Sudan University of Science and technology
College of Medical Laboratory Science

Immunohistochemical Detection of E-cadherin in Urinary Bladder Tumors

الكشف النسيجي الكيميائي المناعي لـ إي – كادرين في أورام المثانة البولية

Dissertation submitted for partial fulfillment of the requirement of the master Degree in Medical Laboratory Science (Histopathology and Cytology)

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Dedication

I dedicate this work to my mother and father how gave me the meaning of life …

My Sweet Sisters and kinds brother ……

My colleagues, my professors and my loyal friends ……

And every one who helped me and whom I respect.
Acknowledgement

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

I am grateful to my Supervisor Dr. Mohammed Siddig Abdelaziz for his perfect Supervision, advice, encouragement and support from the early stage of this research as well as giving me opportunities of experiences through the project.

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Abstract
This analytical retrospective case control is hospital based study was conducted at Epn Sina hospital, and Sudan University of Science and Technology – college of medical laboratory science, during the period from August 2016 to Mayo 2017, aimed to detect the expression of e-cadherin in benign and malignant bladder tumor using immunohistochemistry.

39 paraffin block samples were obtained from patients previously diagnosed as bladder tumor, 20 (51%) of them were malignant and the remaining 19 (49%) were benign samples. From each blocks 3µ section was cut by rotary microtome, then stained by immunohistochemical method (new indirect dextran polymer immune peroxidase technique). Data obtained was analyzed using SPSS program version 20, mean, frequency and chi square test were calculated.

The age of patients ranged between 6-80, with mean age 48 years the study revealed that most patients were older than or equal 55 years representing 21 (53.8%) and the remaining 18 (48.2) were younger than 55 years. The study found most of the patients were male 26 (66.7%) with ratio of 2:1 between male to female.

The study found the expression of E-cadherin in benign tumors was positive in 16 (41%) cases and negative in 3 (7.7%) cases were negative while in malignant tumors 7 (17.0%) cases were negative and 13 (33.3%) cases were positive, with no significant relation between E cadherin expression and type of bladder tumors (P = 0.157).

The study conclude there is no relation between E cadherin expression and type of bladder tumors.
المستخلص

أجرت هذه الدراسة التحليلية التراجعية حالة وحالة ضابطة في مستشفى ابن سينا وجامعة السودان للعلوم والتكنولوجيا، كليه علوم المختبرات الطبية في فتره من أغسطس 2016م إلى مايو 2017 هدفت الدراسة الكشف عن وجود الإي-كاترين في العينات الحمية والخبيثة باستخدام كيمياء الأنسجة المناعية.

جمعت 39 عينة قابلة شمعي من عينات مرضي كانو مشخصين مسبقاً على أنهم مصابون بورام المثانة، 19 منهم كانو مشخصين أورام حمية، 20 أورام مثانة خبيثة تم قطع 3 مايكرون من كل قالب باستخدام المشراح الدوار وصبغها بواسطة طريقة كيمياء الأنسجة المناعية (طريقه البيروكسيدز الجديدة للجزئ المتعدد الغير مباشرة المناعية) واستخدم برامج الحزم الإحصائية للعلوم الاجتماعية. النسخة 20 للتحليل البيانات وحسب المتوسط و التكرار و مربع كاي.

تراوحت اعمار المرضي بين 6 الي 80 بمتوسط عمر 48. أظهرت الدراسة أن معظم المرضي كانت اعمارهم أكبر من 55 سنة وعددهم 21 (53.8%), وما تبقى منهم 18 (46.2%) مريضاً كانت اعمارهم أقل من 55 سنة.

وجدت الدراسة أن معظم المرضي من الذكور 32 (66.7%) ونسبة الذكور إلي الإناث كانа أثناً الي واحد.

اظهرت الدراسة أن الإي-كاترين موجب الظهور في 11 (33.3%)عينة في الورام الخبيثة، وسالب الظهور في 7 (17.0%)عينة، بينما عينات أورام المثانة الحمية موجهة الظهور في 16 (41.0%)، وسالب الظهور في 3 (7.7%)عينة، مع عدم وجود علاقة ذات دلاله إحصائيه بين الإي-كاترين وسرطانات المثانة (p = 0.157).

خلصت الدراسة أن لاتوجد علاقة بين الإي-كاترين وسرطانات المثانة.
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CHAPTER ONE

Introduction
CHAPTER ONE

Introduction

1.1 Introduction

Bladder cancer is a malignant overgrowth of the cells of the bladder. Most commonly, the growth occurs in cells that are in the urothelium called transitional cell cancer. Other types of uncommon cancers in the bladder include squamous cell carcinoma and adenocarcinoma (Pamela and Brett, 2011). Carcinoma of the bladder is the seventh most common cancer worldwide. It comprises 3.2% of all cancers, with an estimated 260,000 new cases each year in men and 76,000 in women. The highest incidence rates in males and females occur in Western Europe, North America, and Australia. The UK annual incidence is over 10,000 new cases, with a male: female ratio of 5:2. Urothelial carcinoma is the most common type of bladder cancer; however, there is significant geographic variation, and in certain regions of the world, such as Egypt and parts of Africa, squamous cell carcinoma (SCC) of the bladder is predominates (David, et al., 2008). In developing countries, particularly in the Middle East and Africa, the majority of bladder cancers are squamous cell carcinomas (SCCs) and most of these cancers are secondary to *Schistosoma haematobium* infection (Maya, et al., 2015). In fact, that squamous cell carcinoma (SCC) is the commonest histological variant of bladder cancer in Sudan (Gouda, et al. 2007).

Risk factors which increase the incidence of bladder cancer are smoking, chemicals in the work place, personal history of bladder cancer, age, life-long bladder irritation, infections and low fluid intake (Bridget, et al. 2014), bladder birth defects, genetics and family history, and prior chemotherapy or radiation therapy (American cancer society, 2016).

Bladder cancer is found due to the presence of signs or symptoms or it might be found because of an accident. The diagnosis includes history and physical examination, urine tests (urinalysis, urine cytology, urine culture and urine tumor marker tests) other tests include cystoscopy, transurethral resection of bladder tumor (TURBT), imaging tests (computed tomography (CT) scan, magnetic resonance imaging (MRI) scan and ultrasound. Depending on the stage of the cancer and other factors...
treatment options for people with bladder cancer can include: surgery, chemotherapy, radiation, therapy and immunotherapy (American cancer society, 2016).

The classical cadherins are calcium-dependent transmembrane glycoproteins found at the adherer's junction and are mediators of cell–cell adhesion in epithelial tissues. E-cadherin is a tumor suppressor. The abnormal expression of other ‘classical’ cadherins (P- and N-cadherin) has been shown to promote a more invasive and malignant phenotype of (UBC). Cadherins, play a fundamental role in the spread of bladder tumors, initially from the urothelium into the lamina propria (through the basement membrane) and subsequently into the detrusor muscle. There for, the classical cadherins and their related molecular pathways represent attractive therapeutic targets for the inhibition of progression in bladder cancer patients (Richard, 2015). Zpopov, et al. reported that E-cadherin down-regulation at the protein level, E-cadherin expression correlated with both stage and grade it status and stage had significant additional prognostic value (Zpopov, et al, 2000). Immunohistochemical interpretation of E-cadherin altered adhesive function is a useful histological prognostic marker in urinary bladder carcinoma (Mona and Rashed, 2004).

1.2 Objectives:

1.2.1 General objective:
To detect the of E-cadherin in bladder tumors.

1.2.2 Specific objectives:
To detect E–cadherin expression in bladder tumors tissue by immunohistochemical method.
To correlate E-cadherin expression with histological diagnosis of bladder tumors.
CHAPTER TWO

Literature review

2.1 Scientific background:
Bladder cancer is a common cancer of the urinary tract. It is the fourth leading cause of cancer-related death among men and the seventh among women. Clinical management of bladder cancer is challenging because of the heterogeneity among bladder tumors with respect to invasion and metastasis and frequent occurrence of new tumors in the bladder among patients treated with bladder preservation treatments. Environmental factors such as cigarette smoking and other carcinogens play a major role in the development of transitional carcinoma of the bladder, where Schistosomiasis, a protozoan infection, results in squamous cell carcinoma of the bladder (Vinata, et al. 2011).

2.2 Histology of bladder:
The bladder is the container in the body that stores urine. The bladder is a soft, round structure that is located in the pelvis. The pubic bone is in front of the bladder; the rectum in men or the uterus in women is behind the bladder. The bladder wall has three separate layers. The inner layer that is in contact with the urine is a thin layer called the urothelium. The middle layer is made of muscle fibers that can squeeze, the outer most layers is a thin but protective layer called serosa. The bladder has two functions: the first is the storage of urine, and the second is the emptying of urine (Pamela and Brett, 2011). Urothelium is a specialized epithelium forming the lining of the calices, renal, pelvis, ureters, bladder and upper urethra (Geoffrey, 1985). Normal urothelium ranges from four to seven cells layers in thickness and contains a basal cell layer, intermediate urothelial cell layers, and a superficial umbrella cell layer (Seth, et al. 2015). Umbrella cells with polyploid nuclei, the cytoplasmic extend to cover several cells of the layer immediately underneath, the surface has a unique angular contour formed by rigidplaque regions in the surface membrane. The membrane in the plaque region is 12 nm thick the other cell which measures 8 nm thick (Geoffrey, 1985). Normal urothelium is characterized by uniform nuclear size, nuclei oriented
towards the luminal surface, nuclear grooves, and regular spacing between cells, in normal urothelium, cytokeratin 20 stains the umbrella cell layer and p53 variably labels the basal and intermediate cell layers. In contrast to the normal urothelium, neoplastic processes may show full-thickness cytokeratin 20 expression, intense nuclear p53 expression in the majority of cells, and loss of CD44, although these findings are often variable and may not be especially helpful in individual cases (Seth, et al. 2015).

2.3 Disorder of bladder:

2.3.1 Benign urothelial changes:

2.3.1.1 Reactive atypia:
Reactive atypia in the urothelium is a benign urothelial change and characterized by distinct cellular atypies and changes in the architecture of the urothelium in combination with a chronic lymphocytic or active granulocytic inflammation. Frequent causes are urocystitis, or a history of stones, instrumentation, or intravesical therapy. Immunohistochemical staining demonstrating a normal CK20 expression pattern in umbrella cells only, a relatively low proliferation activity mostly in the basal cell layer and a negative staining for CD44 and p53 can help to distinguish reactive atypia from dysplasia (Vinata, et al. 2011).

2.3.1.2 Urothelial hyperplasia (UH):
Abnormal thickening of the urothelium, is often evident at low magnification and often extends beyond ten cell layers. Despite the greater number of cells, the urothelium appears otherwise normal to slightly increased in cellularity with retained polarity and absence of nuclear atypia occasionally, mild nuclear enlargement may be present. This finding has been identified both in association with inflammation and adjacent to low grade papillary urothelial carcinomas. Given the latter association, subsequent follow-up of patients with a diagnosis of urothelial hyperplasia may be critical to exclude subsequent neoplastic processes (Seth, et al. 2015). If papillary UH is found in a patient with a history of bladder cancer, this finding is often associated with recurrent tumor growth, and therefore, a close follow-up is recommended (Vinata, et al. 2011).
2.3.1.3 **Urothelial papilloma (UP):**
Urothelial papilloma is characterized by normal appearing urothelium lining thin fibrovascular cores. The umbrella cells may demonstrate prominent vacuolization in this lesion. These lesions are indolent, with only rare cases of recurrence and progression reported (Seth, *et al.* 2015). The lesion is characterized by discrete papillary fronds with occasional branching, but without fusion. The urothelium lacks atypia and superficial cells are often prominent (David, *et al.* 2008).

2.3.1.4 **Inverted urothelial papilloma:**
Inverted papilloma (IP) is a benign urothelial tumor that has an inverted growth pattern with none or minimal cytologic atypia of the neoplastic cells. The lesion has a relatively smooth surface covered by a histologically and cytologically normal urothelium. Cords and nests of urothelial cells invigilate extensively from the surface urothelium into the sub adjacent lamina propria but not into the muscular bladder wall (Vinata, *et al.* 2011).

2.3.1.5 **Papillary urothelial neoplasms of low malignant potential (PUNLMP):**
This lesion is a papillary tumor of urothelium which morphologically resembles its benign papilloma counterpart, but which shows increased cellular proliferation which exceeds the thickness of normal urothelium. Its existence is somewhat controversial as distinguishing this lesion from a benign papilloma or a low grade papillary urothelial carcinoma generates considerable inter observer variation. The lesion may recur but less often than low grade non-invasive papillary urothelial carcinomas (David, *et al.* 2008).

2.3.2 **Malignant bladder cancer:**
There are two broad types of cancers in the bladder: primary and metastatic. Primary bladder cancers are those that begin in the bladder itself. Metastatic cancers are those that originated in another organ and then spread to the bladder through: blood stream, lymphatic system, or by directly extending from a nearby organ. Cancers originating in the bladder are far more common than cancers that metastatic. There are several types of primary tumors: Transitional cell cancer,
squamous cell cancer, adenocarcinoma, and urachal carcinoma (Pamela and Brett, 2011). Bladder cancers are often described based on how far they have invaded into the wall of the bladder: Non-invasive or superficial: cancer cells are still in the inner layer of cells and have not grown into the deeper layers. Invasive: cancer cells have grown into the lamina propria or even deeper into the muscle layer. Metastatic: cancer cells from the main tumor have spread to other parts of the body (Bridget, et al. 2014).

2.3.2.1 Urothelial carcinoma:
Transitional cell (Urothelial) carcinoma is the most common form of bladder cancer. It represents roughly 95% of bladder cancers. Urothelial cells 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. (Bridget, et al. 2014).

2.3.2.2 Squamous carcinoma:
Roughly 1% to 2% of bladder cancers are squamous cell carcinomas. Nearly all squamous cells are invasive (Bridget, et al. 2014).
In certain parts of the world, notably Egypt, there is a high incidence of squamous carcinoma in the bladder. This peculiar geographic incidence has been ascribed to schistosomiasis of the bladder, which is endemic in Egypt (Geoffrey, 1985).
Squamous carcinoma is a carcinoma of urothelial lining origin showing pure squamous morphology throughout. This should primarily be regarded as an urothelial malignancy (David, et al. 2008).

2.3.2.3 Carcinoma in situ:
Carcinoma in situ in other parts of the body, such as the prostate, cervix or testicle, is thought to be a premalignant condition, but in the bladder it is always malignant.
If untreated, 50% of cases will progress to muscle invasive cancer within 5 years. Carcinoma in situ itself is a flat (not papillary) lesion, and thus it can be more difficult to identify during a cystoscopy. It may appear as a red, irritated patch or may be indistinguishable from normal, adjacent bladder. If a patient is at high risk for carcinoma in situ, random biopsies of the bladder to screen for this disease
even though the bladder may appear normal at the time of the cystoscopy. Carcinoma in situ tends to shed cells into the urine, which can usually be detected on a urine sample by urine cytology (Pamela and Brett, 2011).

2.3.2.4 Adenocarcinoma:
Roughly 1% of bladder cancers are adenocarcinomas (Bridget, et al. 2014). Primary pure adenocarcinomas of the bladder are rare, representing no more than 2.5% of all malignant vesical neoplasm. By definition; the tumor should be composed entirely, or virtually entirely, of glandular elements. Grossly, the tumor can be papillary, nodular, or flat and ulcerated. Microscopically, the tumor is most often composed of colonic type glandular epithelium and may contain abundant extracellular mucin (Seth, et al. 2015).

2.3.2.5 Small cell (neuroendocrine) carcinoma:
These are carcinomas derived from the urothelium and which morphologically resemble their lung counterparts. They appear as large, solid, polypoid masses with or without ulceration and often extensively infiltrate the wall of the bladder by the time of presentation they are very aggressive cancers with vascular and detrusor muscle invasion, the importance of diagnosing this type of carcinoma lies in its response to chemotherapy (David, et al. 2008). Neuroendocrine consists of small cells with nuclear molding, scant cytoplasm, and dark nuclei containing finely stippled chromatin and inconspicuous nucleoli, by immunohistochemical studies, most tumors express chromogranin and synaptophysin, neuroendocrine carcinomas should be considered high grade tumors; up to one third of patients have metastases at the time of diagnosis (Seth, et al. 2015).

2.3.2.6 Metaplasia in bladder epithelium:
Abnormalities of the urothelium have been considered to be preneoplastic states and which may ultimately develop into invasive urothelial carcinomas. These are the metaplastic changes; the metaplastic changes which will be considered are : Squamous metaplasia is a change to a keratinizing squamous epithelium, Glandular metaplasia is a metaplastic change in which the normal mucosa is replaced by a mucin-secreting columnar type of epithelium with gland formation
resembling that of the large intestine. In addition to epithelial metaplasia it is known that the stroma of the bladder is capable of metaplastic change into an osseous or chancroid type of matrix (Geoffrey, 1985).

2.4 Bladder cancer risk factors:

2.4.1 Smoking:
Smoking is the most important risk factor for bladder cancer. Smokers are at least 3 times as likely to get bladder cancer as nonsmokers (Bridget, et al. 2014). Many retrospective and prospective studies have shown that there is an increased risk for cigarette smokers of developing bladder cancer (Geoffrey, 1985).

2.4.2 Occupational risk factors:
Exposure to aniline dyes is the most common industrial risk factor for bladder cancer. An increased risk has been reported in all of the following occupations: autoworker, painter, truck driver, drill press operator, leather worker, metal worker, machine operator, drycleaner, paper manufacturer, rope and twine maker, dental technician, barber, hairdresser, physician, apparel manufacturer, and plumber (Pamela and Brett, 2011).

2.4.3 Arsenic in drinking water:
Arsenic in drinking water has been linked with a higher risk of bladder cancer in some parts of the world. People who drink a lot of fluids, especially water, each day tend to have lower rates of bladder cancer (American cancer society, 2016).

2.4.4 Race:
Caucasian (white) Americans are twice likely to develop transitional cell cancer than African Americans. Squamous cell cancer African Americans are twice likely to develop it than white individuals. Of all the different races, Caucasians seem to have the highest rate of bladder cancer (Pamela and Brett, 2011).

2.4.5 Chronic bladder irritation and infections:
Bilharzia is endemic in many regions of Africa and Arabia, and in some of these areas, particularly in Egypt, there is an association with bladder cancer. Very few
human tumors are associated with a possible viral etiology and there is as yet no sound evidence that bladder cancer is caused by a virus (Geoffrey, 1985).

2.4.6 Age:
More than 65% of bladder cancer occurs in patients who are older than 65. Patients in this age group are also more likely to develop more aggressive tumor types than are the younger bladder cancer patients (Pamela and Brett, 2011).

2.4.7 Gender:
Men are almost three times more likely to develop cancer than women (Pamela and Brett, 2011).

2.4.8 Prior chemotherapy or radiation therapy:
Women who have undergone radiation therapy for uterine cancer or ovarian cancer in the past have a twofold to four fold higher risk of developing bladder cancer. Also men who have had radiation therapy for prostate cancer also have an elevated risk of bladder cancer (Pamela and Brett, 2011).

2.5 Signs and symptoms of bladder cancer:
Blood in the urine, feeling pain when you empty your bladder, feeling the need to empty your bladder without results, Needing to strain (bear down) when you empty your bladder (Bridget, et al. 2014).

2.6 Epidemiology of bladder cancer:
Urothelial bladder cancer is the fourth most prevalent cancer in males and the ninth most prevalent cancer in females. An estimated 67,160 new cases of bladder cancer and 13,750 deaths from bladder cancer are expected in 2007 in the United States (Hayat, 2010). In 2006 in Europe, there were an estimated 104,400 incident cases of bladder cancer diagnosed (82,800 in men and 21,600 in women) that represent a 6.6% of the total cancers in men and 2.1% in women. In Egypt, where Bladder Cancer has always been related to bilharziasis, a significance decline of the relative frequency of bladder cancer was observed from 27.63% in the old series to 11.7% in the recent series. (Vinata, et al. 2011). In fact (SCC) the commonest histological variant of bladder cancer in Sudan (Gouda, et al. 2007).
2.7 Diagnosis of bladder cancer:

2.7.1 Urine cytology or other urine screening test:
Urine-Based Tumor Markers: Screening for bladder cancer and detect tumors in patients with a history of cancer. Their use as screening agents is limited due to the invasiveness of cystoscopy and the low sensitivity of cytology. These markers are designed to detect various changes thought to be associated with the development of bladder cancer, such as tumor protein expression or chromosomal abnormalities (Hayat, 2010). Examples of these markers are microsatellite analysis: are highly polymorphic, short tandem DNA repeats found in the human genome (Olaf and Alfred, 2008).

2.7.2 Cystoscopy:
Cystoscopy refers to the direct visual examination of the inside of the bladder using a small telescope called a cystoscope, it has a light at the tip to illuminate the bladder there is a channel through which water flows and fills the bladder, stretching it out to allow better visualization (Pamela and Brett, 2011).

2.7.3 Bladder biopsy:
During your cystoscopy, your doctor will remove any tumors and will take sample cells from the bladder to be looked at under the microscope. These results can take up to five days to complete (Bridget, et al. 2014).

2.7.4 Imaging Tests
2.7.4.1 Computerized tomography (CT) Urogram:
Computed tomography (CT) is a frequently used primary imaging modality for bladder cancer ideally, the bladder should be distended to increase the sensitivity for detecting a lesion and this allows more accurate staging (David, et al. 2008).

2.7.4.2 Chest X-ray:
Chest x-ray may be done to look for bladder cancer which has spread to the lungs (Bridget, et al. 2014).
2.7.4.3 Ultrasound:
Ultrasound uses sound waves to create pictures of internal organs. This is usually an easy test to have, and it uses no radiation. Ultrasound-guided needle biopsy (American cancer society, 2016).

2.7.4.4 Bone scan:
A bone scan looks to see if cancer has spread to the bones. Doctors do not usually order this test unless you have symptoms of bone pain or if blood tests show the cancer might have spread to your bones (Bridget, et al. 2014).

2.7.4.5 MRI (Magnetic resonance imaging):
A MRI looks to see if cancer has spread outside of the bladder into nearby tissues or lymph nodes. A special MRI of the kidneys, ureters, and bladder is known as a MRI Urogram (Bridget, et al. 2014).

2.8 Treatment of bladder cancer:
The stage is very important in determining the treatment that you will receive. There is a good barrier between the urothelium and the muscle of the bladder wall. If the tumor is kept within this barrier, the tumor can usually be completely removed with a transurethral resection of bladder tumor (TURBT) (Pamela and Brett, 2011).

2.8.1 Transurethral resection:
Approximately 70% of all bladder cancer patients present with non-muscle-invasive bladder cancer (NMIBC). The mainstay of treatment for NMIBC is transurethral resection of bladder tumor (TURBT) (Seth, et al. 2015). The therapeutic objectives of (TURBT) are to remove completely the tumor (Geoffrey, 1985).

2.8.2 Chemotherapy:
Chemotherapy is prescribed by a medical oncologist, a patient with muscle-invasive bladder cancer which is found only in the bladder often get chemotherapy before (neo adjuvant therapy) or after (adjuvant therapy) cystectomy to reduce the risk of the cancer spreading to other parts of the body (Bridget, et al. 2011).
2.8.3 Total cystectomy:
The principle of radical cancer surgery is to remove the tumor-bearing organ together with its surrounding tissues and the regional lymph nodes (Geoffrey, 1985).

2.8.4 Immunotherapy:
BCG therapy the immune response to BCG is also able to kill bladder cancer cells. BCG is effective against bladder cancer because it causes an increase of α-interferon in the bladder (Pamela and Brett, 2011). Interferons are natural glycoproteins that mediate host immune responses such as the stimulation of phagocytes, inhibition of nucleotide synthesis, up regulation of tumor antigens, cytokine release, enhanced natural killer cell activity, and activation of T and B lymphocyte (Vinata, et al. 2011).

2.8.5 Radiation:
Radiation therapy uses high-energy beams aimed at your cancer to kill the cancer cells. Radiation therapy can be used after surgery to kill cancer cells that might stay; is sometimes combined with chemotherapy (Bridget, et al. 2011).

2.9 immunohistochemistry stain:
Immunohistochemistry takes advantage of antigen-antibody affinity through its ability to identify and localize proteins of interest via detection with labelled conjugates. A primary antibody which has had a label attached, is allowed to bind. The antibody, and thus the antigen on the tissue surface, is then detected through a series of treatments, which will visualize the label for analysis and quantification. This is called direct IHC. One other important characterization of an antibody that we must consider is the animal that the antibody was developed in. If an antibody against, say, vimentin has been produced in mouse, this is termed “mouse anti vimentin”. This distinction is important when considering another binding assay, called indirect IHC. Indirect IHC involves using an intermediary between the primary antibody and the detection system. (Lee, 2011)
2.10 Cadherins and bladder cancer:


Cadherin expression is very early in epithelial tissue at the two cell stage epithelial differentiation and polarization occur early in ontogeny in the morula stage, when the embryo compacts and each cell polarizes along it’s apicobasal axis to generate an epithelial-like phenotype (Katagiri, et al. 1995).

Somatic mutations is very important to cause cancer in a number of different genes characterizes the process of tumorigenesis. Many genes involved in the process of tumorigenesis are components of one of a great many signal transduction pathways such as E cadherin and beta catenin. It is now apparent that epithelial malignancy can in certain aspects be explained by alterations in the adhesive properties of neoplastic cells, epithelial mesenchymal conversion is also observed in malignant tumors of epithelial origin (Slaus, 2003). Loss of epithelial adhesion and polarity causing mesenchymal cell morphology occurs during mesoderm formation. Heterozygous mutant animals were normal and fertile but abnormal in human (Jeanes, et al. 2008). This loss seems to promote invasive and metastatic properties of neoplastic cells (Mona and Rashed, 2004).

In bladder cancer, altered E-cadherin expression is associated with the degree of invasiveness, lymph node metastasis and increased risk of death from bladder cancer .Loss of E-cadherin expression is considered a critical factor in facilitating the progression of bladder cancer (Maya, et al. 2015).

Several studies have demonstrated that decreased expression of E-CD, as determined by immunohistochemistry, is associated with high grade and advanced stage in transitional cell carcinoma (TCC) of the bladder (Mazaher, et al. 2012). Immunohistochemical interpretation of E-cadherin altered adhesive function is a useful histological prognostic marker in bilharzia associated urinary bladder carcinoma (Mona and Rashed, 2004).
Zpopov, *et al.* reported that E-cadherin down-regulation at the protein level, E-cadherin expression correlated with both stage and grade it status and stage had significant additional prognostic value, The results suggest that the alteration occurs at the transcriptional level and support the clinical and biological relevance of cell adhesion molecules in bladder cancer (Zpopov, *et al.* 2000).

Mazaher *et al* concluded there result there is an association between decreased E-Cadherin immune expression and tumor recurrence in low-grade and non-muscle invasive transitional cell carcinoma of the bladder (Mazaher, *et al.* 2012).

Loss of E-cadherin expression was found to be significantly correlated with high stages of urinary bladder carcinoma and positive lymph nodal metastasis. Also, a significant correlation was detected between E-cad expression and the histological type of the tumors. In conclusions, the application of E-cad immunohistochemical marker could be used as an independent predictor of lymph node metastasis (Maya, *et al.* 2015).
CHAPTER THREE

Materials and Methods
CHAPTER THREE
Materials and Methods

3.1 Materials:
Archived tissue blocks of bladder tumors were selected for this study.

3.2 Methods:
3.2.1 Study design:
This is a hospital based analytical retrospective case control study aimed to detect E-cadherin expression in bladder tumors.

3.2.2 Study samples:
Thirty nine blocks were collected from patients previously diagnosed as bladder tumors, of which 19 were diagnosed as benign tumors, and 20 were diagnosed as malignant tumor. Identification data: (age, histopathological diagnosis, and sex) were obtained from patient's records.

3.2.3 Study area:
This study was conducted at Epn Sina hospital and Sudan University of Science and Technology College of Medical Laboratory Science for partial fulfillment of requirement of the master degree.

3.2.4 Sample processing:
Section of 3µm thickness from each block, was cut by rotary microtome, and placed in positively charged slides for immunohistochemistry stain.

3.2.5 Immunohistochemistry staining:
The sections of 3µm thickness were obtained from formalin fixed paraffin embedded tissue by using a rotary microtome. Following deparaffinization in xylene, slides were rehydrated through a graded series of alcohol (100%, 90%, 70%, and 50%) and were placed in distilled water. The antigens were retrieved using water bath (PT LINK) with tris EDTA buffer (pH 9.0) for 20 minutes at 65°C and boiled at high temperature 95°C for 20 minutes then allowed to cool to 65°C and then washed in phosphate buffer saline (pH 7.4) for five minutes. Then sections were circulated by Dako pen, endogenous peroxidase activity was blocked
with 3% hydrogen peroxide for ten minutes, the slides then treated with anti E-cadherin primary antibody for 20 minutes at room temperature in a humid chamber. Then washed in phosphate buffer saline (pH 7.4) for 3 minutes. Then sections were incubated in dextran labeled polymer (Thermo – ultra vision) secondary antibody for 20 minutes, then washed in three changes of phosphate buffer saline (pH 7.4), after that incubated in 3, 3 diaminobenzidinetetrahy drochloride (DAB) substrate solution for 5 minutes, then washed in running water. Then counter stain in Mayer’s haematoxylin stained for one minute, washed in water. After that dehydrated, cleared and mounted in DPX (Bancroft, et al. 2013).

3.2.6 Result interpretation:
All quality control measures were adopted. Positive and negative control slides were used during immunohistochemical staining. Positive staining for E-cadherin appeared as brown particles at the membrane of cell or in the cytoplasm.

3.2.7 Data analysis:
Data was analyzed using SPSS computer program (version 20). Frequency mean and chi-square test values were calculated.

3.2.8 Ethical consideration:
Samples were collected after talking ethical permission from hospital administration to use the tissue blocks for research purposes.
CHAPTER FOUR

Results
CHAPTER FOUR

4. Results

The study involved 39 samples, previously diagnosed as bladder tumors. Table (4.1) showed frequency of sex, 26 (66.7%) patients were male and 13 (33.3%) patients were female distribution. Table (4.2) showed distribution of age, 21 (53.8%) were more or equal 55 years and 18 (46.2%) were less than 55 years. Table (4.3) showed frequency of immune histopathological of E-cadherin result of benign and malignant tumors. Table (4.4) explains frequency of E-cadherin result in benign and malignant tumor. Table (4.5) showed the Relation between E-cadherin expression in malignant and benign bladder tumors, there was insignificant differences between E-cadherin expression and bladder tumors ($P = 0.157$).
Table 4.1: Frequency of sex among study population:

<table>
<thead>
<tr>
<th>Sex</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>26</td>
<td>66.7%</td>
</tr>
<tr>
<td>Female</td>
<td>13</td>
<td>33.3%</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Table (4.2): Distribution of age group among study population:

<table>
<thead>
<tr>
<th>Histopathology diagnosis</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>19</td>
<td>48.7%</td>
</tr>
<tr>
<td>Malignant</td>
<td>20</td>
<td>51.3%</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Table (4.3): Frequency of histopathology diagnosis among study samples:

<table>
<thead>
<tr>
<th>Age group</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥55 years</td>
<td>21</td>
<td>53.8%</td>
</tr>
<tr>
<td>&lt;55 years</td>
<td>18</td>
<td>46.2%</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Table (4.4): Frequency of E-cadherin result among study samples:

<table>
<thead>
<tr>
<th>E-cadherin result</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>29</td>
<td>74.4%</td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
<td>25.6%</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100.0%</td>
</tr>
</tbody>
</table>
Table (4.5): Relation between E-cadherin expression and histopathological diagnosis of bladder tumors:

<table>
<thead>
<tr>
<th>E-cadherin result</th>
<th>Histopathology</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign</td>
<td>Malignant</td>
</tr>
<tr>
<td>Positive</td>
<td>Count</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>41.0%</td>
</tr>
<tr>
<td>Negative</td>
<td>Count</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>7.7%</td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>48.7%</td>
</tr>
</tbody>
</table>

P.value 0.157
Clear cell carcinoma show positive expression of E-cadherin (40x).
Photograph (4.2):

Clear cell carcinoma show negative expression of E-cadherin (40x).
CHAPTER FIVE

Discussion
CHAPTER FIVE

5. Discussion

In the present study most of the patients were male with ratio of 2:1 to female that agree with study of Shahrokh, *et al.* (2009) found a 6.7 times greater risk of diagnosing Urethral carcinoma of bladder cancer in men than women for both non-muscle invasive and muscle invasive disease, in study of Hassan (2011), who found that the majority of study cases of bladder tumor were males.

In this study most of patients are older than 55 years representing 21 (53.8%) and the remaining 18 (48.2) are younger than 55 years with mean age 48 years that disagree with Hassan (2011), that the mean age was 58.06±12.14 years.

The present study found that, no significant difference between E-cadherin expression in malignant and benign bladder tumors. To our knowledge, there are no studies that agree with us, and this may be due to the size of our sample which is very small. Our study disagree with study of Maya, *et al.* result showed that loss of E-cadherin expression was found to be significantly correlated with high stages of urinary bladder carcinoma (Maya, *et al.* 2015) Mazaher, *et al.* concluded there result there is an association between E-Cadherin immune expression invasive transitional cell carcinoma of the bladder (Mazaher, *et al.* 2012).
CHAPTER SIX

Conclusion and Recommendations
CHAPTER SIX

6. Conclusion and Recommendations

6.1 Conclusion:

On the basis of these results the study concludes that:

There is no association between E cadherin expression and bladder tumors.
6.2 Recommendations:

On the basis of this study we recommended:

Further studies should be done with large sample size.
7.1 References:


7. Appendices

7.2 Appendix

Instruments and materials:
- Disposable gloves.
- Rotary microtome.
- Positively charged slides (thermo).
- Dry oven
- Coplin jars
- Staining racks
- Cover glass
- Water bath
- Humidity chamber
- Pipettors
- Xylene
- Ethanol (100%, 90%, 70%, 50%).
- Mayer's haematoxyline (haematoxyline, Distilled water, potassium alum, sodium iodide, citric acid and chloral hydrate)
- Phosphate buffer saline (pH7.4).
- Tris EDETA buffer (pH9).
- Peroxidase blocker (0.3%hydrogen peroxide in methanol).
- Anti E cadherin antibodies (primary antibody)
- Secondary antibodies (Dextran polymer conjugated secondary antibodies + HRP horse reddish peroxidase )
- DAB (3, 3diaminobenzidine tetrahydrohydrochloride substrate buffer solution)
- DPX mounting media.
- Citrated buffer
FLEX
Monoclonal Mouse
Anti-Human
E-Cadherin
Clone NCH-38
Ready-to-Use
(Dako Omnis)

English
Code GA059

Intended use
For in vitro diagnostic use.

FLEX Monoclonal Mouse Anti-Human E-Cadherin Clone NCH-38, Ready-to-Use (Dako Omnis), is intended for use in immunohistochemistry together with the Dako Omnis instrument. This antibody is useful for the identification of E-cadherin positive cells in normal and neoplastic tissues and for the differential diagnosis between ductal carcinoma and lobular carcinoma of the breast (1). The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

Synonyms for antigen
E-CD, ovomucoid, L-CAM, Arc-1, or cell-CAM 120/180 (3-4)

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

Refer to Dako’s General instructions for Immunohistochemical Staining or the detection system instructions of IHC procedures.

Reagent provided
Ready-to-use monoclonal mouse antibody provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L sodium azide.

Clone: NCH-38, Isoform: IgG1, kappa.

Immunogen
E-cadherin (ovomucoid) (6).

Specificity
Anti-E-cadherin, NCH-38 recognizes the 120 kDa mature form and 82 kDa fragment of E-cadherin in Western blots of A431 cells lysates (6).

Precautions
1. For professional users.
2. This product contains sodium azide (NaNo2), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
3. As with any product derived from biological sources, proper handling procedures should be used.
4. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
5. Unused solution should be disposed of according to local, State and Federal regulations.

Storage
Store at 2-8 °C. During storage the cap should be closed. Do not use after expiration date stamped on vial. Onboard stability is 30 hours. Remaining on-board stability is tracked by the Dako Omnis software. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.
### Quick guide

<table>
<thead>
<tr>
<th>Step</th>
<th>Fixation/Embedding</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Formalin-fixed, paraffin-embedded</td>
<td>Embedding, deparaffinization</td>
</tr>
<tr>
<td>Pre-treatment</td>
<td>EnVision™ FLEX, High pH (Code GV804)</td>
<td>30 min HIER</td>
</tr>
<tr>
<td>Antibody</td>
<td>Ready-to-use</td>
<td>25 min incubation</td>
</tr>
<tr>
<td>Negative Control</td>
<td>FLEX Negative Control Mouse (Code GA759)</td>
<td>25 min incubation</td>
</tr>
<tr>
<td>Visualization</td>
<td>EnVision™ FLEX (Code GV800) + EnVision™ FLEX Mouse LINKER (Code GV821)</td>
<td>30 min; Link: 10 min; Polymer: 20 min; Chromogen: 5 min</td>
</tr>
<tr>
<td>Counterstain</td>
<td>Hematoxylin (Code QC608)</td>
<td>3 min incubation</td>
</tr>
<tr>
<td>Control/Tissue</td>
<td>Colon and Liver</td>
<td>Cytoplasm/membranous staining</td>
</tr>
<tr>
<td>Slides</td>
<td>FLEX IHC Microscope Slides (Code K802)</td>
<td>Recommended for greater adherence of tissue sections to glass slides</td>
</tr>
<tr>
<td>Mounting</td>
<td>Non-aqueous, permanent mounting required</td>
<td>After staining, the sections must be dehydrated, cleared, and mounted using permanent mounting medium</td>
</tr>
<tr>
<td>Instrumentation</td>
<td>Dako Omnis</td>
<td>Reagents are provided in instrument-specific vials</td>
</tr>
</tbody>
</table>

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

### Specimen preparation

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

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

The tissue sections should not dry out during the pre-treatment process or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of FLEX IHC Microscope Slides, Code K802, is recommended.

### Staining procedure

**Program:** The staining slips and incubation times are pre-programmed into the Dako Omnis software. Please refer to the Dako Omnis Basic User Guide for detailed instructions on loading slides and reagents. If the protocols are not available in the Dako Omnis system, please contact Dako Technical Services. All incubation slips are performed at 37°C onboard Dako Omnis.

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

**Counterstaining:** The recommended counterstaining is Hematoxylin (Dako Omnis), Code QC609. The counterstaining is performed onboard Dako Omnis.

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

**Controls:** Positive and negative controls should be run simultaneously using the same protocol as the patient specimens. The positive control tissue should include colon and liver as the reference tissues should display reaction patterns as described for this tissue in the "Performance characteristics" section. The recommended negative control reagent is FLEX Universal Negative Control, Mouse (Dako Omnis), Code GA759.

### Staining Interpretation

The cellular staining pattern is cytoplasmic and membranous.

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

### Summary of Normal Tissue Reactivity

<table>
<thead>
<tr>
<th>Tissue Type (of tested)</th>
<th>Positive Tissue Elements</th>
<th>Tissue Type (of tested)</th>
<th>Positive Tissue Elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal (3)</td>
<td>3/3 epithelium (50-96%), membrane and cytoplasm</td>
<td>Pancreas (3)</td>
<td>3/3 epithelium (100%), membrane and cytoplasm</td>
</tr>
<tr>
<td>Bone marrow (1)</td>
<td>0/1</td>
<td>Parathyroid (3)</td>
<td>3/3 epithelium (100%), membrane and cytoplasm</td>
</tr>
<tr>
<td>Breast (3)</td>
<td>3/3 epithelial cells (100%), membrane and cytoplasm</td>
<td>Pituitary (3)</td>
<td>0/3</td>
</tr>
<tr>
<td>Cerebellum (3)</td>
<td>0/0</td>
<td>Prostate (3)</td>
<td>3/3 epithelium (100%), membrane and cytoplasm</td>
</tr>
<tr>
<td>Cervix (1)</td>
<td>1/1 epithelium (100%), membrane and cytoplasm</td>
<td>Spleen (3)</td>
<td>3/3 small round endothelium (60%), membrane and cytoplasm</td>
</tr>
<tr>
<td>Colon (3)</td>
<td>3/3 epithelial cells (100%), membrane and cytoplasm</td>
<td>Small intestine (3)</td>
<td>3/3 epithelium (100%), membrane and cytoplasm</td>
</tr>
<tr>
<td>Esophagus (3)</td>
<td>3/3 epithelium (100%), membrane and cytoplasm</td>
<td>Stomach (3)</td>
<td>3/3 epithelium (100%), membrane and cytoplasm</td>
</tr>
<tr>
<td>Kidney (3)</td>
<td>3/3 tubular epithelium (60%), membrane and cytoplasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (3)</td>
<td>3/3 hepatocytes, membrane and cytoplasm 3/3 bile ducts, membrane and cytoplasm</td>
<td>Testis (3)</td>
<td>0/0</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------------------------------------------------------</td>
<td>------------</td>
<td>-----</td>
</tr>
<tr>
<td>Lung (3)</td>
<td>3/3 alveolar bronchial epithelium cells (100%), membrane and cytoplasm</td>
<td>Thyroid (3)</td>
<td>3/3 epithelium (100%), membrane and cytoplasm</td>
</tr>
<tr>
<td>Muscle, cardiac (3)</td>
<td>0/3</td>
<td>Thymus (3)</td>
<td>Hassall's corpuscles - cortical reticular (100%), membrane and cytoplasm</td>
</tr>
<tr>
<td>Muscle, skeletal (3)</td>
<td>0/3</td>
<td>Tonsil (3)</td>
<td>3/3 epithelium (100%), membrane and cytoplasm</td>
</tr>
<tr>
<td>Nerve, peripheral (2)</td>
<td>2/2 nerve (60%), cytoplasm</td>
<td>Uterus (3)</td>
<td>3/3 epithelium (100%), membrane and cytoplasm</td>
</tr>
<tr>
<td>Ovary (3)</td>
<td>2/3 epithelium in primary follicles (&lt;1-100%), membrane and cytoplasm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abnormal tissues: The antibody labeled 203/204 invasive ductal breast carcinoma, 549 invasive lobular breast carcinoma, 3/10 pleomorphic lobular carcinoma, 4/4 cases of tubulolobular breast carcinomas; and 51/5 invasive breast carcinomas, with uncertain classification between lobular and ductal type (1).