer. It represents roughly 95% of bladder cancers. Urothelialcells also line other parts of the urinary tract as well as
the kidneys, the ureters, and the urethra. Patients with bladder cancer sometimes have cancer in the lining of the kidneys, ureters, or urethra. It is important to have the whole urinary tract checked for cancer (BridgetandJulie,2014).

2.3.1.1Non-invasive transitional cell carcinoma:

Cancer cells are still in the inner layer of cells and have not grown into the deeper layers (Mark and Saad, 2012).

2.3.1.2Invasive transitional cell carcinoma:

Cancer cells have grown into the lamina propria or even deeper into the muscle layer(Mark and Saad, 2012).

2.3.1.3Metastatic transitional cell carcinoma :

Cancer cells from the main tumor have spread to other parts of the body.

Bladder cancer can be described as superficial or non-muscle invasive, it means the cancer has not spread into the muscle and is non-invasive (Mark and Saad, 2012).

2.3.2 Papillary carcinomas:

It grow in finger like projections from the inner surface of the bladder toward the hollow center.

Urothelial papilloma Non-cancerous (benign) tumor. Papillary urothelial neoplasm of low malignant potential (PUNLMP) very slow growing and unlikely to spread.

Low grade papillary urothelial carcinoma slow growing and unlikely to spread.

High grade papillary urothelial carcinoma more quickly growing and more likely to spread (Bridget and Julie, 2014).

2.3.3 Flat carcinomas:

It dose not grow toward the hollow part of the bladder. It is a flat tumor ,it called non-invasive flat carcinoma or a flat carcinoma in situ (CIS)(Silvia, *etal*. 2012).

2.3.4 Squamous cell carcinoma:

It is uncontrolled growth of abnormal cell arising in the squamous cell, roughly 1% to 2% of bladder cancers are squamous cell carcinomas. Nearly all squamous cells are invasive (Silvia, *etal*. 2012).

2.3.5 Adenocarcinoma:

It is a cancer originating in glanduler tissue, 1% of bladder cancers are adenocarcinomas(Mark and Saad, 2012).

2.3.6 Small cell carcinoma:

It is a type of highly malignant cancer cell. It has a shorter doubling time, higher growth fraction, and earlier development of metastases. 1% of bladder cancers are small cell carcinomas(Arnulf, *et al*. 2011).

2.3.7 Sarcoma:

Sarcomas start in the muscle of the bladder. This cancer is very rare in the bladder (Bridget and Julie, 2014).

2.4 Epidemiology of bladder cancer :

Bladder cancer (BC) is the 11th most commonly diagnosed cancer in the world. The worldwide agestandardisedincidence rate (per 100,000 person-years) is 8.9 for men and 2.2 for women. In the european union (EU), the age-standardised incidence rate is 27 for men and six for women. In europe,thehighest age-standardised incidence rate has been reported in Spain (41.5 in men and 4.8 in women) and thelowest in Finland (18.1 in men and 4.3 in women)(Marko,*etal*.2015).

Worldwide, BC is the 14th leading cause of cancer deaths, the age-standardisedmortality rate was 8 for men and 3 for women, respectively. BC incidence and mortality rates vary across the countries due to differences in risk factors,detection and diagnostic practices, and availability of treatments. The variations are however partly caused by the different methodology and quality of data collection (Marko,*etal*.2015).

The incidence and mortality of BC has decreased in some registries, possibly reflecting thedecreased impact of causative agents.Approximately 75% of patients with BC present with a disease confined to the mucosa (stage Ta,CIS) or submucosa (stage T1). They have a high prevalence due to long-term survival in many cases and lowerrisk of cancer specific mortality compared to T2-4 tumours (Marko,*etal*.2015).

2.5Risk factor of bladder cancer:

2.5.1 Hereditary conditions:

People with a family history of bladder cancer are at increased risk for getting bladder cancer, this is because they inherited a genetic syndrome(David and Joseph, 2006).

. 2.5.2 Age:

Bladder cancer is more common in people aged 55 and older(Bridget and Julie, 2014).

2.5.3 Treatment of bladder cancer:

Treatment with alkylating agent chemotherapy drugs such as Cytoxan (cyclophosphamide) increase risk of bladder cancer(Arnulf, *et al*. 2011).

2.5.4 Smoking:

Smoker are three times more likely to get bladder cancer than a non-smoker (Sandr ,*et al*. 2006).

2.5.5 Medical conditions:

Urinary infections, kidney and bladder stones, and other causes of chronic bladder irritation have been linked with bladder cancer (David and Joseph, 2006).

2.5.6 Chemicals in the workplace:

Some people have a higher risk of bladder cancer because they have worked around cancer-causing chemicals (Bridget and Julie, 2014).

2.5.7 Dietary factors:

A diet low in fruits and vegetables increase risk of bladder cancer(Arnulf, *et al*. 2011).

2.5.8 Exposure to hair Dye:

There issignificant association between both frequency of use and duration of exposure to permanent hair dye and increased risk of bladdercancer(Sandra, *et al* .2006).

2.6 Diagnosis of bladder cancer:

2.6.1 Medicalhistory:

The medical history looks for any risk factors for bladder cancer and the physical examgives more information about signs of bladder cancer(Patcharin and Sudarat, 2014).

2.6.2 Signs and symptoms:

Haematuria is the most common finding in non metastatic invasive bladder cancer(NMIBC). Present with lower urinary tract symptoms. CIS might be suspected in patients who do complain of these symptoms, particularly if they are refractory to symptomatic treatment (Marko,*etal*.2015).

2.6.3 Physical examination:

Physical examination give more information about signs of bladder cancer (Bridget and Julie, 2014).

2.6.4 Cystoscopy:

A slender tube with a lens and a light is inserted into the bladder through the urethra. It allows to view the inside of the bladder and any suspicious growths that could be removed for microscopic examination (Adami,*etal*. 2002).

2.6.5 Bladder biopsy:

During the cystoscopy, the taking of sample should take place from the bladder to be looked at under the microscope. These results can take up to five days to complete(Patcharin and Sudarat, 2014).

2.6.6 Urine cytology:

The examination of voided urine or bladder-washing specimens for exfoliated cancer cells has high sensitivityin G3 tumours, but low sensitivity in G1 tumours.

Cytology is useful, particularly as an adjunct to cystoscopy, when a G3 malignancy or CIS is present. Positivevoided urinary cytology can indicate an urothelial tumour anywhere in the urinary tract; negative cytology,however, does not exclude the presence of a tumour(Marko,*etal*.2015).

2.6.7 Urine culture:

It is done if having urinary symptoms, this test performed to see a urinary tract infection. This test can take up to two to three days for results (Adami,*etal*. 2002).

2.6.8 Urinary molecular marker tests:

Driven by the low sensitivity of urine cytology, numerous urinary tests were developed . None ofthese markers have been accepted for diagnosis or follow-up in routine urology (Marko,*etal*.2015).

2.6.9.FISH (Fluorescence in situ hybridization):

It is a urine based genetic test for the diagnosis and surveillance of bladder cancer. It gives the most sensitive detection of bladder cancer available today, and it can spot bladder cancer up to six months sooner than other tests (Sandra,*et al* .2006).

2.6.10 Imaging Tests:

2.6.10.1 Computerized tomography (CT) Urogram:

It is a CT of the kidneys, ureters, and bladder.This gives detailed information about the size, shape and position of any tumors in the urinary tract(David and Joseph, 2006).

2.6.10.2 Intravenous pyelogram (IVP):

Itis an x-ray of the urinary system taken after a special dye has been injected into the vein. The dye outlines these organs on the x-ray and helps find urinary tract tumors (Patcharin and Sudarat, 2014).

2.6.10.3 Retrograde pyelogram:

A catheter is placed through the urethra and up into the bladder or into a ureter. Then a dye is injected through the catheter to make the lining of the bladder, and show the abnormality (David and Joseph, 2006).

2.6.10.4 Chest X-ray:

Chest x-ray may be done to look for bladder cancer which has spread to the lungs (Sandra, *et al.* 2006).

2.6.10.5 Ultrasound:

Ultrasound can be useful in deciding the size of the tumor and whether it has spread beyond the bladder to nearby organs or tissues (Sandra, *et al*. 2006).

2.6.10.6 Bone scan:

A bone scan done to testid a cancer has spread to the bones (Patcharin and Sudarat, 2014).

2.6.10.7 MRI (Magnetic resonance imaging):

A MRI looks to any spread of tumors outside of the bladder into nearby tissues or lymph nodes(Sandra, *et al* .2006).

2.7 Treatment of bladder cancer

2.7.1 Surgery:

Transurethral surgery the operation is done through a cytoscope that is placed into the urethra while the patient is under anesthesia. The operation takes out the bladder cancer(Adami,*etal*. 2002).

Cystectomy when the bladder cancer has spread, the diseased area needs to be removed by cutting through the bladder wall. This surgery needs to be performed through the abdomen. This operation can remove a part of or the whole bladder wall. A radical cystectomy is done when the entire bladder and prostate or reproductive system is removed (Adami,*etal.* 2002).

2.7.2 Immunotherapy:

It is a treatment that causes the body's own natural defenses (immune system) to attack the bladder cancer. Intravesical therapy is a delivery system where the treatment is placed directly into the bladder through a catheter rather than being given by mouth or injected into a vein. This is the most common way that the immunotherapy is given to the patient for bladder cancer (David and Joseph, 2006).

Interferon therapy a type of substances that is naturally produced by several types of cells. Interferon-alpha is the kind most often used when treating bladder cancer.

Bacillus Calmette-Guerin − BCG is the bacterium that is used in a tuberculosis vaccination. The presence of the BCG is activate the immune system to attack the foreign agent in the bladder as well as the cancer cells that are present (David and Joseph, 2006).

2.7.3 Chemotherapy:

It can be delivered either through the bladder (intravesical) or in the vein (systemic). Neoadjuvant chemotherapy doing the treatment before surgery in order to shrink down the tumor. Intravesical chemotherapy it tends to get used for earlier stages of bladder cancer due to the localized administration of the chemotherapy.

Systemic chemotherapy this tends to be used when there are possible cancer cells in other areas of the body. The chemotherapy drugs can be several different combinations of drugs (Patcharin and Sudarat, 2014).

2.7.4 Radiation therapy:

External beam radiation therapy focuses radiation from outside of the body on the cancer. Local or interstitial radiation therapy uses a small pellet of radioactive material placed directly into the cancer. Radiation can also be used before surgery in order to shrink the size of the tumor.

Radiation can be used in conjunction with chemotherapy to make sure that the cancer has been gotten without doing more surgery (Patcharin and Sudarat, 2014).

2.8 Cytokeratin5/6 and it is relation with bladder cancer:

Cytokeratin (CK) is an intermediate filament protein,reflect the epithelial cell type, state of tissue growth, differentiation,functional status and is used for the fingerprinting of various carcinomas (Amarpreet,*et al*.2010).

Cytokeratin 5/6 are intermediate sized basic keratins. In normal tissue, CK 5/6 are mainly expressed in keratinizing(epidermis) and non keratinizing (mucosa), squamous epithelium,as well as in basal myoepithelial cell layer prostate, salivary gland and breast (Peiguo and Lawrance, 2002).

CK 5/6 are also seen in benign and malignant tumors of epidermal,squamous mucosal and myoepithelial origins (Peiguo and Lawrance, 2002).

Urothelium is a stratified epithelium, which demonstrates similarities with squamous cell carcinoma, resulting in markedly overlapping immunoprofiles. One characteristic is the exepression of CK 5/6 and other squamous association marker, CK5/6 is expressed in 65% to 97% of benign urothelial tumors, 35% to 60% of urothelium carcinoma (Mahul , *etal* .2014).

The CK staining pattern and intensity varied between well diffrentiation and poorly diffrentiation transitional cell carcinoma. In low grade papillary transitional cell carcinoma, the CK5/6 positive cell were observed at the basal layer of the papillary, where as in high grade transitional cell carcinoma, tumor cell were diffusely positive for CK5/6 in some cases (Peiguo and Lawrance, 2002).

Mahul *et al* reported that (22/25) of malignant lesions showed negative expression, and all benign lesions of bladder showed positive CK5/6 expression (Mahul , *etal* .2014).

Peiguo and Lawrance reported that all malignent lesions showed negative expression, and positive rate of CK5/6 in benign bladder lesions was 100% (Peiguo and Lawrance, 2002).

CHAPTER THREE

MATERIALS AND METHODS

Materials and methods

3.1 Materials:

Archived tissue block of bladder tumors were selected for this study.

3.2 Methods:

3.2.1 Study design:

This is hospital based analytical retrospective case control study aimed to detect CK5/6 expression in bladder tumors.

3.2.2 Study samples:

Tissue blocks obtained from thirty sample were previously diagnosed as malignant bladder tissue and ten samples were previously diagnosed as benign tumor. Pateint's data (age, histopathological diagnosis , malignant tumor grade) were obtained from patients files.

3.2.3 Study area:

This study was held in Ibn Sena hospital during period from April to August 2016.

3.2.4 sample processing:

Section to be stained were cut at 3µm thichness by rotary microtome, mounted in positively charged glass slides and put at 60˚C oven for 30 minutes.

3.2.5 Immunohistochemical staining:

Immunohistochemical staining was carried out using indirect streptoavidin- biotin immune peroxidase technique. Tissue sections (3µm) were deparaffinized in xylene and rehydrated in graded alcohol (100% , 90% , 70% , 50%) slide were incubated for 10 minutes in 0.3 % hydrogen peroxide to block endogenous peroxidase activity. Antigen retrieval was performed by using PT link water path with citrate buffer (pH 6.8).

The slides then were treated with anti CK5/6 primary antibody for 30 minutes. Then section were incubated in biotinylated secondary antibody for 15 minutes then washed

in phosphate buffer saline (pH7.4), incubated in streptavidin-HRP (horseradish peroxidase) for 15 minutes ,washed in phosphate buffer saline (pH 7.4), incubated in diaminobenzidine tetra hydrochloride (DAB) substrate solution, washed in running water. Then counterstained in Mayer's hematoxylin stain for 1 minute. Dehydrated, cleared and mounted in DPX mounting media (Bancroft, *et al*. 2013).

3.2.6 Data analysis:

Data analysis was done using SPSS 20 computer program. Frequencies mean and Chi –square test values were calculated.

3.2.7 Result interpretation:

All quality control measures were adopted, positive and negative control slides were used during immunohistochemical staining. Detection of more than 5 cell with cytoplasm per one field considered as positive result.

3.2.8 Ethical consideration:

Samples were collected after taking ethical acceptance from hospital administration.

CHAPTER FOUR

RESULTS

Results4.

The age of study population range between 49 and 70 years with mean age of 59 years, and standard deviation 8.1 .

Most patient's were more than 55 years representing 22 (55%), and the remaining 18 (45%) were less than 55 years as indicated in table (4.1).

The study includes forty samples ,30 (75%) samples were malignant and 10 (25%) samples were benign.The diagnosis of malignant samples include transitional cell carcinoma in 28(70%) samples, adenocarcinoma in 2 (5%) samples as indicated in table (4.2).

Cytokeratin 5/6 positive exepression was found (20/30) in malignant samples ,while (10/30) samples showed negative exrpression , while all benign samples (10/10) showed positive exepression for CK5/6 . This result showed significant association (P. value =0.035) as indicated in table (4.3).

Table (4.1): Distribution of age group among the study population

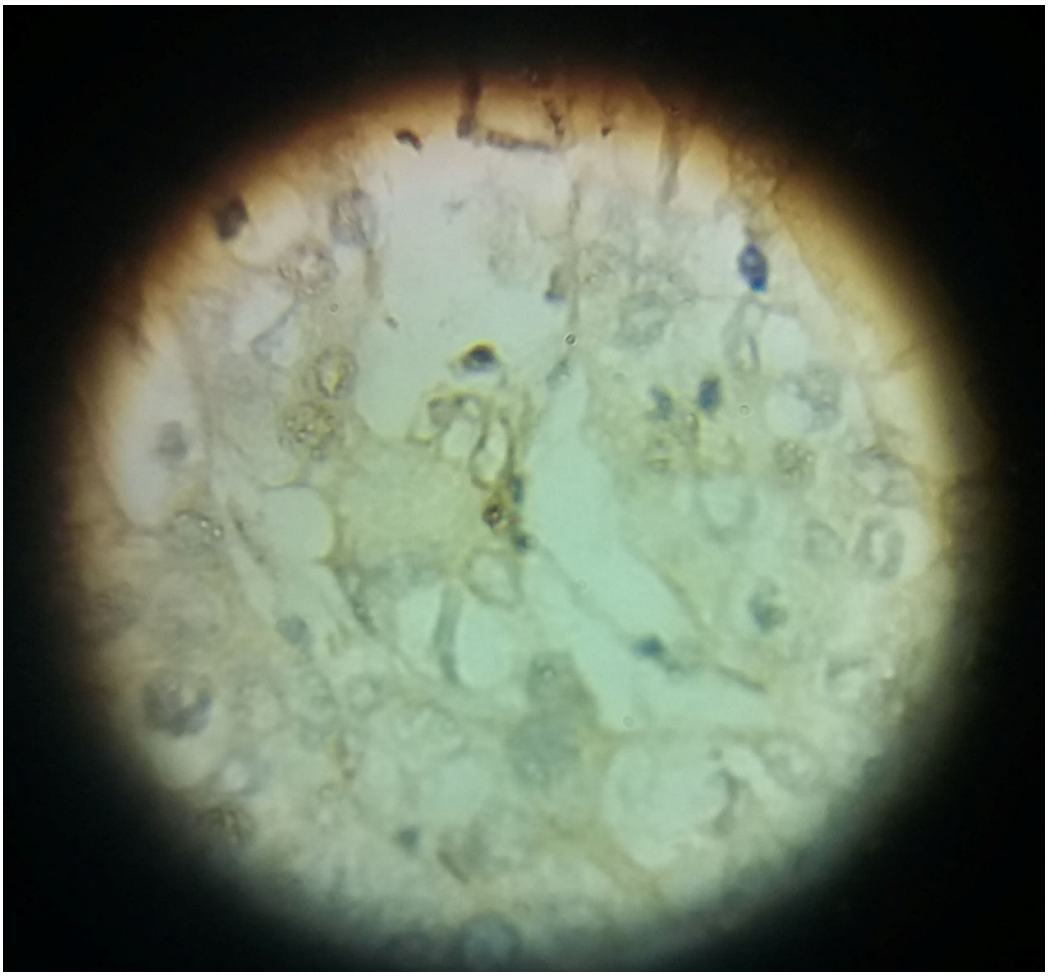
Age group	Frequency	Percentage
Less than 55 years	18	45%
More than 55 years	22	55%
Total	40	100%

Table (4.2): Distribution of histopathological diagnosis among the study population

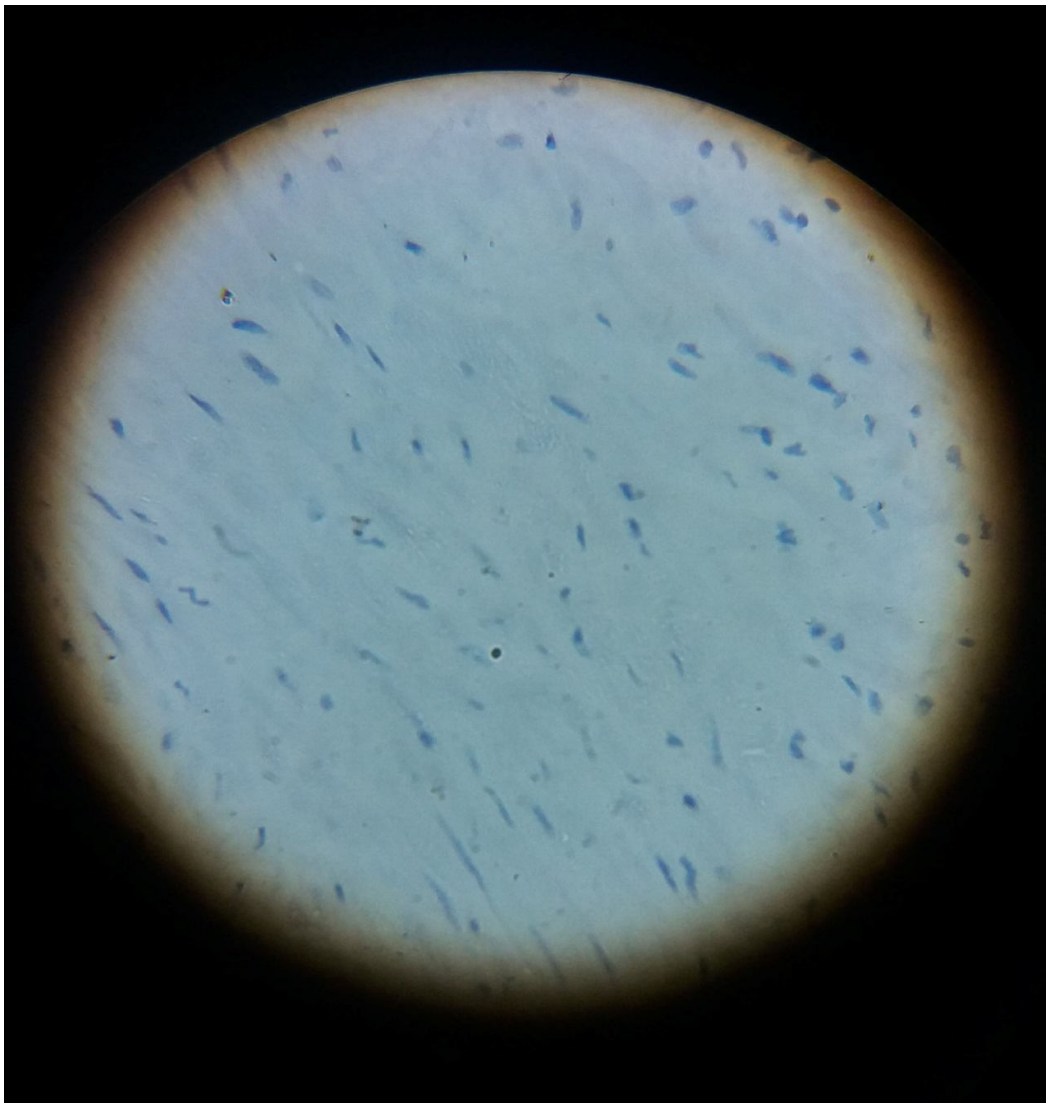
Histopathological diagnosis	Type	Frequency	Percentage
Bengin		10	25%
Malignant	Transitional cell carcinoma	28	70%
	Adeno carcinoma	2	5%
Total		40	100%

Table (4.3): Relation between histopathological diagnosis of bladder tumor and CK5/6 expression

Histopathological diagnosis	CK5/6 expression		P. value
	Positive	Negative	
Bengin	10 (100%)	0 (0.0%)	0.035
Malignant	20 (66.7%)	10 (33.3)	
Total	30 (75%)	10 (25%)	



Photograph (4.1) Benign bladder tumor show positive expression of CK5/6 (40×).



Photograph (4.2) Transitional cell carcinoma of bladder show negative expression of CK5/6(40×).

CHAPTER FIVE

DISCUSSION

Chapter Five

5.Discussion

The present study involves 40 cases of bladder lesions for immunohistochemical staining by cytokeratin 5/6. Regarding the age group of stuy population, the study revealed that most of patients were more than 55 years, indicating that older patients are more susceptible to bladder cancer due to change in gene expression and hormonal change by age. This result is compatible with Yoshiyuki *et al*. (1980), who reported that risk of developing bladder cancer increases with age. Also agree with Hayam *et al*. (2014), who reported that number of the patient was increased with age. This result also agree with Arshad *et al*. (2015), who reported that the older patients, the higher risk throughout his life.

The histopathological diagnosis of the study population revealed that more frequent type was transitional cell carcinoma, this result is compatible with Arshad *et al*. (2015), who reported that (28 / 30) cases of malignant tumors were diagnosed as transitional cell carcinoma. Also combatable with Irfan *et al*. (2014), who reported that (20 / 25) cases of malignant lesions were diagnosed as transitional cell carcinoma. Also agree with Silvia *etal*. (2012), who reported that 90% of cases is transitional cell carcinoma.

The exepression of CK 5/6 revealed that (20/30) of malignant lesions showed positive expression and (10/30) showed negative expression, and all benign cases of bladder lesion showed positive expression for CK 5/6 because CK5/6 normally present in the basal layer of the bladder tissues. This relation showed significant association (P.valuo =0.035), this result is compatible with Mahul *et al*.(2014), who reported that (22/25) of malignant lesions showed negative expression, and all benign lesions of bladder showed positive CK5/6 expression. Also agree with Peiguo *et al* . (2002), who reported that all malignent lesions showed negative expression, and positive rate of CK5/6 in benign bladder lesions was 100% .The appearance of positive among malignant may be due to samples may contain benign tissue.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

Chapter Six

Conclusion and Recommendations

6.1Conclusion:

From this study we conclude that

The age of the bladder cancer in Sudanese patients is commonly more than 55 years.

Most histological type of bladder cancer is transitional cell carcinoma

CK5/6 expression is associated with benign forms of bladder tumors

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6.2 Recommendations:

From this study we recommended that:

Further research should be done on expression of CK5/6 in bladder tumors tissue with large sample size.

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Appendices 1

Appendix 1:

Materials and instruments used for processing and staining of the specimens include :

Disposable gloves.

Rotary microtome.

Microtome knives.

Positively charged slides (Thermo).

Cover glasses.

Dry oven.

Water path (Dako water path).

Coplin jars.

Humidity chamber.

Ethanol(100%, 90 %, 70 %, 50 %).

Xylene.

Mayer,s haematoxylin.

Citrate buffer (PH6.8).

Phosphate buffer (PH7.4).

0.3 Hydrogen peroxidase.

Primary antibody (CK5/6).

Secondary antibody (biotinylated secondary antibody).

Streptavidin-HRP

Substrate chromogen (DAB).

DPX