Sudan University of Science and Technology

College of Graduate Studies

Diagnostic Significance of CK20 and CD15 Expression in Gallbladder Tumors

الاهمية التشخيصية لافراز CD15 و CK20 في اورام المرارة.

A Dissertation Submitted in Partial Fulfilment of the Requirements of M.Sc. Degree in Medical Laboratory Sciences (Histopathology and Cytology)

Submitted by:

Amal Ibrahim Ali Mohammed Hayder

B.Sc. in Medical Laboratory Sciences - Histopathology and Cytology-2002

Omdurman Ahlia University

Supervisor:

Prof. Mohammed Siddig Abdalaziaz

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استهلال

الاية

بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

قَالَ تَعَالَى:

(وَقُل رَّبِّ زِدْنِي عِلْمًا)

سورة طه الاية, (114)

Dedication

This research study is wholeheartedly dedicated…… to my mother, ….to the soul of my father who left us on 15th of December 2020,….. to my brother, …..to my sisters,….to Ali & Omer Salah ,…… to my husband and my lovely kids ,who have been the source of inspiration , support and encouragement.

Acknowledgements

With all respect, gratitude, appreciation and deepest heartful prayer to you, to you my greatest teachers, the river of tender which never ending.

I would like to thank Prof. Mohammed Siddig Abdalaziaz for his great effort, support & encouragement. He was always available despite the unusual situation of COVID-19 & curfew.

I am also grateful to the teaching staff of SUST in Histopathology & Cytology Department, thank you for your dedication & sharing your knowledge with us.

Finally, I consider myself to be extremely fortunate to have had the opportunity to be one of SUST Master degree holder.

All the words of thanks cannot describe my feeling towards you. Being a teacher is a great responsibility & great duty.

Abstract

This is descriptive retrospective study conducted at International Medical Center in Jeddah, Saudi Arabia. From September 2019 to January 2020. The study aimed to detect CK20& CD15 expression in Gallbladder tumors, using immunohistochemistry staining technique. Samples were collected from51 patients previously diagnosed as bladder tumors, 16 (31.3%) were benign and 35 were malignant (68.7%). their age ranges from 25 to 97 years with mean age of 60 years. (26) of them were females with percentage (51%) and 25 patients were males with a percentage (49%).

The benign tumors samples 16 with percentage (31.3%) show negative result with CK20 & CD15.

CK20 was positive in 31 (60.8%) samples and negative in 20 (39.2%) samples.

Positive results in 13 samples of Adenocarcinoma with percentage (37.1%) and 18 in Transitional Cell Carcinoma with percentage (51.4%).CK20 was negative in 4 samples with percentage (11.5%). There is a significant correlation of CK20 & malignant gallbladder tumors (P-value=0.000)

CD15 was positive in 9 samples with percentage (17.6%) and negative in 42 samples with percentage (82.4%), all the positive CD15 results were in Transitional Cell carcinoma.

There is a significance correlation between CD15 expression and malignant tumors of gallbladder (P-value= 0.000).

المستخلص

اجريت هذه الدراسة الوصفية التراجعية في مستشفى المركز الطبي الدولي في مدينة جدة في المملكة العربية في الفترة من سبتمبر ٢٠١٩ الى يناير ٢٠٢٠ .هدفت هذه الدراسة الى الكشف عن الواسمتين الورميتين CD15و CK20 في اورام المرارة باستخدام كيمياء ومناعة الانسجة، تم جمع العينات من 51 مريضا مشخصين مسبقا باورام المرارة ، موزعين على النحو التالي ؛ 19 منها اورام حميدة ، 16 اورام سرطانية غدية المصدر و 16 اورام سرطان الخلايا الانتقالية.تراوحت اعمار المرضى بين 25 الى 97 سنة بمتوسط عمر 60 سنة.

تم تحليل البيانات التي تم الحصول عليها باستخدام برنامج التحليل الاحصائي (الحزم الاحصائية للدراسات الاجتماعية) من51مريضا, 26 مريضا كانو من الاناث بنسبة(51%) و25 مريضا كانو من الذكور بنسبة (49%) .اعطى ظهور ال CK20 نتيجة ايجابية في 31 عينة بنسبة(60.8%) وكانت النتائج سلبية في 20 عينة بنسبة(39,2%).

اعطى ظهور الواسمتين المرضيتين CK20 و CD15 نتائج سلبية في الاورام الحميدة للمرارة.

كانت النتائج الايجابية لل CK20 في 31 عينة في الاورام الخبيثة بنسبة (100%) موزعة كالتالي 13 منها في الاورام السرطانية الغدية المصدر بنسبة (42%) و 18 منها في سطان الخلايا الانتقالية بنسبة (58%).

اعطى ظهور الواسمة المرضية CD15 نتائج ايجابية في9 عينات بنسبة (17.6%) كانت في الاورام الخبيثة وجميعها في اورام الخلايا الانتقالية.

خلصت الدراسة الى انه يوجد ارتباط بين التعبير النسيجي للCK20 واورام الحويصلة المرارية حيث القيمة الاحتمالية (0.000) ويوجد ايضا ارتباط بين التعبير النسيجي لل CD15 واورام الحويصلة المرارية حيث لقيمة الاحتمالية (0.000), ما يدل على وجود ارتباط بين افراز CD15 وCK20 والانواع الفرعية لسرطان المرارة الخبيث.

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Chapter One

Introduction

Chapter One

1.1.Introduction

Gallbladder cancer (GBC) is one of the most common and aggressive malignant neoplasms of the biliary system. Early-stage GBC lacks typical clinical manifestations, leading to a 5-year survival. Most patients are at the advanced stage at the time of diagnosis, and thus lose the chance of radical cure. It is therefore important to diagnose GBC earlier. Currently, the diagnosis of GBC mainly depends on non-invasive auxiliary imaging and invasive examination such as laparoscopy and biopsy. However, there is no ideal single tumor marker for the diagnosis and prognosis of GBC (Yun-Feng *et al*., 2014)

During the last four decades, since the introduction of diagnostic immunopathology, tumor markers have been widely accepted and essential rules in the management of patients with cancer. In recent years definition of tumor marker has been expanded to include, in addition to those markers circulating in the blood, marker measured either quantitatively or qualitatively in tissue and other body fluids including urine and cerebrospinal fluid and even the assay of genes and oncogenes. (Kumar *et al* .,2014)

Gallbladder cancer accounts for 1.2% of all global cancer diagnoses, but 1.7% of all cancer deaths. Only 1 in 5 GBC cases in the United States is diagnosed at an early stage, and median survival for advanced stage cancer is no more than about a year (Rawla *et al*.,2000).

The great advance in the uses of laparoscopic surgery in Sudan, especially in biliary apparatus interference, makes the need to revise the anatomical data of the gallbladder more important. Congenital anomalies and anatomical variations of extra-hepatic biliary tree are not common but can be of clinical importance and surprising, if present. Awareness of these anomalies will decrease morbidity, conversion, and re-exploration in these patients (Aballa *et al*.,2012).

1.2. Rational

Gallbladder cancer is disproportionately deadly because it is rarely found before it has advanced or metastasized. A better understanding of the etiology, risk factors and diagnosis for the disease will allow patients to make modifications to prevent the disease (Rawla *et al*.,2000).

In tumors it is reported, there is a marked difference in the expression of CK 20 within different carcinomas. Neoplasms expressing CK 20 are derived from normal epithelia which themselves expressed CK20 (Leong *et al.,* 2003).

CD15 is a sensitive and specific marker for intraepithelial and invasive neoplasias of the bile duct (Walter *et al.*,2016).

CD15 expression and its contents could be involved in aggressiveness of carcinoma of the gallbladder and might be a useful indicator to evaluate the malignancy and biological features and could be considered as a good prognostic predictor for patients with carcinoma of the gallbladder. (Gu *et al*.,2000)

The tumor markers CK20 & CD15 will be used in this study to test their diagnostic Value in Gallbladder cancers. The significance of these markers will lead to better understanding of the gallbladder tumors.

1.3. Objectives

1.3.1. General Objective

To study the expression of CK20 & CD15 in Gallbladder tumors.

1.3.2. Specific Objectives:

1-To detect CK 20 and CD15 in Gallbladder tumors using immunohistochemistry.

2-To associate the expression pattern of CK 20 and CD15 with Histological diagnosis (benign, Adenocarcinoma and Transitional Carcinomas).

Chapter Two

Literature Review

Chapter Two

2. Literature Review

2.1. Anatomy & Physiology of Gallbladder:

The gallbladder is 8–10 cm (3–4 in) long and is nested in a shallow area on the posterior aspect of the right lobe of the liver. This muscular sac store concentrates, and, when stimulated, propels the bile into the duodenum via the common bile duct. It is divided into three regions. The fundus is the widest portion and tapers medially into the body, which in turn narrows to become the neck. The neck angles slightly superiorly as it approaches the hepatic duct. The cystic duct is 1–2 cm (less than 1 in) long and turns inferiorly as it bridges the neck and hepatic duct (Gordon *et al.*, 2013).

The wall of the gallbladder has the same layers of issue as hose of the basic structure of he alimentary canal, with some modifications. The upper surface of the gall bladder is in direct contact with the liver and held in place by the visceral peritoneum hat covers the liver. There is an additional layer of oblique muscle fibers and mucous membrane which displays small rugae when the gall bladder is empty that disappear when it is distended with bile. The gallbladder concentrates the bile by up to 10 or 15fold, by absorption of water through the walls of the gall bladder to be released in need (Waugh and Grant, 2014).

2.2. Epidemiology of Gallbladder Cancer:

Gallbladder carcinoma (GB CA) is the most common malignancy of the biliary tree and the fifth most common gastrointestinal cancer following malignant tumors of the colon, pancreas, stomach, liver, and esophagus. The incidence of gallbladder cancer is approximately 1–3 cases per 100,000 persons. It represents approximately 6,000 new diagnoses per year in the United States alone. (Brady *et al.*,2014).

Countries with the top five highest age-standardized incidence rates per 100,000 for males in 2018 are Bolivia (12.8), Thailand (9.0), Republic of Korea (8.4), Chile (6.6) and Nepal (6.0). Countries with the top five highest age-standardized incidence rates per 100,000 for females in 2018 are Bolivia (15.1), Chile (11.7), Bangladesh (7.3), Nepal (7.3) and Peru (6.0). Rawla *et al*.,2000).

Marked geographic differences seen in frequency of gallbladder carcinoma suggest a possible environmental cause besides race or ethnicity (Yalcin, 2004).

2.3Pathology of Gallbladder

1-Cholecystitis

- **Acute cholecystitis :**

  In many cases, gallstones block the bile ducts. Bile builds up and can push on the walls of the gallbladder, causing inflammation. Other causes of acute cholecystitis include infection, trauma, diabetes, or blockage of the bile ducts due to a tumor (Parmet *et al*.,2003).

- **Chronic cholecystitis:**

  The thickening of the gallbladder wall occurs as a result of extensive fibrosis. Chronic inflammation is frequently complicated by gallstones. Cholelithiasis (gallstones**)** has a higher incidence in women and is often associated with obesity and multiple pregnancies (Arthur *et al.*, 2014).

2-Tumors of gallbladder

It is not always clear whether bladder tumors are benign or malignant. Tumors are often multiple and recurrence is common.. Tumors of the gallbladder can be divided as following:

A- Benign tumors

- Adenomas are the most common benign neoplasms of the gallbladder.
- Cholesterol polyps are the most common pseudotumor of the gallbladder, the polyps can be single or multiple, usually less than 10 mm in size (Pejić and Milić ,2003)

B- Malignant tumors:

- **Solid tumors:**

  These are all malignant to some degree. At an early stage, the more malignant and solid tumors rapidly invade the bladder wall and spread in lymph and blood to other parts of the body. If the surface ulcerates there may be hemorrhage and necrosis **(**Waugh & Grant ,2014).

- **Adenocarcinoma:**
  It is the most common primary tumor of the gallbladder, which is often associated with gallstones. (Rawla *et al*.,2000).

- **Carcinoma of the extrahepatic biliary ducts and the ampulla of Vater:**

  It is less common than carcinoma of the gallbladder and almost always adenocarcinoma. Typical presenting features include a progressive, relentless obstructive jaundice. Clinical characteristics include the combination of jaundice and a palpably enlarged gallbladder**.** Tumors that obstruct the common bile duct (Arthur *et al.*, 2014).

- **Transitional cell carcinomas**:

  It is also known as papillomas, arise from the transitional epithelium and are often benign. They consist of a stalk with fine-branching fronds, which tend to break off causing painless bleeding and hematuria. Papillomas commonly recur, even when benign. Sometimes the tumor cells are well differentiated and non-invasive but in other cases, they behave as carcinomas and invade surrounding blood and lymph vessels **(**Waugh & Grant ,2014).

2.4 Risk Factors of Gallbladder Cancers

1-Gallstones:

Gallstones increases the risk of this cancer. Inflammation associated with gallstones decreases the speed at which bile empties from the gallbladder; gallstones may also have a direct effect by blocking the transit of bile or by causing direct mechanical irritation to the surrounding mucosal surface (Norat *et al*.,2018).

2-Age:

Gallbladder cancer (GBC) is usually diagnosed after 60 years of age. Behavior of GBC in young patients is largely unknown (Gupta *et al.*, 2014).

3-Gender:

Gallbladder cancer occurs very frequently in patients with pancreaticobiliary maljunction without biliary dilatation, and women significantly higher risk than men (Kamisawa *et al* .,2008)

4-Pregnancy:

Fertility and the number of births are associated with gallbladder cancers. Pregnancy increases the risk of gallstones, but is less associated with gallbladder cancer (Mahdavifar *et al* .,2018).

5-Ethnicity:

Mexican Americans and Native Americans, particularly in the southwestern United States, are more likely to develop gallbladder cancer than the general population (Lora *et al*., 2011).

6- Smoking:

Tobacco use may increase the risk of gallbladder cancer. Predisposing factors include cigarette smoking, prolonged use of certain analgesics, and occupational exposure to some chemicals, e.g., aniline dyes used in the textile and printing industries. (Arthur *et al.*, 2014).

7-Family history& dietary factors:

Among other risk factors, family history of gallbladder cancer, a number of genetic, dietary factors, have been associated with the development of gallbladder cancer. (Eduardo *et al*.,2001).

8-Infections:

Other reported risk factors include congenital biliary cysts, infectious factors (Salmonella typhi), primary sclerosing cholangitis (PSC), and genetic factors. (Brady *et al.*,2014).

9-Peutz- Jeghers syndrome

Gallbladder cancer has also been associated with multiple familial polyposis/Gardner syndrome, Peutz- Jeghers syndrome, "porcelain" gallbladder, and anomalous pancreato-biliary ductal union. (Eduardo *et al*.,2001).

10-Exposing to chemicals

Other factors may also be involved, and many toxins, whether they come from diet, smoke inhalation or other environmental sources (and their metabolic products), are excreted and concentrated in the bile (Norat *et al*.,2018).

2.5. Diagnosis of Gallbladder Cancer

1-Blood tests:

Blood tests to evaluate the liver function may help the doctor determine what's causing the signs and symptoms. (American Cancer Society ,2018).

2-Ultrasound &CT scan:

Ultrasound is the most sensitive and informative initial test, as most patients being evaluated have benign gallbladder disease; however, CT provides better anatomic detail in evaluating the liver and porta hepatis for adenopathy in patients with advanced disease. (Wanebo & Avradopoulos ,2002).

3- The combination of MRI with MRA:

The combination of MRI with MRA (magnetic resonance angiography) and MRCP (magnetic resonance cholangiopancreatography) is useful in detecting vascular invasion and biliary tract involvement (Prasad & Sen ,2021).

4-Exploratory surgery:

In recent years, laparoscopic cholecystectomy (LC) has become the gold standard management for gallbladder disease. It is a routine in the treatment of benign gallbladder disease worldwide. LC that is performed for benign gallbladder disease rarely results in a diagnosis of unexpected gallbladder carcinoma (Utsumi *et al*.,2017).

5-Cytology:

Imprint cytology of the gallbladder mucosa is an easy, rapid, and high-quality method for detecting gallbladder carcinoma . Ultrasound-guided fine-needle aspiration cytology is also a safe diagnostic modality for gallbladder carcinoma. (Sandberg,2012).

2.6. Symptoms of Gallbladder Cancer:

Gallbladder cancer doesn't usually cause signs or symptoms until later in the course of the disease, when the tumor is large and/or has spread. Sometimes symptoms can appear and include abdominal pain, nausea, vomiting, Jaundice, yellow skin, yellow color in the white part of the eye and lumps in the belly, If the cancer blocks the bile ducts, the gallbladder will swell (American Cancer Society ,2018).

2.7. Treatment of Gallbladder Cancers

1-Open surgery:

Open surgery has been recommended even for suspected early-stage gallbladder cancer However, with the increasing incidental detection of gallbladder cancer resulting from widespread application of laparoscopic cholecystectomy, reports have shown that laparoscopic surgery does not adversely influence the prognosis of patients with early-stage GBC (Han *et al*.,2019).

2-Targeted therapy:

Targeted therapy for cancers based on the concept of specific chemicals block a number of molecular targets that are keenly associated with tumor cell proliferation, differentiation, migration, cancer stemness, vascular angiogenesis, and antitumor immune responses (Song *et al*., 2020).

3-Radeotherapy:

Moreover, several studies also reported that adjuvant RT with or without chemotherapy is associated with better survival rates than those of surgery alone reported in historical studies (Jeong *et al*., 2014).

4-Chemoghrapy:

Reported response rates with chemotherapy in patients with gallbladder cancer are in the range of 50 to 60 percent. There is limited data assessing the impact of treatment on overall survival (Sirohi *et al*.,2014).

2.8. CK20

Cytokeratin 20 (CK20) is a type I acidic LMWCK. It is found in normal tissues of the stomach, intestine, urothelium, and in Merkel cells. It is expressed in most adenocarcinomas of the large and small intestines, in some mucinous tumors of the ovary, and in Merkel cell carcinomas. It is frequently present in urothelial carcinoma and in adenocarcinoma of the stomach, pancreas, and bile ducts. CK20 is a useful marker for primary mucinous tumors of the ovary and for various types of metastases found in the ovaries. Most primary no mucinous ovarian epithelial tumors are CK20 negative (Dabbas ,2019).

2.9. CD15:

CD15, the X hapten or Lewis X antigen, originally noted as a monocyte/myeloid cell marker, it was recognized as a marker for the Reed-Sternberg cells of classical Hodgkin lymphoma. (Dabbas,2019).

Myeloid cells and eosinophils; activated B and T cells (including infectious mononucleosis); variable monocytes and basophils. Kidney proximal convoluted tubules (Nat ,2014).

It is negative in most non-Hodgkin lymphomas, with the exception of some primary cutaneous anaplastic large cell lymphomas and other peripheral T-cell lymphomas. It is also used to stain adenocarcinoma (Dabbas,2019).

CD15 antibody reacted with a high proportion of both normal and metaplastic Paneth cells. Paneth cell immunoreactivity to CD15, however, was less intense and less extensive than to antilysozyme antibody, though the latter also stained many other cell types and was more commonly associated with nonspecific background staining. (Ariza *et al*.,1996)

Chapter Three

Materials &methods

Chapter Three

3.Materials &methods

3.1 Study Design

This is a descriptive retrospective case study aiming at evaluating the expression of CK20 & CD15 tumor marker in the gallbladder using immunohistochemistry technique.

3.2Study area

This study was held in Jeddah –Saudi Arabia in International Medical Center Hospital during the period from September 2019 to January 2020.

3.3 Study population

Fifty-one gallbladder formalin-fixed paraffin-embedded archival blocks were taken and primarily diagnosed by Hematoxylin and Eosin; they have been selected as 16 benign,16 adenocarcinoma & 19 transitional cell carcinoma.

3.4 Sample collection

Samples were collected from tissue blocks which were already diagnosed by pathologist as malignant and benign Gallbladder tumors. The selected tissue blocks sent for subsequent procedures.

3.5 Sample processing :

3.5.1 Tissue processing:

All the blocks were processed by the Sakura VIP 5Jr. It is a vacuum infiltration tissue processer**.**

3.5.2 Hematoxylin & Eosin Staining:

The H& E staining was performed automatically by Leica 4040 linear Stainer which includes dewaxing, rehydration, Gills hematoxylin staining bluing by running tap water, staining by aqueous Eosin Y, dehydration through ascending grades of ethanol and clearing by Xylene. The mounting of the slides was done manually using commercially prepared Synthetic mounting media

3.5.3 Automated immunohistochemistry incubation methods:

Automated IHC systems such as the Leica Bond III use a flatbed system with unique Covertile technology facilitating uniform staining of the tissue.

(Bancroft *et al* ,2018)

The full IHC procedure, including dewaxing and antigen retrieval, is carried out using this automation. Two sections (4µm) from formalin-fixed paraffin-embedded tumor was cut and mounted onto coated slides (Leica). Following deparaffinization in xylene, slides was rehydrated through a graded series of alcohol and placed in distilled water. Samples were steamed for antigen retrieval for E cadherin using Leica Bond Max strainer, slides was placed in the staining rack which has the capacity of 10slides the slides where covered with tris buffer (ph9.0) to cover the sections, then the machine was turn on and programmed as follow, 20 minutes to start heating from 65ºc till it reach 95ºc and then boiled at high temp (95ºc) for 20 minutes then will allow sections to cool to 65ºc . Endogenous peroxidase activity was blocked with peroxidase blocking reagent (3% hydrogen peroxide and methanol) for 10 minutes.

3.6 Sample Staining

The CK20 slides were incubated with 100-200µl of primary antibody (CK 20) (Leica) for 20 minutes at room temperature in moisture chamber, also the same incubation period was applied for CD15 and then all slides will be rinsed in phosphate buffer saline, after washed with PBS for 3minutes, binding of antibodies will be detected by incubating for 20 minutes with dextran labeled polymer (Leica Polymer Refine detection). Finally, the sections will be washed in three changes of PBS, followed by adding 3,3 diaminobenzidine tetra hydrochloride (DAB) as chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 minutes. Slides were counter stain with hematoxylin (Gill's) for one minute, Dehydrate, clear and mounted in DPX.

3.7 Statistical analysis

To achieve the objectives of the study and to verify hypothesis, statistical methods were used are Charts, frequency distribution of the pathological changes, percentages, Chi-square test for the significance differences between the Expression of Ck20 & CD15 in subtypes of malignant and Benign gallbladder tumors. To obtain results as accurate as possible, SPSS (version23) statistical software has been used, which indicates a shortcut to Statistical Package for Social Sciences.

3.8 Ethical consideration

All samples and information were taken after legal permission and within the consent authority of the International Medical Center Hospital in Jeddah.

Chapter Four

Results

Chapter Four

4. Results

The study involved 51 subjects, already diagnosed by pathologist as malignant and benign gallbladder tumors. Table (1) displays the distribution gallbladder tumors patients according to histopathological diagnosis, (35) malignant gallbladder tumors (68.7%) distributed as follow (16) Adenocarcinoma (31.3%) and (19) Transitional Cell Carcinoma (37.4 %) ,the remaining (16) benign gallbladder tumors (31.3%).

The study subjects age was ranged from 25to 97 years, all subjects were grouped into two age groups.

Figure (1) shows the age distribution of the study population;(14) of the study samples with percentage (72.5%) were more than or equal to 60 years old while (37) were less than 60 years old with percentage (27.5%).

Figure (2) shows the gender distribution of the study population, (26) patient which is more than half of the study population were females with percentage (51%),the remining (25) patient were males with percentage (49%)

Table (2) shows the relation between CK20 expression and tumor types ,where it was negative in all benign gallbladder (16) tumors. In malignant tumors (31) positive with percentage (60.8%) and (4) negative with percentage (7.8%). The correlation is significant at (0.05) level. That means CK20 secretion is significantly associated with the type of the tumor, positive CK20 expression was more frequent in patient with malignant tumors (P-value = 0.000).

Table (3) shows the relation between CD15 expression and gallbladder tumors, (9) positive among malignant tumors with percentage (17.6%) while it was totally negative in benign tumors. The CD15 secretion is significantly associated with the type of the tumor, positive CD15 was more frequent in patient of malignant tumor of (Transitional Cell Carcinoma(P-value = 0.000).

Table (4) shows the relation of CK20 and subtypes of malignant gallbladder tumors : Out of total(16) Adenocarcinoma patient ,(13)subjects positive with percentage (37.1), while (3) were negative with percentage (8.6%).

Out of ((19) subjects od Transitional Cell Carcinoma (18) subjects were positive with percentage (51.4%),while (1) subject was negative with percentage of (2.9%). the P-value (0.212) which means that there is no significant association with the subtype of the malignant tumors.

Table (5) shows the relation between CD15 expression and subtypes of malignant gallbladder tumors. Out of (35) samples (9) samples were positively expressed with percentage (25.7%) in Transitional cell carcinoma, and it was negative in the remaining (10) samples of the transitional cell carcinoma with percentage (28.6%). All the (16) samples of the Adenocarcinoma were negatively expressed with percentage (45.7%), the P-Value (0.001) that means CD15 secretion significantly associated with the subtypes of the malignant tumors, positive with the Tractional Cell Carcinoma type o tumors.

Table 4.1: Distribution of patients according to histopathological diagnosis

Histopathological Diagnosis		Frequency	Percentage
Benign		16	31.3%
malignant	Adenocarcinoma	16	31.3%
	Transitional Cell Carcinoma	19	37.4%
Total		51	%100

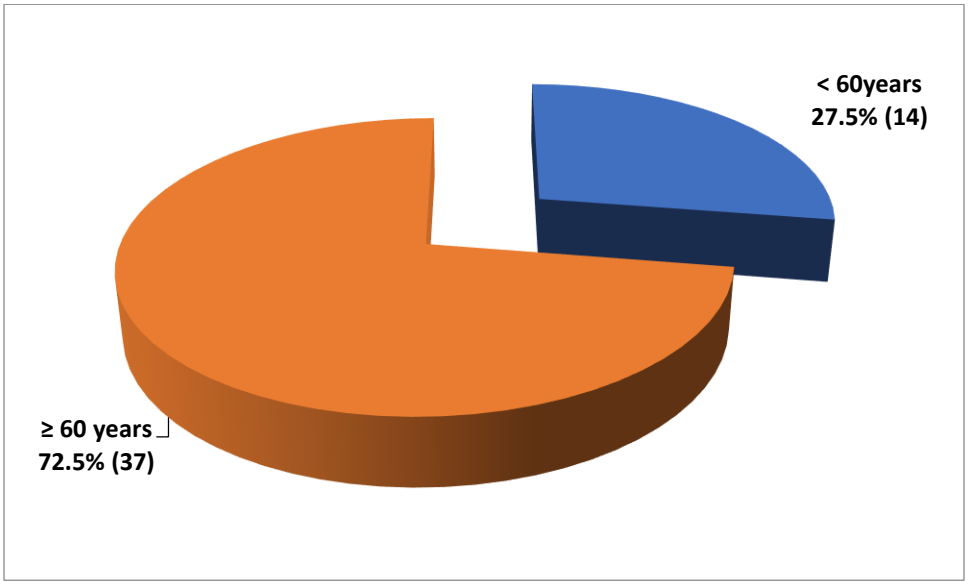

Figure 4.1: Age Distribution of the study population.

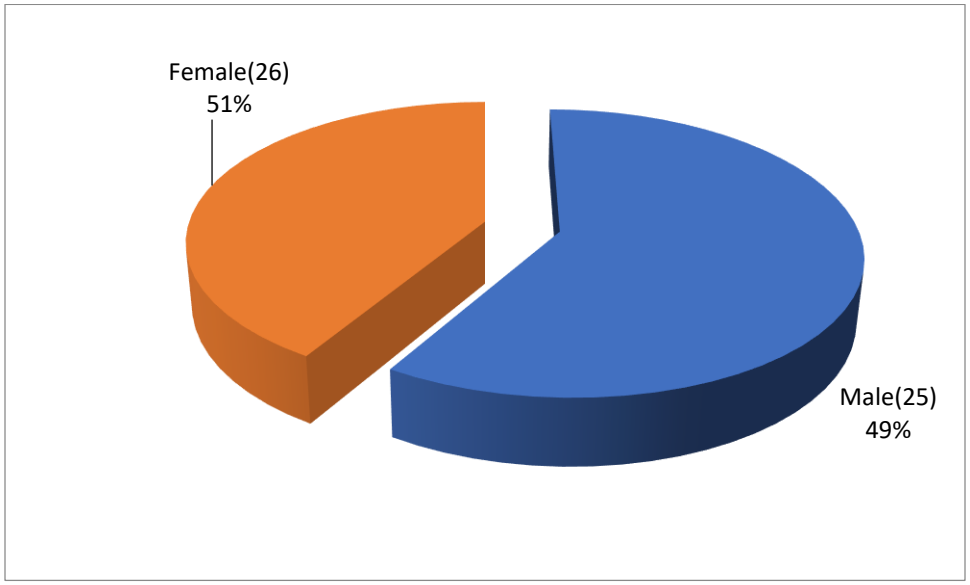

Figure 4.2: Gender Distribution of the study population.

Table 4.2: Relation between CK20 and tumor types.

Histopathological Diagnosis	CK20				Total		p-value
	Positive		Negative				
	f	*%*	*f*	*%*	*f*	*%*	
Benign	0	0%	16	31.4%	**16**	31.4%	**0.000**
malignant	31	60.8%	4	7.8%	35	**68.6%**	
Total	**31**	**60.8%**	**20**	**39.2%**	**51**	**100%**	

Table 4.3: Relation between CD15 and tumor types.

Histopathological Diagnosis	CD15				Total		p-value
	Positive		Negative				
	f	*%*	**0.000**	*%*	*f*	*%*	**0.000**
Benign	0	0%	16	31.4%	**16**	31.4%	
malignant	9	17.6%	26	51%	35	**68.6%**	
Total	**9**	**17.6%**	**42**	**82.4%**	**51**	**100%**	

Table 4.4: Relation between CK20 and subtypes of malignant tumors.

Malignant Subtype	CK20				Total		P-value
	Positive		Negative				
	f	*%*	*f*	*%*	*f*	*%*	
Adenocarcinoma	13	37.1%	3	8.6%	**16**	**45.7%**	**0.212**
Transitional Cell Carcinoma	18	51.4%	1	2.9%	**19**	**54.3%**	
Total	**31**	**88.5%**	**4**	**11.5%**	**35**	**100%**	

Table 4.5: Relation between CD15 and subtypes of the malignant tumors

Malignant Subtype	CD15				Total		p-value
	Positive		Negative				
	f	*%*	*f*	*%*	*f*	*%*	
Adenocarcinoma	0	0%	16	45.7%	**16**	**45.7%**	**0.001**
Transitional Cell Carcinoma	9	25.7%	10	28.6%	**19**	**54.3%**	
Total	**9**	**25.7%**	**26**	**74.3%**	**35**	**100%**	

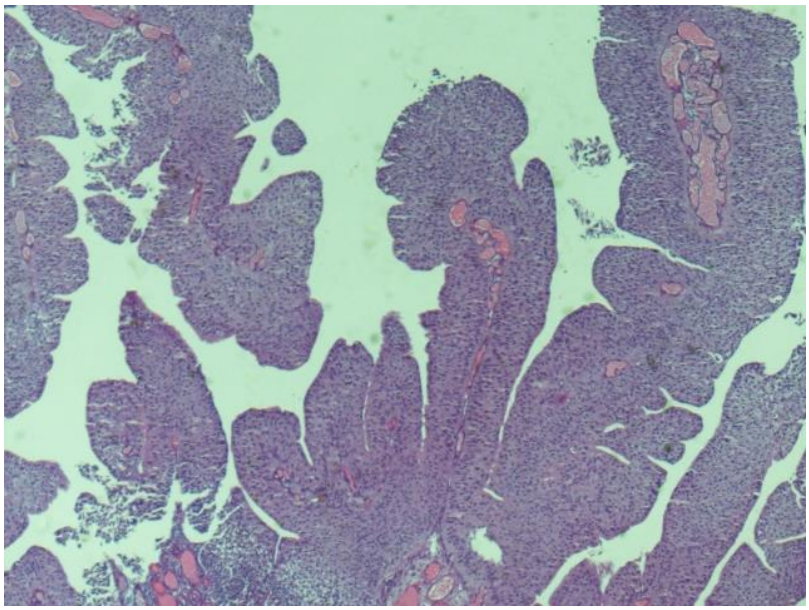

Photo 4.1 H&E staining of adenocarcinoma of the gallbladder (40X)

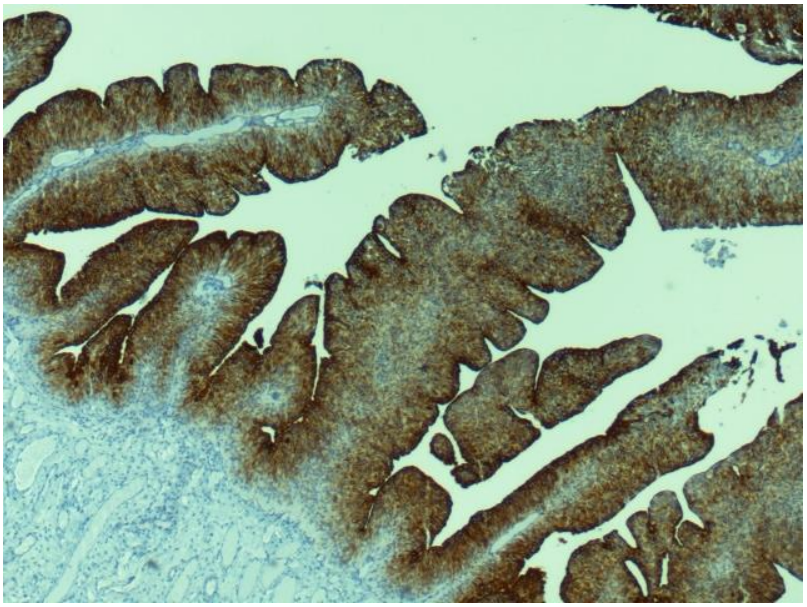

Photo 4.2 Positive immunohistochemical staining of CK20 marker in adenocarcinoma of Gallbladder (40X)

Chapter Five

Discussion, Conclusion & Recommendations

Chapter Five

5. 1Discussion

The immunohistochemical markers most often employed in the workup of adenocarcinomas of different sites usually include CK20 and CK7, However, there are very few reports in the literature regarding CD15 and CK20 expression in gallbladder carcinoma This study aimed to correlate the expression pattern of CK20 & CD15 to clinicopathological features of Gallbladder tumors. A group of (16) benign urinary bladder patients was studied, there were 11 patients constitute (68.6%) of this sample their age more than 45 years. of average age was (51 ± 12.4), the majority (62.5%) were females, which agree with the literature review that Women are about twice as likely to develop gallbladder cancer as men (Brady *et al.*,2014) .

All of the patients with benign tumors were negatively expressed for both CK20 and CD15 immunohistochemical markers, this result agrees with that found by (Jiang *et al.*,2001) CK20 is restricted to superficial and occasional intermediate cells of the normal urothelium of the bladder. Aberrant CK20 expression has been documented in urothelial carcinoma and has proved useful as an ancillary diagnostic aid for urinary bladder tumors. Another group consisted of (16) Adenocarcinoma patients was also studied, the results showed that (11) patients represented (68.8%) of this sample their age more than 65 years of average age (70±7.1), the majority were females. All patients of the Adenocarcinoma group showed positive expression for CK20, but a negative expression for CD15, this result agreed with (Krafft *et al*., 2018) findings in which analysis of a total of 116 urachal adenocarcinomas, only 4 cases were negative for CK20, an overall positive rate of (97%).

A group of (19) of transitional cell patients was studied, out of the total (19), there were 10 patients constitute (52.6%) of this sample their age more than 65 years, the

group average age was (was (66.8 ± 7.1). a great majority of this group (84.2%) were females. Most of the group patients (94.7%) showed positive expression for CK20. And less than half of them (47.4%) showed positive CD15. The results of the study come in line with that effective systematic use of appropriate immunohistochemical panels enables accurate classification of most of the undifferentiated carcinomas, as well as careful preservation of tissues for potential molecular or other ancillary tests (Selves, 2018) Also, agree with (Wegelin *et al*., 2015), who found (37) patients (46.3%) underwent transurethral resection or ureterorenoscopy procedures, in (30) patients (37.5%) tumor presence was confirmed by histopathology.

This result agrees with (Jiang *et al*., (2001) study which found in all cases, there was a concordant expression of CK20 in primary cancer and its matched lymph node metastasis.

Based on this study we found that out of 19 pteints of transitional Cell Carcinoma (9)of these patient (47.%) were positively expressed with CD15.

5.2 Conclusion:

The tumor markers CK20 & CD15 could be used to distinguish between benign and malignant tumors of the gallbladder.

On the other there is a significant relation between CD15 and subtypes of malignant gallbladder tumors, specifically transitional cell carcinoma, so it could be used in the differential diagnosis of the transitional cell carcinoma of the gallbladder.

5.3 Recommendations

The tumor marker CK20 has limited significance with subtypes of the gallbladder tumors more studies should be done with large sample size to demonstrate the use of Ck 20 in the subtypes of the malignant gallbladder tumors, the researches are very limited in Sudan & there is lack of published data .

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Appendices

Appendix (1)

1-Instrument and materials:

1-Instrument:

-Rotary microtome

-Oven

-Leica Liner ST40 H&E stainer

-Leica Bond Max III IHC stainer

-Stainless microtome blade

-Leica charged slides

-PT link

-Cover glass

-Water bath

-Work station

-Sony camera

-Microscope

2. Materials:

-Xylene

-Ethyl alcohol

-Gill‘s hematoxylin

-Distilled water

-Citrate buffer

-Peroxidase blocker

-Anti CK20 antibodies (primary antibody)

-Anti CD15 antibodies (primary antibody)

-Leica Bond Max Polymer detection Kit

-DPX mounting media

Appendix (2)

IMC hospital legal permission form.

المركز الطبي الدولي
International Medical Center

To : Ms. Amal A. Haidar
Principal Investigator

Project: Significance of CK20 & Cathepsin Expression in Human Gallbladder Carcinoma

IMC-IRB #: 2020-05-120

From : Research Center, International Medical Center

Date : 18 May 2020

International Medical Center (IMC) IRB[1] has reviewed and approved your retrospective study proposal submission titled **"Significance of CK20 & Cathepsin Expression in Human Gallbladder Carcinoma"** and the below related documents:

1. IMC Retrospective Application form

The submission was reviewed by all IRB members and got the full approval with a super majority of members (without conflicts of interest) voted in favor of projects with no major/minor concerns.

Good luck and we wish you all the success.

Best Regards,

Prof. Ezzeldin M. Ibrahim
Executive Director, Research Center

[1]International Medical Center IRB is an Institutional Review Board, established in accordance with 7 CFR lc.107, 10 CFR 745.107, 14 CFR 1230.107, 15 CFR 27.107, 16 CFR 1028.107, 21 CFR 56.107, 22 CFR 225.107, 24 CFR 60.107, 28 CFR46.107, 32 CFR 219.107, 34 CFR 97.107, 38 CFR 16.107, 40 CFR 26.107, 45CFR 46.107, 45 CFR 690.107, or 49 CFR 11.107 and in compliance to ICH GCP.

[2]Independent affiliated IRB member

المركز الطبي الدولي
International Medical Center

APPENDIX 1

INTERNATIONAL MEDICAL CENTER (IMC) IRB MEMBERS

S.no	Name	Position/Title
1	**Abdul Hameed Hassan**	Consultant, Family Medicine
2	**Ezzeldin Ibrahim**	Consultant, Oncology
3	**Ibrahim Mansoor**	Consultant, Anatomical Pathology & Clinical Chemistry
4	**Mohammad A. Albar**[4]	Consultant, Islamic Medicine
5	**Mohammed Janish**	Clinical Research Coordinator
6	**Raheel Shariff**	Consultant, Orthopedic
7	**Nashaat S. Hamza**	Consultant, Infection Control
8	**Hatem S. Bayoumy**	Clinical Pharmacist
9	**Abdullah E. Khalil**	Consultant, Emergency Medicine

[4]Independent affiliated IRB member from other institution, consulted on the applicable Islamic sharia

Appendix (2)

Leaflet of the Kits

Leica
BIOSYSTEMS

BOND™ Ready-to-Use Primary Antibody CD15 (MMA)

Catalog No: PA0473

Leica Biosystems Newcastle Ltd
Balliol Business Park
Benton Lane
Newcastle Upon Tyne NE12 8EW
United Kingdom
+44 191 215 4242

CE

EN FR IT DE ES PT SV EL DA NL
NO TR BG HU RO RU PL SL CS SK AR

Instructions for Use
Please read before using this product.

Mode d'emploi
Á lire avant d'utiliser ce produit.

Istruzioni per l'uso
Si prega di leggere, prima di usare il prodotto.

Gebrauchsanweisung
Bitte vor der Verwendung dieses Produkts lesen.

Instrucciones de uso
Por favor, leer antes de utilizar este producto.

Instruções de Utilização
Leia estas instruções antes de utilizar este produto.

Instruktioner vid Användning
Var god läs innan ni använder produkten.

Οδηγίες Χρήσης
Παρακαλούμε διαβάστε τις οδηγίες πριν χρησιμοποιήσετε το προϊόν αυτό.

Brugsanvisning
Læs venligst før produktet tages i brug.

Gebruiksinstructies
Lezen vóór gebruik van dit product.

Bruksanvisning
Vennligst les denne før du bruker produktet.

Kullanım Talimatları
Lütfen bu ürünü kullanmadan önce okuyunuz.

Инструкции за употреба
Моля, прочетете преди употреба на този продукт.

Használati utasítás
A termék használatba vétele előtt olvassa el.

Instrucțiuni de utilizare
Citiți aceste instrucțiuni înainte de a utiliza produsul.

Инструкция по применению
Прочтите перед применением этого продукта.

Instrukcja obsługi
Przed użyciem tego produktu należy przeczytać instrukcję.

Navodila za uporabo
Preberite pred uporabo tega izdelka.

Návod k použití
Čtěte před použitím tohoto výrobku.

Návod na použitie
Prosím, prečítajte si ho pred použitím produktov.

إرشادات الاستعمال
يُرجى القراءة قبل استخدام هذا المنتج.

Check the integrity of the packaging before use.
Vérifier que le conditionnement est en bon état avant l'emploi.
Prima dell'uso, controllare l'integrità della confezione.
Vor dem Gebrauch die Verpackung auf Unversehrtheit überprüfen.
Comprobar la integridad del envase, antes de usarlo.
Verifique a integridade da embalagem antes de utilizar o produto.
Kontrollera att paketet är obrutet innan användning.
Ελέγξτε την ακεραιότητα της συσκευασίας πριν από τη χρήση.
Kontroller, at pakken er ubeskadiget før brug.
Controleer de verpakking vóór gebruik.
Sjekk at pakningen er intakt før bruk.
Kullanmadan önce ambalajın bozulmamış olmasını kontrol edin.
Проверете целостта на опаковката преди употреба.
Használat előtt ellenőrizze a csomagolás épségét.
Verificați integritatea ambalajului înainte de a utiliza produsul.
Перед применением убедитесь в целостности упаковки.
Przed użyciem należy sprawdzić, czy opakowanie jest szczelne.
Pred uporabo preverite celovitost embalaže.
Před použitím zkontrolujte neporušenost obalu.
Pre použitím skontrolujte, či balenie nie je porušené.
تحقق من سلامة العبوة قبل الاستخدام.

www.LeicaBiosystems.com

Rx Only

BOND™ Ready-To-Use Primary Antibody CD15 (MMA)

Catalog No: PA0473

Intended Use

This reagent is for *in vitro* diagnostic use.

CD15 (MMA) monoclonal antibody is intended to be used for the qualitative identification by light microscopy of human CD15 protein in formalin-fixed, paraffin-embedded tissue by immunohistochemical staining using the automated BOND system (includes Leica BOND-MAX system and Leica BOND-III system).

The clinical interpretation of any staining or its absence should be complemented by morphological studies and proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation

Immunohistochemical techniques can be used to demonstrate the presence of antigens in tissue and cells (see "Using BOND Reagents" in your BOND user documentation). CD15 (MMA) primary antibody is a ready to use product that has been specifically optimized for use with BOND Polymer Refine Detection. The demonstration of human CD15 protein is achieved by first allowing the binding of CD15 (MMA) to the section, and then visualizing this binding using the reagents provided in the detection system. The use of these products, in combination with the automated BOND system (includes Leica BOND-MAX system and Leica BOND-III system), reduces the possibility of human error and inherent variability resulting from individual reagent dilution, manual pipetting and reagent application.

Reagents Provided

CD15 (MMA) is a mouse anti-human monoclonal antibody produced as a tissue culture supernatant, and supplied in Tris buffered saline with carrier protein, containing 0.35 % ProClin™ 950 as a preservative.

Total volume = 7 mL.

Clone

MMA.

Immunogen

BALB/C mice injected with U937 histiocytic cell line.

Specificity

Human CD15 antigen.

Ig Class

IgM.

Total Protein Concentration

Approx 10 mg/mL.

Antibody Concentration

Not applicable.

Dilution and Mixing

CD15 (MMA) primary antibody is optimally diluted for use on the BOND system (includes Leica BOND-MAX system and Leica BOND-III system). Reconstitution, mixing, dilution or titration of this reagent is not required.

Materials Required But Not Provided

Refer to "Using BOND Reagents" in your BOND user documentation for a complete list of materials required for specimen treatment and immunohistochemical staining using the BOND system (includes Leica BOND-MAX system and Leica BOND-III system).

Storage and Stability

Store at 2–8 °C. Do not use after the expiration date indicated on the container label.

The signs indicating contamination and/or instability of CD15 (MMA) are: turbidity of the solution, odor development, and presence of precipitate.

Return to 2–8 °C immediately after use.

Storage conditions other than those specified above must be verified by the user[1].

Precautions

- This product is intended for *in vitro* diagnostic use.
- The concentration of ProClin™ 950 is 0.35 %. It contains the active ingredient 2-methyl-4-isothiazolin-3-one, and may cause irritation to the skin, eyes, mucous membranes and upper respiratory tract. Wear disposable gloves when handling reagents.
- To obtain a copy of the Material Safety Data Sheet contact your local distributor or regional office of Leica Biosystems, or alternatively, visit the Leica Biosystems' Web site, www.LeicaBiosystems.com

- Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions[2]. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents or specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Seek medical advice.
- Consult Federal, State or local regulations for disposal of any potentially toxic components.
- Minimize microbial contamination of reagents or an increase in non-specific staining may occur.
- Retrieval, incubation times or temperatures other than those specified may give erroneous results. Any such change must be validated by the user.

Instructions for Use

CD15 (MMA) primary antibody was developed for use on the automated BOND system (includes Leica BOND-MAX system and Leica BOND-III system) in combination with BOND Polymer Refine Detection. The recommended staining protocol for CD15 (MMA) primary antibody is IHC Protocol F. Heat induced epitope retrieval is recommended using BOND Epitope Retrieval Solution 1 for 20 minutes.

Results Expected

Normal Tissues

Clone MMA detected the CD15 protein on the cell membrane and in the cytoplasm of epithelial cells and granulocytes in a variety of tissues evaluated. Staining was also observed in neuropil of the cerebrum and cerebellum, Leydig cells in testis and Hassall's corpuscles in thymus (Total number of normal cases evaluated = 123).

Tumor Tissues

Clone MMA stained 36/195 hematological malignancies (including 34/40 Hodgkin's lymphomas, 1/107 diffuse large B-cell lymphomas, 1/1 peripheral T-cell lymphoma, 0/11 chronic lymphocytic lymphomas, 0/11 follicular lymphomas, 0/7 T-cell anaplastic large cell lymphomas, 0/6 mantle cell lymphomas, 0/4 angioimmunoblastic T-cell lymphomas, 0/3 T/NK cell lymphomas, 0/1 B-cell acute lymphoblastic lymphoma, 0/1 primitive B/T cell acute lymphoblastic lymphoma, 0/1 T-cell lymphoma, 0/1 marginal zone lymphoma and 0/1 non-Hodgkin's B-cell lymphoma). Clone MMA also stained 8/9 bowel tumors, 3/4 lung tumors, 2/3 adenocarcinomas of the stomach, 2/2 clear cell carcinomas of the kidney, 2/2 ovarian tumors, 2/2 endometrial tumors, 1/5 brain tumors, 1/5 metastatic tumors, 1/3 squamous cell carcinomas of the esophagus, 1/2 tumors of the adrenal gland and 1/2 tumors of the head and neck. Except for infiltrating granulocytes, no staining was detected in a variety of additional abnormal tissues evaluated, including breast tumors (0/5), liver tumors (0/5), tumors of the thyroid (0/5), bladder tumors (0/2), prostatic tumors (0/2), melanomas (0/2), seminomas (0/2), cervical tumors (0/2), a tongue tumor (0/1), a pancreatic tumor (0/1), a prostatic hyperplasia (0/1), a tumor of the salivary gland (0/1), a skin tumor (0/1) and a bone tumor (0/1). (Total number of abnormal cases evaluated = 265).

CD15 (MMA) is recommended for the detection of CD15 protein in normal and neoplastic tissues, as an adjunct to conventional histopathology using non-immunologic histochemical stains.

Product Specific Limitations

CD15 (MMA) has been optimized at Leica Biosystems for use with BOND Polymer Refine Detection and BOND ancillary reagents. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results under these circumstances. The protocol times may vary, due to variation in tissue fixation and the effectiveness of antigen enhancement, and must be determined empirically. Negative reagent controls should be used when optimizing retrieval conditions and protocol times.

Troubleshooting

Refer to reference 3 for remedial action.

Contact your local distributor or the regional office of Leica Biosystems to report unusual staining.

Further Information

Further information on immunostaining with BOND reagents, under the headings Principle of the Procedure, Materials Required, Specimen Preparation, Quality Control, Assay Verification, Interpretation of Staining, Key to Symbols on Labels, and General Limitations can be found in "Using BOND Reagents" in your BOND user documentation.

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Date of Issue

13 March 2019

BOND™ Ready-to-Use Primary Antibody Cytokeratin 20 (Ks20.8)

Catalog No: PA0022

Leica Biosystems Newcastle Ltd
Balliol Business Park
Benton Lane
Newcastle Upon Tyne NE12 8EW
United Kingdom
+44 191 215 4242

CE

EN FR IT DE ES PT SV EL DA NL
NO TR BG HU RO RU PL SL CS SK AR

Instructions for Use
Please read before using this product.

Mode d'emploi
Á lire avant d'utiliser ce produit.

Istruzioni per L'uso
Si prega di leggere, prima di usare il prodotto.

Gebrauchsanweisung
Bitte vor der Verwendung dieses Produkts lesen.

Instrucciones de Uso
Por favor, leer antes de utilizar este producto.

Instruções de Utilização
Leia estas instruções antes de utilizar este produto.

Instruktioner vid Användning
Var god läs innan ni använder produkten.

Οδηγίες Χρήσης
Παρακαλούμε διαβάστε τις οδηγίες πριν χρησιμοποιήσετε το προϊόν αυτό.

Brugsanvisning
Læs venligst før produktet tages i brug.

Gebruiksinstructies
Lezen vóór gebruik van dit product.

Bruksanvisning
Vennligst les denne før du bruker produktet.

Kullanım Talimatları
Lütfen bu ürünü kullanmadan önce okuyunuz.

Инструкции за употреба
Моля, прочетете преди употреба на този продукт.

Használati utasítás
A termék használatba vétele előtt olvassa el.

Instrucțiuni de utilizare
Citiți aceste instrucțiuni înainte de a utiliza produsul.

Инструкция по применению
Прочтите перед применением этого продукта.

Instrukcja obsługi
Przed użyciem tego produktu należy przeczytać instrukcję.

Navodila za uporabo
Preberite pred uporabo tega izdelka.

Návod k použití
Čtěte před použitím tohoto výrobku.

Návod na použitie
Prosím, prečítajte si ho pred použitím produktov.

إرشادات الاستعمال
يُرجى القراءة قبل استخدام هذا المنتج.

Check the integrity of the packaging before use.
Vérifier que le conditionnement est en bon état avant l'emploi.
Prima dell'uso, controllare l'integrità della confezione.
Vor dem Gebrauch die Verpackung auf Unversehrtheit überprüfen.
Comprobar la integridad del envase, antes de usarlo.
Verifique a integridade da embalagem antes de utilizar o produto.
Kontrollera att paketet är obrutet innan användning.
Ελέγξτε την ακεραιότητα της συσκευασίας πριν από τη χρήση.
Kontroller, at pakken er ubeskadiget før brug.
Controleer de verpakking vóór gebruik.
Sjekk at pakningen er intakt før bruk.
Kullanmadan önce ambalajın bozulmamış olmasını kontrol edin.
Проверете целостта на опаковката преди употреба.
Használat előtt ellenőrizze a csomagolás épségét.
Verificați integritatea ambalajului înainte de a utiliza produsul.
Перед применением убедитесь в целостности упаковки.
Przed użyciem należy sprawdzić, czy opakowanie jest szczelne.
Pred uporabo preverite celovitost embalaže.
Před použitím zkontrolujte neporušenost obalu.
Pre použitím skontrolujte, či balenie nie je porušené.
تحقق من سلامة العبوة قبل الاستخدام.

www.LeicaBiosystems.com

Rx Only

BOND™ Ready-To-Use Primary Antibody Cytokeratin 20 (Ks20.8)

Catalog No: PA0022

Intended Use

This reagent is for *in vitro* diagnostic use.

Cytokeratin 20 (Ks20.8) monoclonal antibody is intended to be used for the qualitative identification by light microscopy of human cytokeratin 20 intermediate filament protein in formalin-fixed, paraffin-embedded tissue by immunohistochemical staining using the automated BOND system (includes Leica BOND-MAX system and Leica BOND-III system).

The clinical interpretation of any staining or its absence should be complemented by morphological studies and proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation

Immunohistochemical techniques can be used to demonstrate the presence of antigens in tissue and cells (see "Using BOND Reagents" in your BOND user documentation). Cytokeratin 20 (Ks20.8) primary antibody is a ready to use product that has been specifically optimized for use with BOND Polymer Refine Detection. The demonstration of human cytokeratin 20 intermediate filament protein is achieved by first allowing the binding of Cytokeratin 20 (Ks20.8) to the section, and then visualizing this binding using the reagents provided in the detection system. The use of these products, in combination with the automated BOND system (includes Leica BOND-MAX system and Leica BOND-III system), reduces the possibility of human error and inherent variability resulting from individual reagent dilution, manual pipetting and reagent application.

Reagents Provided

Cytokeratin 20 (Ks20.8) is a mouse anti-human monoclonal antibody produced as a tissue culture supernatant, and supplied in Tris buffered saline with carrier protein, containing 0.35 % ProClin™ 950 as a preservative.

Total volume = 7 mL.

Clone

Ks20.8

Immunogen

Cytoskeletal preparation isolated from microdissected villi of human duodenal mucosa.

Specificity

Human cytokeratin 20 intermediate filament protein.

Ig Class

IgG2a, kappa

Total Protein Concentration

Approx 10 mg/mL.

Antibody Concentration

Greater than or equal to 0.09 mg/L.

Dilution and Mixing

Cytokeratin 20 (Ks20.8) primary antibody is optimally diluted for use on the BOND system (includes Leica BOND-MAX system and Leica BOND-III system). Reconstitution, mixing, dilution or titration of this reagent is not required.

Materials Required But Not Provided

Refer to "Using BOND Reagents" in your BOND user documentation for a complete list of materials required for specimen treatment and immunohistochemical staining using the BOND system (includes Leica BOND-MAX system and Leica BOND-III system).

Storage and Stability

Store at 2–8 °C. Do not use after the expiration date indicated on the container label.

The signs indicating contamination and/or instability of Cytokeratin 20 (Ks20.8) are: turbidity of the solution, odor development, and presence of precipitate.

Return to 2–8 °C immediately after use.

Storage conditions other than those specified above must be verified by the user[1].

Precautions

- This product is intended for *in vitro* diagnostic use.
- The concentration of ProClin™ 950 is 0.35 %. It contains the active ingredient 2-methyl-4-isothiazolin-3-one, and may cause irritation to the skin, eyes, mucous membranes and upper respiratory tract. Wear disposable gloves when handling reagents.
- To obtain a copy of the Material Safety Data Sheet contact your local distributor or regional office of Leica Biosystems, or alternatively, visit the Leica Biosystems' Web site, www.LeicaBiosystems.com

- Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions[2]. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents or specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Seek medical advice.
- Consult Federal, State or local regulations for disposal of any potentially toxic components.
- Minimize microbial contamination of reagents or an increase in non-specific staining may occur.
- Retrieval, incubation times or temperatures other than those specified may give erroneous results. Any such change must be validated by the user.

Instructions for Use

Cytokeratin 20 (Ks20.8) primary antibody was developed for use on the automated BOND system (includes Leica BOND-MAX system and Leica BOND-III system) in combination with BOND Polymer Refine Detection. The recommended staining protocol for Cytokeratin 20 (Ks20.8) primary antibody is IHC Protocol F. Heat induced epitope retrieval is recommended using BOND Epitope Retrieval Solution 2 for 20 minutes.

Results Expected

Normal Tissues

Clone Ks20.8 detects cytokeratin 20 in the cytoplasm of normal gastric, small and large bowel epithelium, urothelium and Merkel cells of the skin. (Total number of normal cases evaluated = 106).

Tumor Tissues

Clone Ks20.8 stained 10/19 urothelial carcinomas, 2/2 gastric adenocarcinomas, 2/2 colon adenocarcinomas, 1/2 rectal adenocarcinomas, and 1/1 Merkel cell tumor. No staining was observed in breast tumors (0/32), lung tumors (0/4), liver tumors (0/4), ovarian tumors (0/4), papillary carcinomas of the thyroid (0/4), brain tumors (0/2), squamous cell carcinomas of the esophagus (0/2), soft tissue tumors (0/2), squamous cell carcinomas of the tongue (0/2), metastatic tumors of unknown origin (0/2), renal cell carcinomas (0/2), squamous cell carcinomas of the cervix (0/2), seminomas (0/2), skin tumors (0/2), a squamous cell carcinoma of the larynx (0/1), or an atypical carcinoid tumor of the thymus (0/1). (Total number of tumor cases evaluated = 94).

Cytokeratin 20 (Ks20.8) is recommended for the detection of human CK20 protein in normal and neoplastic tissues, as an adjunct to conventional histopathology using non-immunologic histochemical stains.

Product Specific Limitations

Cytokeratin 20 (Ks20.8) has been optimized at Leica Biosystems for use with BOND Polymer Refine Detection and BOND ancillary reagents. Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results under these circumstances. The protocol times may vary, due to variation in tissue fixation and the effectiveness of antigen enhancement, and must be determined empirically. Negative reagent controls should be used when optimizing retrieval conditions and protocol times.

Troubleshooting

Refer to reference 3 for remedial action.

Contact your local distributor or the regional office of Leica Biosystems to report unusual staining.

Further Information

Further information on immunostaining with BOND reagents, under the headings Principle of the Procedure, Materials Required, Specimen Preparation, Quality Control, Assay Verification, Interpretation of Staining, Key to Symbols on Labels, and General Limitations can be found in “Using BOND Reagents” in your BOND user documentation.

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BOND Polymer Refine Detection

Catalog No:DS9800

Leica Biosystems Newcastle Ltd
Balliol Business Park West
Benton Lane
Newcastle Upon Tyne NE12 8EW
United Kingdom
+44 191 215 4242

EN FR IT DE ES PT SV EL DA NL
NO TR BG HU RO RU PL SL CS SK AR

Instructions for Use
Please read before using this product.

Mode d'emploi
Á lire avant d'utiliser ce produit.

Istruzioni per L'uso
Si prega di leggere, prima di usare il prodotto.

Gebrauchsanweisung
Bitte vor der Verwendung dieses Produkts lesen.

Instrucciones de Uso
Por favor, leer antes de utilizar este producto.

Instruções de Utilização
Leia estas instruções antes de utilizar este produto.

Instruktioner vid Användning
Var god läs innan ni använder produkten.

Οδηγίες Χρήσης
Παρακαλούμε διαβάστε τις οδηγίες πριν χρησιμοποιήσετε το προϊόν αυτό.

Brugsanvisning
Læs venligst før produktet tages i brug.

Gebruiksinstructies
Lezen vóór gebruik van dit product.

Bruksanvisning
Vennligst les denne før du bruker produktet.

Kullanım Talimatları
Lütfen bu ürünü kullanmadan önce okuyunuz.

Инструкции за употреба
Моля, прочетете преди употреба на този продукт.

Használati utasítás
A termék használatba vétele előtt olvassa el.

Instrucțiuni de utilizare
Citiți aceste instrucțiuni înainte de a utiliza produsul.

Инструкция по применению
Прочтите перед применением этого продукта.

Instrukcja obsługi
Przed użyciem tego produktu należy przeczytać instrukcję.

Navodila za uporabo
Preberite pred uporabo tega izdelka.

Návod k použití
Čtěte před použitím tohoto výrobku.

Návod na použitie
Prosím, prečítajte si ho pred použitím produktov.

إرشادات الاستعمال
يُرجى القراءة قبل استخدام هذا المنتج.

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Před použitím zkontrolujte neporušenost obalu.
Pre použitím skontrolujte, či balenie nie je porušené.
تحقق من سلامة العبوة قبل الاستخدام.

www.LeicaBiosystems.com

Rx Only

BOND Polymer Refine Detection
Catalog No: DS9800

Intended Use

This detection system is for *in vitro* diagnostic use.

BOND Polymer Refine Detection is a biotin-free, polymeric horseradish peroxidase (HRP)-linker antibody conjugate system for the detection of tissue-bound mouse and rabbit IgG and some mouse IgM primary antibodies. It is intended for staining sections of formalin-fixed, paraffin-embedded tissue on the BOND automated system (includes Leica BOND-MAX system and Leica BOND-III system).

The clinical interpretation of any staining or its absence should be complemented by morphological studies and proper controls. They should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

The BOND Polymer Refine Detection Kit must be used with laboratory best practice in the use of tissue controls. For assurance, laboratories should stain each patient sample in conjunction with positive, negative, and other tissue specific controls as needed.

Summary and Explanation

Immunohistochemical techniques can be used to demonstrate the presence of antigens in tissue and cells (see "Using BOND Reagents" in your BOND user documentation).

BOND Polymer Refine Detection utilizes a novel controlled polymerization technology to prepare polymeric HRP-linker antibody conjugates. The detection system avoids the use of streptavidin and biotin, and therefore eliminates non-specific staining as a result of endogenous biotin.

BOND Polymer Refine Detection works as follows:

- The specimen is incubated with hydrogen peroxide to quench endogenous peroxidase activity.
- A user-supplied specific primary antibody is applied.
- Post Primary IgG linker reagent localizes mouse antibodies.
- Poly-HRP IgG reagent localizes rabbit antibodies.
- The substrate chromogen, 3,3'-Diaminobenzidine tetrahydrochloride hydrate (DAB), visualizes the complex via a brown precipitate.
- Hematoxylin (blue) counterstaining allows the visualization of cell nuclei.

Using BOND Polymer Refine Detection in combination with the BOND automated system reduces the possibility of human error and inherent variability resulting from individual reagent dilution, manual pipetting and reagent application.

Reagents Provided

Reagents sufficient for 200–300 tests

1. Peroxide Block (30 mL) 3–4% (v/v) Hydrogen peroxide.
2. Post Primary (30 mL) Rabbit anti mouse IgG (<10 µg/mL) in 10% (v/v) animal serum in tris-buffered saline/0.1% ProClin™ 950.
3. Polymer (30 mL) Anti-rabbit Poly-HRP-IgG (<25µg/mL) containing 10% (v/v) animal serum in tris-buffered saline/0.1% ProClin™ 950.
4. DAB Part 1 (2.4 mL) 66 mM 3,3'-Diaminobenzidine tetrahydrochloride hydrate, in a stabilizer solution.
5. DAB Part B (30 mL) ≤0.1% (v/v) Hydrogen Peroxide in a stabilizer solution.
6. DAB Part B (30 mL) ≤0.1% (v/v) Hydrogen Peroxide in a stabilizer solution.
7. Hematoxylin (30 mL) <0.1% Hematoxylin.

Dilution and Mixing

BOND Polymer Refine Detection is optimized for use on the BOND system. Reconstitution, mixing, dilution, or titration of these reagents is not required.

Materials Required But Not Provided

Refer to "Using BOND Reagents" in your BOND user documentation for a complete list of materials required for specimen treatment and immunohistochemical staining using the BOND system.

Storage and Stability

Store at 2–8 °C. Do not freeze. Do not use after the expiration date indicated on the tray handle label. Return to 2–8 °C immediately after use.

There are no obvious signs to indicate instability of this product, therefore positive and negative controls should be run simultaneously with unknown specimens (refer to "Quality Control" in the "Using BOND Reagents" section of your BOND user documentation).

If unexpected staining is observed that cannot be explained by variations in laboratory procedures, and a problem with the detection system is suspected, contact your local distributor or the regional office of Leica Biosystems immediately.

Storage conditions other than those specified above must be verified by the user[1].

Precautions

- Restricted to professional users.
- This detection system is intended for *in vitro* diagnostic use.
- To obtain a copy of the Material Safety Data Sheet contact your local distributor or regional office of Leica Biosystems, or alternatively, visit the Leica Biosystems' web site, www.LeicaBiosystems.com
- Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions[3]. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents or specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Seek medical advice.
- Consult Federal, State or local regulations for disposal of any potentially toxic components.
- Minimize microbial contamination of reagents or an increase in non-specific staining may occur.
- Incubation times or temperatures other than those specified may give erroneous results. Any such change must be validated by the user[1].
- Do not mix reagents from different detection systems.

Instructions for Use

BOND Polymer Refine Detection was developed for use on the BOND automated system. Operating parameters for application of the detection system reagents on the BOND Processing Module have been optimized at Leica Biosystems. These can be displayed by following the instructions in your BOND user documentation.

Product Specific Limitations

BOND Polymer Refine Detection has been optimized at Leica Biosystems for use with BOND ancillary reagents. Laboratories may use their own primary antibodies provided they have been diluted to an appropriate concentration with BOND Primary Antibody Diluent (Catalog No. AR9352). Users who deviate from recommended test procedures must accept responsibility for interpretation of patient results under these circumstances.

The appropriate concentration of user's own primary antibodies may vary, due to variation in tissue fixation and the effectiveness of antigen enhancement, and must be determined empirically. Negative reagent controls should be used when optimizing retrieval conditions and primary antibody concentrations.

The clinical interpretation of any staining or its absence should be complemented by morphological studies and proper controls. They should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Troubleshooting

Refer to reference 4 for remedial action.

If the patient result does not correspond to the expected results with the use of controls, the test should be repeated.

If the staining result is not as expected, and you wish to troubleshoot performance of the instrument and detection system independently, your local Leica representative can provide specific protocols. The detection kit must be used in conformance to the package instructions and within the shelf life indicated on the product itself.

Further Information

Further information on immunostaining with BOND reagents, under the headings Principle of the Procedure, Materials Required, Specimen Preparation, Quality Control, Assay Verification, Interpretation of Staining, Key to Symbols on Labels, and General Limitations can be found in "Using BOND Reagents" in your BOND user documentation.

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