Immunohistochemical Expression of P53 and Bcl-2 In Esophageal Tumors

الكشف النسيجي الكيميائي المناعي عن P53 و Bcl-2 في أورام المرئ

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

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2015
بسم الله الرحمن الرحيم

قال تعالى:

(يَوْمَ يُتْرِكُ الْحُكْمَةُ مِنْ كِتَابِهِ وَمِنْ هَذِهِ الْحُكْمَةُ فَقَدْ أَوْحَيْتُهَا لِحَسَبِيٌّ حَسَبًا وَمَا يُعَذَّبُونَ إِلَّا أَوْلَاهَا الْأَبَابِ)

صدق الله العظيم

سورة البقرة الآية (269)
DEDICATION

To my mother
To the soul of my father
To my sister, brother and friends…
I dedicate this work.
Acknowledgement

I would like to thank Allah for giving me the knowledge and support me to complete this research.

Special thanks to my supervisor Dr. Abu Elgasim Abass for his support to finish this research.

I would like to thank everyone who supported me throughout this research by providing samples and reagents, I am sincerely grateful to them for sharing their views on a number of issues related to this project.
Abstract

This is a descriptive retrospective hospital based study conducted in Khartoum state during the period from March to December 2014 aimed to detect the expression of P53 and Bcl-2 proteins in esophageal tumors using immunohistochemical method.

Fifty paraffin embedded blocks previously diagnosed as esophageal tumors were collected from Soba university hospital. Samples include 40 (80%) malignant tumors, 34 (68%) from those 40 samples were squamous cell carcinoma and 6 (12%) were adenocarcinoma, and 10 samples (20%) were benign tumors.

The study samples includes 32 (64%) males and 18 (36%) females. with mean of age was 60.7 years.

Two sections were cut from each paraffin block by rotary microtome and stained by immunohistochemical method using modified new indirect method (Dako technique) for detection of P53 and Bcl-2. Data collected from patients files and results obtained and analyzed using statistical package of social science program (SPSS).

The immunohistochemical expression of P53 was detected in 23 (46%) samples, all of them were malignant and negative in 27 (54%) samples, 17 samples (34%) of them were malignant and the remaining 10 samples (20%) were benign, and there was statistical association between P53 expression and malignant tumors of the esophagus (P.value =0.001).

Out of 23 positive samples, 19 (38%) samples were squamous cell carcinoma, and 4 (8%) samples were adenocarcinoma with no statistical association between the type of tumor and P53 expression (P.value = 0.622). The expression of p53 was
compared with degree of histological differentiation and it has been detected in 11(22%) samples of moderately differentiated tumors, and 11(22%) samples of poorly differentiated tumors with statistical association with the grade of tumor (P.value =0.002).

Immunohistochemical expression of Bcl2 was detected in 5 (10%) samples and negative in 45 (90%) samples, 35 (70%) of them were malignant and 10 (20%) were benign with no statistical association between Bcl-2 expression and esophageal tumors (P.value=0.239).

Out of five samples expressing Bcl-2, four (8%) of them were squamous cell carcinoma and only one (2%) was adenocarcinoma with no statistical association between Bcl-2 expression and type of cancer (P.value=0.738). Comparing the expression of bcl-2 and the grade of the tumor the positive result was detected in 2 (4%) samples of moderately differentiated tumors and 3 (6%) samples of poorly differentiated tumors with no relation between the histologic grade of tumor and Bcl-2 expression (P.value=0.857).

In this study the expression of the two markers was P53 -/bcl-2- in 26 (52%) samples, P53+/bcl-2 - in 19 (38%) samples, P53-/bcl2 + in 1(2%) samples, P53+/bcl2 + in 4 (8%) samples .We found that no correlation between the expression of p53 and bcl-2 in esophageal tumor.

This study concludes that there is association between P53 expression and malignant tumors of esophagus, with no association with type of tumor while the expression of P53 is associated with histological differentiation of tumor.

Bcl-2 expression is not associated with both type of tumor and the histological grade of tumor and there is no correlation between the expression of P53 and Bcl-2 in esophageal tumors.
الخلاصة

أجريت هذه الدراسة الوراثية التراجعية في مستشفى سوبا الجامعي ولاية الخرطوم خلال الفترة من مارس إلى ديسمبر 2014، وتهدف الدراسة للكشف عن P53 و Bcl-2 في أورام المريء باستخدام كيمياء الأنسجة المناعية.

تم جمع خمسين عينة مثبت بالفورمالين مغمر في نسيج الفرومة، بشرح البارفين من عيادات مرضى تم تشخيصهم مسبقًا على انهم مصابون بأورام المريء، وتكون أفراد العينة من 32 (64٪) من الذكور و 18 (36٪) من الإناث مع متوسط العمر 60.7 عامًا.

تتكون العينات من 40 (80٪) عينة لأورام خبيثة، 34 (68٪) عينة تم تشخيصها على أنها سرطان الخلايا الحشرسفية، و 6 (12٪) عينة لسرطان الخلايا الحمراء، و 10 (20٪) عينة لأورام حميدة.

قطرت العينات ثم صبعت بواسطة كيمياء الأنسجة المناعية باستخدام طريقة داكور المعده غير المباشرة الجديدة للكشف عن P53 و Bcl-2 ثم استخدم برنامج الحزمة الإحصائية للأعمال الإجتماعية (SPSS) لتحليل البيانات.

أظهرت الدراسة أن الكشف عن التعبير المناعي للواسم P53 و Bcl-2 في الكشف عن الأورام الخبيثة. وسالب الظهور في 27 (54٪) من العينات، 17 (34٪) عينة منها كانت لأورام خبيثة و 10 (20٪) عينة بها أورام حميدة، ووجد أن هناك علاقة بين ظهور P53 والأورام الخبيثة في المريء (القيمة الإحتمالية = 0.001).

من أصل 23 عينة إيجابية، وجد أن 19 (83٪) عينة لسرطان الخلايا الحشرسفية، و 4 (8٪) عينة لسرطان الخلايا الحمراء، ومع عدم وجود علاقة إحصائية بين نوع الورم وظهور P53 (القيمة الإحتمالية = 0.622). كما أظهرت الدراسة أن P53 موجب الظهور في 22 (44٪) عينة كانت من الأورام متوسطة التمايز، و 11 (22٪) عينة من الأورام ضعيفة التمايز. و أن هناك علاقة بين ظهور الواسمة P53 و درجة التمايز النسيجي (القيمة الإحتمالية = 0.002).

كما تم الكشف عن Bcl-2 في المريء، الذي كان موجب الظهور في 5 (10٪) عينة تشخيصها كأورام خبيثة وسالب الظهور في 45 (90٪) عينة، 35 (70٪) منها لأورام خبيثة و 10 (20٪) عينات لأورام حميدة مع عدم وجود علاقة إحصائية بين ظهور الواسمة Bcl-2 وأورام المريء (القيمة الإحتمالية = 0.239).

أربعة (8٪) من أصل خمسة عيادات موجب لظهور Bcl-2 شخصت كسرطان الخلايا الحشرسفية و كانت
واحدة (2%) لسرطان الخلايا الغدية مع عدم وجود علاقة إحصائية بين ظهور الواسم Bcl-2 ونوع السرطان (القيمة الإحتمالية = 0.738). كما وجد أن العينات التي أعطت نتيجة إيجابية هي 2 (4%) عينات من الأورام متوسطة التماثز و 3 (6%) عينات من الأورام ضعيفة التماثز و أنه لا توجد علاقة بين درجة تماثز الورم و الواسم Bcl-2 (القيمة الإحتمالية = 0.857).

و بمقارنة نتائج الكشف عن الواسمات P53 و Bcl-2، اظهرت النتائج - Bcl-2/p53 في 26 (52%) عينة - Bcl-2 في 19 (38%) عينة، Bcl-2 + P53 في 1 (2%) عينة، Bcl-2 + P53 في 4 (8%) عينة. و وجد أنه لا يوجد ارتباط بين ظهور الواسم Bcl-2 و الواسم P53 في أورام المرء.

و تخلص هذه الدراسة إلى أن هناك علاقة بين ظهور P53 و الأورام الخبيثة في المرء مع عدم وجود علاقة مع نوع الأورام السرطانية. و كما أن هناك علاقة مع التماثز النسيجي للورم. و أن ظهور Bcl-2 لا يرتبط بنوع الورم أو درجة التماثز النسيجي. كما أنه لا يوجد علاقة بين ظهور الواسمين Bcl-2 و p53 في أورام المرء.
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Chapter one

Introduction
1.1 Introduction:
Esophageal cancer is cancer arising from the food pipe known as the esophagus that runs between the throat and the stomach (Montgomery, et al. 2014), and it is one of the least studied and deadliest cancers worldwide because of its extremely aggressive nature and poor survival rate. It ranks sixth among all cancers in mortality (Yuwei, 2013). There are two main types of esophageal cancer: squamous cell carcinoma and adenocarcinoma (Das, 2010). The incidence of the two main types of esophageal cancer varies greatly between different geographical areas (Napier and Scheerer, 2014). In general, ESCC is more common in the developing world, and EAC is more common in the developed world (Montgomery, et al. 2014), and it is one of the top ten most common cancers in Khartoum among all registered cancer cases with available information ($N = 6548, 96.7\%$), cancer of esophagus rate = 5.8 per 100,000 (Intisar, et al. 2014).

Smoking and alcohol consumption, hot tea drinking, red meat consumption, poor oral health, low intake of fresh fruit and vegetables, and low socioeconomic status have been associated with a higher risk of esophageal squamous cell carcinoma. Barrett’s esophagus is clearly recognized as a risk factor for esophageal cancer, and dysplasia remains the only factor useful for identifying patients at increased risk, for the development of esophageal adenocarcinoma in clinical practice. (Yuwei, 2013).

For different types of esophageal cancer, the risk increases with age, with a mean age at diagnosis 67 years (Cummings, et al. 2008).

The majority of patients presenting with esophageal cancer have locally advanced disease producing dysphagia. Endoscopic evaluation generally reveals partial luminal obstruction with
resultant patient discomfort and distress, as well as impairment in nutritional intake (Holland, 2003).

The diagnosis should be made from an endoscopic biopsy with histopathology to be classified according to the World Health Organization criteria (Stahl, et al. 2013).

Curative treatment options for esophageal cancer include surgical resection, external beam radiotherapy, chemotherapy with cisplatin and 5-FU, or combinations of two or three of these options (Hostetter, et al. 2002), and the main factors for selecting primary therapy are tumor stage and location, histological type and the medical condition, as well as considerations from patients (Rice, et al. 2010).

The tumor suppressor P53, encoded by the P53 gene located at chromosome 17q13.1, is highly associated with a poor prognosis in human cancers (Murata, et al. 2013). P53 is the most frequently mutated gene in human tumors. Mutations occur in almost every type of tumor and in over 50% of all tumors. p53 mutations are found in ~30%–50% of lung, esophageal, colorectal, head and neck, and ovarian cancers, and in ~5% of leukemia, sarcoma, melanoma, testicular cancer, and cervical cancer (Hollstein, et al. 1991, Olivier, et al. 2004).

The Bcl-2 gene is located in chromosome 18q21 (Zavrides, et al. 2005). The Bcl-2 family of proto-oncogenes block apoptosis (Reed, et al. 2004). It was found to be increased in reflux esophagitis, non dysplastic Barrett’s and low grade dysplastic Barrett’s epithelium, but or virtually absent in high grade dysplasia or carcinomas (Chatelain and Flejou, 2003), (Woude, et al. 2002).

There are many reports indicating an inverse relationship between mutant p53 over expression and apoptosis (Masuda, et al. 2003). Numerous preclinical and clinical studies have suggested that impact of p53 status on responses to chemotherapy or radiotherapy depends on status of other genes such as Bcl-2 (Skirmisdottir, et al. 2002). There are evidences referring to the development of a multi-drug resistance phenomenon as a result of over expression of Bcl-2 protein (Tamm, et al. 2001).
Our aim from the study is to evaluate the importance of two apoptosis regulators as prognostic markers in Sudanese esophageal cancer patients using immunohistochemical techniques to determine the expression of p53 and Bcl-2 proteins in patients with esophageal cancer.
1.2 Objectives of the study:

1.2.1 General objective:
- To detect the expression of p53 and Bcl-2 proteins in esophageal tumors using immunohistochemical method.

1.2.2 Specific objective:
- To correlate between the p53 and Bcl-2 expression and histological diagnosis and tumor grade.
Chapter two

Literature review
CHAPTER TWO
LITERATURE REVIEW

2.1 Anatomy of the esophagus:
The esophagus is a pliable muscular tube that transports food bolus from the pharynx to the stomach, it is about 25cm long beginning at the inferior border of the cricoid cartilage in front of the C6/C7 and opening at the level of T10/T11 in to the cardial orifice (Fritsch and Kuehnel, 2011).
The esophagus divided to three parts based on the respective regions of the body through which it passes the first part is the cervical part; the posterior wall of the short cervical part of the esophagus rest against the vertebral column, and the anterior wall against the trachea. And the second part is the thoracic part which during its course the 16cm part gradually moves away from the vertebral column, it runs parallel to trachea in front of it as far as the tracheal bifurcation at the level of the T4, and at this point the aortic arch crosses over it. And the third part is the Abdominal part; is very short part only 1-3cm, it extends from the esophageal hiatus of the diaphragm to which it is connected by loose connective tissue that allow movements of the stomach (Fritsch and Kuehnel, 2011).

2.2 Histology:
The wall of the esophagus consists of four layers: mucosa, submucosa, muscular layer and adventitia. Unlike other areas of the gastrointestinal tract, the esophagus does not have a distinct serosal covering. This allows esophageal tumors to spread more easily and makes them harder to treat surgically (Boyce, 2003).
Its mucosa is lined by stratified non keratinized squamous epithelium beneath the connective tissue lamina proparia contain a prominent muscularis mucosa this stratified nonkeratinized squamous epithelium of the esophagus end abruptly at the jejunum with the cardial orifice and is replaced by the columnar epithelium of the gastric mucosa. The submucosa consists of layer of loose connective tissue containing venus plexus and nerves as well as mixed scattered
glands known as esophageal glands. The muscular layer is composed of an inner layer of circular muscles which help propel the bolus toward the stomach by means of wavelike muscular contraction and an outer longitudinal layer which is responsible for longitudinal tension and for shortening segments of the esophagus (Fritsch and Kuehnel, 2011).

In the upper two third of the esophagus the muscular layer contain striated muscles fiber from the pharyngeal muscle, in the lower one third it composed entirely of smooth muscle. The esophagus is connected to its surroundings by the adventitia (Fritsch and Kuehnel, 2011).

### 2.3 Esophageal disorders:

#### 2.3.1 Esophgitis:

Esophagitis caused by cytotoxic chemotherapy, irradiation, viruses, bacteria, and fungi. Other causes include acid-peptic esophagitis, pill-induced injury, trauma caused by nasogastric tubes, and graft-versus-host disease (GVHD) in hematopoietic cell transplant recipients (Baehr and McDonald, 1997, Otero, et al. 1997).

In nontransplant patients, *Candida albicans* and herpes simplex virus are the most common pathogens, but bacterial organisms and other viruses, including cytomegalovirus (CMV), can also be responsible (Baehr and Mcdonald, 1997).

#### 2.3.2 Barrett’s Esophagus:

Barrett’s esophagus is a chronic active inflammatory condition in which the normal squamous epithelium of the esophagus is replaced by a metaplastic columnar epithelium, usually as a consequence of chronic gastroesophageal reflux disease (GERD) and it is the only known precursor to esophageal adenocarcinoma and the strongest risk factor (Winters, et al.1987).

#### 2.3.3 Malignant tumors:

##### 2.3.3.1 Esophageal squamous cell carcinomas:

It is the most common form of esophageal cancer worldwide which occurred largely in the upper two-thirds of the esophagus (*Blot, 1994*).
Develop through the progression of premalignant or dysplastic lesions as does its counterpart in skin and cervix and it’s commonly graded as low, moderate or high grade depending on the extent of involvement of the epithelium (Lewin, 1996).

The survival of ESCC is very poor, largely due to late development of symptoms and consequent late diagnosis (Siegel, 2012)

### 2.3.3.2 Esophageal adenocarcinoma:

Barrett’s esophagus is the only known precursor to esophageal adenocarcinoma and the strongest risk factor forming 80% of cancers in the lower third of the esophagus (Winters, 1987, Lewin, 1996). Other non-Barrett’s associated adenocarcinomas are extremely rare and they may rise in association with gastric heterotopia or the esophageal submucosal glands which usually occur in the upper third of the esophagus and often show papillary growth pattern (Ednoh, 1999).

### 2.4 Risk factors of esophageal cancer:

It is likely that lifestyle and environmental factors play important roles in the development of esophageal adenocarcinoma along with genetic factors. Although GERD, obesity, and smoking have been identified as modifiable risk factors of esophageal adenocarcinoma (Kamangar, et al., 2009)

#### 2.4.1 Familial susceptibility to esophageal cancer:

Esophageal cancer is typically considered a sporadic disease however familial clusters have been found, and there is an increased risk of esophageal cancer in individuals with family history of esophageal cancer both ESCC and EAC (Hu, et al. 2003, Ji and Hemminiki, 2006). Further familial association of risk factors of esophageal cancer; particularly gastroesophageal reflux disease and Barrett’s esophagus are also associated with elevated risk of EAC (Chak, et al. 2006, Alcbari, et al. 2006).

#### 2.4.2 Tobacco:

In esophageal squamous cell carcinoma tobacco use regardless of form, is a major risk factor for esophageal cancer in most parts of the world. Several case-control studies have reported
strong positive dose-response effect with duration and/or intensity of cigarette smoking (Brown, et al. 2002)

2.4.3 Alcohol:
There are clear cut epidemiologic data indicating that alcoholic beverages are a major cause of SCC (Lee, et al. 2005). However alcohol consumption has not been shown to be a risk factor in some developing countries. Variability in risks by type of alcoholic beverage may reflect culturally or economically determined drinking habits (Brown, et al. 2002)

2.4.4 Diet and nutrients:
Diets high in starch and low in fiber contribute to the development of SCC of esophagus (Chadirian, 2007).

2.4.5 Body mass index (BMI) and obesity:
The risk of the ESCC increases when the BMI decreases while the adenocarcinoma in contrast to SCC where high-risk population are generally poorly nourished, AC risk tends to rise as BMI increases (Hasibe, et al. 2007).

2.4.6 Medical condition and medications:
2.4.6.1 Gastroesophageal reflux disease and Helicobacter Pylori:
Helicobacter pylori infection associated with an increased risk of ESCC in some studies and a protective effect on others (Kamangar, et al. 2007) H.pylori infection appear to be associated with an increased risk of SCC when it induces atrophic gastritis but a decreased risk when induces antral-predominant, non atrophic gastritis (Mccoll, 2007).

While significance of 2-fold or greater risk of AC have been associated with the presence of GERD, a major risk factor that predispose Barrett’s esophagus, a precursor lesion for AC (Brown, et al. 2002).

2.4.6.2 Human papilloma virus:
HPV particularly HPV-16 and HPV-18 is an oncogenic virus that appear to play an oncogenic role in some high risk areas with an exceptionally high incidence of esophageal cancer (Brown, et al. 2002).
2.4.6.3 Other medical conditions:
elevated risk of esophageal squamous cell cancer have been reported with certain medical conditions, such as pernicious anemia, achalasia, some autoimmune disease, gastrectomy and chemical injuries to the esophagus (Brown, et al. 2002).

2.4.7 Socioeconomic statuses:
Highest rate of SCC are generally found in areas of the world where the population is impoverished. Within various populations the risk of esophageal cancer is greatest among those with the lowest socioeconomic status whether measured by income, education or occupation. While low socioeconomic status has been related to excess risk of adenocarcinoma (Wu, et al.2006).

2.4.8 Radiation:
Ionizing radiation has been linked to esophageal cancer, particularly among patients radiated for ankylosing spondylitis and for breast cancer (Brown, et al. 2002).
In one study postmastectomy radiation therapy was associated with greater than 2–fold risk of SCC. And no significant risk of AC was found following either postmastectomy or postlumpectomy radiation (Zablotska, 2005).

2.4.9 Occupation and industry:
Esophageal SCC cancer is not usually considered to be an occupational disease, although elevated risks have been reported for several exposures. Increased risk of esophageal cancer have been observed among metal workers exposed to metal working fluids, metal polishers, dry cleaners, gas station attendants (Brown, et al. 2002).

2.5 Diagnosis:
Clinically, the patients usually presented with dysphagia, the majority being in very poor nutritional state and marked dehydration. The duration of symptoms at presentation ranged from 6-12 months (Malik, et al. 1 976). The most commonly used diagnostic and staging modalities are:
2.5.1 Barium swallows study:
Barium swallows followed by endoscopy with biopsy.

2.5.2 Endoscopy:
Endoscopy remains the method of choice for confirmation of esophageal cancer, used in detection of early lesions is improved by the use of staining technique toluidine blue, a metachromatic stain has particular affinity for RNA and DNA and therefore stains suspected areas in the mucosa, which are richer in nuclei than normal mucosa (Bergman, et al. 2011).

2.5.3 ComputeTomography (CT):
Computed tomography scan staging of the esophageal cancer include assessment of the extent of involvement of the esophageal wall by tumor and tumor invasion of adjacent structures and metastases (Saunders, et al. 1997).

2.5.4 Magnetic resonance imaging (MRI):
MRI appears an alternative to CT for evaluation of esophageal cancer, However the practical application of MRI are limited by the lack of reliable contrast agents for the gastrointestinal tract and the substantially longer imaging time required for this procedure (Takashima, et al. 1991).

2.5.5 Endoscopic ultrasound (EUS):
one of the newer modalities used in the staining of esophageal cancer which utilizes the technologies of flexible endoscopy and ultrasonic imaging. By virtue of its ability to depict the various layers of the esophageal wall and periesophageal tissue, EUS has proved useful in staging esophageal cancer (Tio, 1998). The use of EUS - guided FNA was reported in the diagnosis of esophageal cancer recurrence after distal esophageal resection (Tio, et al. 1989).

2.5.6 Tumor markers:

2.5.6.1 P53:
The P53 gene at chromosome 17p13 encodes a protein that monitor the integrity of the genome and halts cell cycle progression at G1 via P21 if the genome is damaged, allowing time for DNA repair. When DNA damage occurs and P53 is functioning correctly, it leads to
cell cycle arrest to allow for DNA repair or apoptosis. If the damage is excessive (tetraploidy and aneuploidy ) loss or a mutation of P53 is probably the most common single genetic change in all cancers , including esophageal adenocarcinoma ( Hemelin, et al.1994, Prevo, et al.1999, Reid, et al .2001).

The tumor suppressor gene P53 has several critical functions within the cell, the most important is its role in signaling cells for repair or apoptosis , so-called guardian of the genome(Koppert, et al. 2005).

In general tumor suppressor gene provide antiprolifiration signals for cells . mutation include missense mutation , deletion , or insertion and promoter methylation rendering nonfunctional protein product (Koppert, et al. 2005).

Loss of heterozygosity of the P53 locus has been found in 75%-80% of esophageal adenocarcinoma as well as in 79% of patients of high-grade dysplasia, 42% of low-grade dysplasia , and 14% of Barrett’s metaplasia (Reid, et al. 2001).

Mutation of P53 were found in 29%-66% of patients with Barrett’s metaplasia and in 40-88% with high-grade dysplasia / adenocarcinoma (Bian, et al. 2001). 17p loss of heterozygosity analysis performed on endoscopic biopsies identified patients with Barrett’s esophagus at risk of neoplastic progression within surveillance programs; therefore it could supplement histology in determining the frequency that surveillance endoscopy should be performed (Reid, et al. 2001, Dolan, et al. 2003).

The p53 protein prevents cells with DNA damage from dividing, and activates the apoptosis pathway, thereby preventing the propagation of cells with such alterations. Disruption of native p53 function inhibits apoptosis and thereby allows expansion of abnormal cell population. Lesions in p53 have been documented in 85%-95% of esophageal adenocarcinomas, but almost never in normal esophageal tissues from the same patient .more over their prevalence increases significantly with advancing histologic grade of dysplasia (Muzeau and Flejou ,1996, Barrett, et al. 1999).
2.5.6.2 Bcl2:
The Bcl-2 gene is located in chromosome 18q21 and its product is a 24 KD protein localized to the nuclear envelope, endoplasmic reticulum and outer mitochondrial membrane (Zavrides, et al. 2005). The Bcl-2 family of proto-oncogenes block apoptosis (Reed, et al. 2004), it was found to be increased in reflux esophagitis, non dysplastic Barrett’s and low grade dysplastic Barrett’s epithelium, but or virtually absent in high grade dysplasia or carcinomas (Woude, et al. 2002, Chatelain and Flejou, 2003).

It has been proposed that an apoptotic balance must be upset for transformation of metaplasia to adenocarcinoma, such that the cell switches toward an antiapoptotic phenotype due to increased Bcl-xl and decreased Bax expression (Woude, et al. 2002, Rouf, et al. 2003). Inhibition of apoptosis by over expression of Bcl-2 protein occurs mainly early in the neoplastic progression and latter diminishes perhaps due to loss of normal cellular processes as tumor cells take one more mutation. therefore as malignancy appears, cells acquire other ways of avoiding apoptosis. For example Bcl-xl expression demonstrated early progression in the metaplasia to low-grade to high-grade dysplasia sequence without further expression in adenocarcinoma (Iravani, et al. 2003) and loss of expression was associated with poor survival (Rouf, et al. 2003).

2.6 Treatments:
Despite the progress in chemotherapy in recent years, the benefits of chemotherapy in esophageal cancer remain modest. The most widely used combination in esophageal cancer, 5-FU and cisplatin, has shown objective responses in 20-40% of cases, but had no impact on overall survival (Manion and Hockenberry, 2003)

In addition to surgical resection, multimodality treatment has general favor among many oncologists in order to improve response to traditional therapy such as chemotherapy, radiation and resection (Mork, et al. 1998).
Chapter three

Materials and methods
CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials:
Archived tissue blocks of esophageal tumors were used in this study.

3.2 Methods:

3.2.1 Study design:
This is a hospital based descriptive retrospective study aimed to detect P53 and Bcl-2 tumor markers in esophageal tumors using immunohistochemical method.

3.2.2 Study samples:
Fifty esophageal tissue blocks were obtained from tissues previously diagnosed as esophageal tumors. 40 samples were esophageal cancer and 10 samples were diagnosed as benign tumors collected from Soba university hospital. Patient identification and other information were obtained from patients files.

3.2.3 Sample processing:
Two sections each one of 5 µm in thickness were obtained from each formalin fixed paraffin wax embedded tissue using rotary microtome.

3.2.3.1 Immunohistochemical staining:
Sections required for immunohistochemistry retrieved by water bath retrieval technique, and then stained using monoclonal antibodies by new indirect techniques as follow:
Sections were dewaxed in oven and cleared in two changes of xylene for two minutes in each and then hydrated through descending concentrations of ethanol (100%, 90%, 70%, 50%) and water two minutes for each then antigen retrieved using water bath technique for thirty minutes at 97°C in citrate buffer, then treated with hydrogen peroxide solution for fifteen minutes then washed in phosphate buffer saline (PH7.4) for five minutes.
Then the first section was treated with anti Bcl-2 (Bcl-2 alpha Ab-1) primary antibody for thirty minutes, and the second section was treated with anti P53 primary antibody also for thirty minutes.

Then sections washed in phosphate buffer saline (PH 7.4) for 5 minutes then treated with secondary polymer conjugated antibody for thirty minutes, then rinsed in phosphate buffer saline (PH 7.4), then treated with DAB for seven minutes, then washed in phosphate buffer saline (PH 7.4), then counter stained in Mayer’s haematoxylin for one minute then washed and blued in running tap water, then dehydrated through ascending concentrations of ethanol (50%, 70%, 90%, 100%), then cleared in xylene and mounted in DPX mountant.

3.2.4 Result Interpretation:
All quality control measures had been adopted, positive and negative control slides used during histopathological and immunohistochemical staining.

For Bcl-2 detection of more than 5 cells with brown cytoplasm per one field considered as positive result.

For P53 detection of more than 5 cells with brown nucleus per one field considered as positive result.

3.2.5 Data Analysis:
Data analysis was done using SPSS 11.5 program. Frequencies mean and chi-square test and correlation values were calculated.

3.2.6 Ethical Considerations:
Samples collected after ethical acceptance from Soba university hospital.
Chapter four

Results
CHAPTER FOUR

RESULTS

A total of 50 samples of patients with esophageal disorders were investigated, 40 of them were malignant esophageal tumors representing 80%, and the remaining 10 (20%) were benign as indicated in table (3.1).

The age of study population ranged between 8 to 85 years old with mean of age 60.7 years. The first group less than 60 years were 23 (46%), the second group were older than 60 years was 27 (54%), as indicated in table (3.2).

The description of sex show 32(64%) were male and 18 (36%) were female. As indicated in table (3.3).

The description of tumor grade revealed that well differentiated tumor in one sample (2.5%) sample, moderately differentiated tumor in 14 (35%) sample, poorly differentiated tumors in 16 (40%) sample. And other samples that not graded were 9 (22.5%) as indicated in table (3.4).

Malignant esophageal tumors revealed positive expression of P53 in 23 (46%) patients and negative expression in 17(34%) patients, while all benign tumors 10 (20%) sample show negative expression of P53. This result show significant statistical association (P. value=0.001) as indicated in table (3.5).

As P53 has a nuclear expression;positive result appear as brown color in the nucleus as in graph (1) and not appear in negative results as in graph (2)

Immunohistochemical detection of p53 yield positive result in 23 sample , 19 were diagnosed as ESCC and 4 were diagnosed as AC and negative in 17 sample , 15 of SCC and 2 were AC.

The analysis showed that no relation between expression of P53 and the type of tumor (P.value =0.622) as indicated in Table (3:6).
The comparison between P53 expression and the grade of the tumor we found that p53 expression was positive in 11 moderately differentiated tumors and 11 poorly differentiated tumors. And there is significant relation between p53 expression and the grade of tumor (P.value =0.002) as indicated in Table (3:6).

Malignant esophageal tumors revealed positive expression of Bcl-2 in 5 (10%) sample and negative expression in 35 (70 %) sample , while all benign tumors show negative expression of Bcl-2 .This result show in significant statistical association (P. value= 0.239). as indicated in table (3.7).

Samples that were express Bcl-2 appear as brown color in the cytoplasm as in graph (3) and not appear in negative results as in graph (4)

Immunohistochemical detection of Bcl-2 showed positive results in 5 samples, 4 were diagnosed as ESCC and 1 was diagnosed as AC and negative in 35 sample, 30 of SCC and 5 were AC. The analysis showed that no relation between expressions of bcl-2and the type of tumor (P.value =0.738) as indicated in Table (3.8).

The comparison between Bcl-2 expression and the grade of the tumor we found that Bcl-2 expression was positive in 2 moderately differentiated tumors and 3 poorly differentiated tumors. And there is insignificant relation between Bcl-2 expression and the grade of tumor (P.value =0.857) as indicated in Table (3.8).

The co-expression of the two markers was P53-/ bcl2- in 16 samples (52%), P53+/bcl2 –in 19samples (38%), P53-/bcl2 + in 1sample (2%) , P53+/bcl2 + in 4samples (8%). we found that no correlation between the expression of p53 and bcl-2 in esophageal tumor (P. value =0.288).
Table (4.1): distribution of sample among the study population:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant</td>
<td>40</td>
<td>80%</td>
</tr>
<tr>
<td>Benign</td>
<td>10</td>
<td>20%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (4.2): Distribution of age group among study population:

<table>
<thead>
<tr>
<th>Age group</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 60 years</td>
<td>23</td>
<td>46%</td>
</tr>
<tr>
<td>More than 60 years</td>
<td>27</td>
<td>54%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (4.3): Distribution 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>32</td>
<td>64%</td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>36%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (4.4): Distribution of cancer grade among malignant esophageal tumor:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated tumors</td>
<td>1</td>
<td>2.5%</td>
</tr>
<tr>
<td>Moderately differentiated tumors</td>
<td>14</td>
<td>35%</td>
</tr>
<tr>
<td>Poorly differentiated tumors</td>
<td>16</td>
<td>40%</td>
</tr>
<tr>
<td>Not graded</td>
<td>9</td>
<td>22.5%</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table (4.5): Immunohistochemical expression of P53 among the study samples:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>23</td>
<td>17</td>
<td>40</td>
<td>0.001</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>27</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>
Table (4.6): Relation between P53 expression and type of tumor and the grade of tumor:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Type of tumor</th>
<th>Grade of tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCC</td>
<td>AC</td>
</tr>
<tr>
<td>Positive</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>6</td>
</tr>
<tr>
<td>P.value</td>
<td>0.622</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Table (4.7): Immunohistochemical expression of Bcl2 among the study samples:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Positive</th>
<th>negative</th>
<th>Total</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>5</td>
<td>35</td>
<td>40</td>
<td>0.239</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>45</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>
Table (4.8): Relation between Bcl-2 expression and type of tumor and the grade of tumor:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Type of tumor</th>
<th>Grade of tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCC</td>
<td>AC</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>6</td>
</tr>
<tr>
<td>P.value</td>
<td>0.738</td>
<td></td>
</tr>
</tbody>
</table>
Graph (1): Moderately differentiated esophageal squamous cell carcinoma show positive expression of P53
Graph (2): Moderately differentiated esophageal squamous cell carcinoma show negative expression of P53
Graph (3): Moderately differentiated esophageal squamous cell carcinoma show positive expression of Bcl-2
Graph (4): Moderately differentiated esophageal squamous cell carcinoma show negative expression of Bcl-2
Chapter five

Discussion
CHAPTER FIVE
DISCUSSION

Esophageal cancer is the 8th most common cancer and the 6th most common cause of cancer death in the world (Jemal, et al. 2011)

This study was conducted by collecting 50 formalin fixed paraffin embedded tissue blocks of esophageal sample received at Soba university hospital and all data were collected from patients files.

Our study includes samples include 32(64%) samples from males and 18 (36%) samples from females. patients age ranging between 8-85 years, with mean age 60.7 years. A similar result was observed by Makoto et al (1997) their study reported that the mean of age of patient ranging between 42-83 years was 61 years.

The samples revealed that 34 (68%) were squamous cell carcinoma, while 6 (12%) were adenocarcinoma , esophageal squamous cell carcinoma (ESCC) is the most frequent esophageal cancer subtype internationally (Agrawal, et al. 2012) and 10 samples (20%) were benign tumors used in this study to evaluate the expression of the two markers P53 and Bcl-2 in non malignant esophagus tissue.

Immunohistochemical detection of p53 show positive result in 23(46%) samples all of them were malignant and negative result in 27(54%) samples 17 (34%) samples were malignant and 10 (20%) samples were benign. The result show a significant statistical association between the positive expression of P53 and malignant tumor (P. value =0.001).This result is similar to results observed by Arbabi et al (2005) reported that nuclear p53 expression in the neoplastic epithelial cells of esophagus was observed in 67.6% of tumor samples.

P53 positive samples when compared with the type of tumor although we found that 19/23 positive samples were diagnosed as esophageal squamous cell carcinoma, and 4/23 were adenocarcinoma with no statistical association between the p53 expression and the type of tumor (P.value =0.622) and this was contradicted with results obtained by Huang et al (2014)
who reported that the expression of P53 in the ESCC tissue was significantly high \( P_{\text{value}} = 0.01 \)

The comparison between the expression of P53 and the grade of tumor positive result was not observed in well differentiated tumors, while it had been observed in 11 moderately differentiated, 11 poorly differentiated, and there was significant relation between the P53 positive expression and the poor differentiation of tumor \( (P_{\text{value}} = 0.002) \), similar results has been reported by Huang et al (2014) showed the level of P53 protein expression was found to correlate with the pathological grade \( (P_{\text{value}} = 0.001) \).

Immunohistochemical detection of Bcl-2 showed positive result in 5 (10%) of samples all of them were malignant, and negative result in 45 (90%) sample 10 (20%) were benign and 35 (70%) were malignant and there was insignificant association between Bcl-2 expression and malignancy \( (P_{\text{value}} = 0.239) \) compatible with results observed by Sarbia (1996) in their study in Bcl-2 expression in carcinomas of the esophagus only 48 (32.0%) out of 150 showed cytoplasmatic Bcl-2 expression.

Bcl-2 positive samples were compared with the type of tumor and we found that 4/5 positive samples were diagnosed as esophageal squamous cell carcinoma, and 1/5 was Adenocarcinoma. With insignificant relationship between each type of malignancy and the Bcl-2 positive results \( (P_{\text{value}} = 0.738) \).

Comparing the expression of Bcl-2 and the grade of the tumor the positive result was not observed in well differentiated while it has been observed in 2 moderately differentiated, 3 poorly differentiated with insignificant relation between the grade of tumor and expression of Bcl-2 \( (P_{\text{value}} = 0.857) \), as observed by Sarbia (1996) study in Bcl-2 expression correlated inversely with tumor differentiation, occurring more frequently in G3 and G4 carcinomas (47.1%) than in G1 and G2 no correlations were found between bcl-2 expression and stage.

On this study the expression of the two markers was P53-/Bcl2- in 16 (52%) samples, P53+/bcl-2- in 19 (38%) samples, P53-/bcl2 + in 1(2%) sample, P53+/bcl2 + in 4 (8%) samples. we find that no correlation between the expression of p53 and bcl-2 in esophageal
tumor (p value =0.288), and this result is contradicted with the results of Arbabi et al (2005) in Iran who observed over expression of mutated p53 protein and wild type Bcl-2 in esophageal tumor samples with lower histological differentiation. Co-expression of p53 and Bcl-2 implies that functional alterations in p53 protein may affect transcription regulation of Bcl-2 and consequently the over expression of Bcl-2 protein (Blagosklonny, 2001).
Chapter six

Conclusion and recommendations
CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion:
On the basis of this study we conclude that:

There is association between P53 expression and malignant tumors of esophagus, with no association with type of cancer.
The expression of P53 is associated with histological differentiation of cancer.
Bcl-2 expression is not associated with both type of tumor and the histological grade of cancer.
There is no correlation between the expression of P53 and Bcl-2 in esophageal tumors.

6.2 Recommendations:
On the basis of this study we recommend that:

Carry out further study in larger sample size.
Molecular detection of tumor markers should be done.
References
References


American Joint Committee on Cancer/Union Against Cancer staging manuals. Cancer;116:3763-3773.


Appendices
APPENDICES

**Materials and Instruments:**
Materials and instruments used for processing and staining of the specimens include:
- Disposable gloves
- Rotary microtome
- Microtome knives
- Coated slides
- Cover glasses
- Dry oven
- Water bath
- Coplin jars
- Humidity chamber
- Ethanol (100%, 90%, 70%, 50%)
- Xylene
- Mayer’s haematoxylin
- Citrate buffer (PH 6.8)
- Phosphate buffer (PH 7.4)
- Primary antibody P53
- Primary antibody Bcl-2
- Secondary antibody
- Substrate
- Chromogen
Staining Interpretation

Cells labeled by the antibody display cytoplasmic staining.

Performance characteristics

Normal tissue: The antibody labels most of peripheral blood lymphocytes. In lymphoid tissues, small lymphocytes in the mantle zones and T-cell areas are positive whereas very few cells in germinal centers are labeled. In the spleen, many cells in both T- and B-cell areas and the red pulp are labeled by the antibody. In the thymus, many cells in the medulla are labeled, whereas most cells in the cortex show weak or negative staining. In skin, the lymphocytes in the peripheral nerve zone and interfollicular lymphocytes show a moderate to strong staining reaction, whereas basal epithelial cells show a weak to moderate staining reaction.

Abnormal findings: The antibody labeled many neoplastic cells including 31/38 of cases of diffuse lymphoma and lymphomatous disorders of low and high grade, including chronic lymphocytic leukemia, hairy cell leukemia, T-cell lymphomas, B- and T-cell large cell lymphomas, and angiocentric large cell Ki-1 lymphoma, and 37/43 of neoplastic follicles in cases of follicular lymphoma (1). Expression of BCL-2 oncogene was also detected in 15/19 of non-Hodgkin sarcomas (7), and in macrophage derived tumors (6). Labeling was only observed in 5/21 Burkitt lymphomas, while 32/52 diffuse large B-cell lymphomas were positive with the antibody (2).
Intended use
For in vitro diagnostic use.

Summary and explanation
BCL2 Oncoprotein is a blocker of apoptotic cell death. Some tumor experiments have shown that elevated levels of this protein can protect a wide variety of cells from diverse cell death stimuli ranging from growth factor withdrawal and cytotoxic lymphocytes to virus infection and DNA damaging, anticancer drugs and radiation. BCL2 Oncoprotein resides on the cytoplasmic side of the mitochondrial outer membrane, cytoplasmic reticulum and nuclear envelope and has a molecular mass of 25 kDa. The BCL2 gene is involved in the lymphomagenesis of the large B-cell lymphomas (5). In this translocation, the BCL2 gene at chromosome segment 18q21 is juxtaposed with the Ig heavy chain locus at 14q32, resulting in deregulated expression of BCL2 Oncoprotein (5). Refer to Dako's General Instructions for Immunohistochemical Staining or the detection system instructions of IHC procedures for: 1) Principle of Principle, 2) Materials Required, 3) Staining, 4) Specimen Preparation 5) Staining Procedures, 6) Quality Control, 7) Troubleshooting, 8) Interpretation of Staining, 9) General Limitations.

Reagent provided
Ready-to-use monoclonal mouse antibody provided in liquid form in a buffer containing stabilizing protein and 0.015 M, sodium azide. Clone: 124 (lgG1, kappa).

Immunogen
Synthetic peptides comprising amino acids 61-54 of human BCL2 oncoprotein (1).

Specificity
In Western blotting of extracts of normal human spleen (1, 6), 14q32-positive follicular lymphoma (1), and myeloid leukemic cell lines (8), the antibody labels solely a band of 25 kDa corresponding to BCL2 Oncoprotein under both reducing (1) and reductive conditions (1, 6). The antibody labels the myeloid leukemic cell lines, HL-60 (myelomonocytic), KG-1 (myeloblastic), M1 (myeloblastic) and K562 (erythroblastic) (6).

Precautions
1. For professional users.
2. The product contains sodium azide (Na35), 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 buildups of metal azides. Upon disposal, flush with large volumes of water to prevent azide buildup 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. Do not use after expiration date shown on vial. 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 is a problem in the antibody is suspected, contact Dako Technical Support.

Specimen preparation including materials required but not supplied
The antibody can be used for labeling formalin-fixed, paraffin-embedded tissue sections. Tissue sections should be cut into sections of approximately 4 µm.

Pre-treatment with heat-induced epitope retrieval (HIER) is required using Dako PT Link (Code PT1000/PT101). For details, please refer to the PT Link User Guide. Optimal results are obtained by pretreating tissues using Envision™ FLEX Target Retrieval Solution, High pH (50x) (Code K8001/K8004).

Paraffin-embedded sections: Pre-treatment of formalin-fixed, paraffin-embedded tissue sections is recommended using the 2×10-minute pretreatment sections of Dako PT Link. Follow the pre-treatment procedure outlined in the package insert for Envision™ FLEX Target Retrieval Solution, High pH (50x) (Code K8001/K8004). Note: After staining the sections must be dehydrated, cleared and mounted using permanent mounting medium.

Deparaffinized sections: Pre-treatment of deparaffinized formalin-fixed, paraffin-embedded tissue sections is recommended using Dako HIER Link and following the same procedure as described for paraffin-embedded sections. After staining the sections should be mounted using aqueous or permanent mounting medium.

The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of FLEX INC Microscope Slides (Code K8020) is recommended.

Staining procedure including materials required but not supplied
The recommended visualization system is Envision™ FLEX, High pH. (Dako Autostainer/Autostainer Plus) (Code K8015). The staining steps and incubation times are pre-programmed into the software of Dako Autostainer/Autostainer Plus instruments, using the following protocols: Template protocol: FLEXRT01 (200 µl dispersed volume) or FLEXRT03 (400 µl dispersed volume)

Auxiliary protocol: FLEXRT03 (200 µl dispersed volume) or FLEXRT04 (400 µl dispersed volume)

The Auxiliary step should be set to “rinse buffer” in staining runs with ≤10 slides. For staining runs with >10 slides the Auxiliary step should be set to “none”. This prevents comparable wash times.

All incubation steps should be performed at room temperature, For details, please refer to the Operator’s Manual for the dedicated instrument. If the protocols are not available on the used Dako Autostainer instrument, please contact Dako Technical Services.

Optimal conditions may vary depending on specimen and preparation methods, and should be determined by each individual laboratory. If the evaluation pathway should desire a different staining intensity, a Dako Application Specialist/Technical Service Specialist can be consulted for information on re-programming of the protocol. Verify that the performance of the adjusted protocol is still valid by evaluating that the staining pattern is identical to the staining pattern described in “Performance characteristics”.

Countersmae in hematoxylin is recommended using Envision™ FLEX Hematoxylin, (Dako Autostainer/Autostainer Plus) (Code K8161). No aqueous, permanent mounting medium is recommended.
Les anticorps marquent la présence du p53 de type sauvage et existent de la même manière que des tests de cytopathologie. Le p53 est une protéine qui, lorsqu'elle est détectée, peut entraîner la prolifération cellulaire. Il est également possible de détecter la présence de la protéine p53 dans les cellules cancéreuses. La technique de cytopathologie permet d'identifier ces anticorps et de détecter la présence de la protéine p53.

En conclusion, la cytopathologie permet de détecter la présence de la protéine p53 dans les cellules cancéreuses, ce qui peut aider au diagnostic et au traitement des maladies cancéreuses. Il est important de noter que la cytopathologie est un outil précieux dans le诊断 et le traitement des maladies cancéreuses.

Références

Précautions
1. Ne pas utiliser les anticorps dans des conditions qui pourraient entraîner une réaction allergique.
2. Ne pas utiliser les anticorps dans des conditions qui pourraient entraîner une réaction infectieuse.
3. Ne pas utiliser les anticorps dans des conditions qui pourraient entraîner une réaction immunologique.

Conseils pour l’utilisation
1. Utiliser les anticorps dans des conditions qui pourraient entraîner une réaction allergique.
2. Utiliser les anticorps dans des conditions qui pourraient entraîner une réaction infectieuse.
3. Utiliser les anticorps dans des conditions qui pourraient entraîner une réaction immunologique.

Les anticorps marquent la présence de la protéine p53 dans les cellules cancéreuses, ce qui peut aider au diagnostic et au traitement des maladies cancéreuses. Il est important de noter que la cytopathologie est un outil précieux dans le diagnosis et le traitement des maladies cancéreuses. Il est donc essentiel de s'assurer que les anticorps utilisés sont adaptés à la situation clinique.

En conclusion, la cytopathologie permet de détecter la présence de la protéine p53 dans les cellules cancéreuses, ce qui peut aider au diagnostic et au traitement des maladies cancéreuses. Il est important de noter que la cytopathologie est un outil précieux dans le diagnosis et le traitement des maladies cancéreuses. Il est donc essentiel de s'assurer que les anticorps utilisés sont adaptés à la situation clinique.
FLEX Monoclonal Mouse Anti-Human p53 Protein
Clone DO-7
Ready-to-Use
(Dako Autostainer/Autostainer Plus)
Code IS616
ENGLISH

Intended use
For in vitro diagnostic use.

Summary and explanation
p53 is a nuclear phosphoprotein with a molecular mass of 53 kDa. Wild-type p53 protein is present in a wide variety of normal cells, but the protein has a very short half-life and thus is present in only minute amounts (1), generally below the detection level of immunohistochemical methods (4). Somatic mutation of the p53 gene is a very frequent event in the development of human neoplasia, and because mutant p53 proteins often are much more stable than wildtype p53 protein, the mutant p53 protein accumulates to a high level (1). As examples, p53 protein accumulation was observed in 70% of 215 human malignant tumors, including breast, ovarian and stomach carcinomas, melanomas, embryonal carcinomas of the testis, transitional carcinomas of the urinary bladder, ovarian carcinoma and soft tissue sarcomas (5).

Wild-type p53 protein functions as a transcription factor, i.e. as a modifier, which can turn crucial genes either on or off. It also inhibits DNA replication and is a checkpoint control molecule for progression of the cell cycle. Furthermore, p53 protein is involved in the regulation of apoptosis (4). In in vivo research, wildtype p53 behaves as a tumor suppressor, while mutant p53 behaves as a dominant transforming oncogene (1).

Reagents provided
Ready-to-use monoclonal mouse antibody provided in liquid form in a buffer containing stabilizing protein and 0.01% methyl cellulose, sodium azide.

Storage: DO-7 (1) buffer, lyophilized, keeps

Instruments
For Dako's General instructions for immunohistochemical Staining or the detection system instructions of IHC procedures by 1) Principle of Procedure, 2) Materials Required, Not Supplied, 3) Staging, 4) Specimen Preparation, 5) Staining Procedure, 6) Quality Control, 7) Troubleshooting, 8) Interpretation of Staining, 9) General Limitations.

Precautions
1. For professional use only.
2. This product contains sodium azide (NaN₃), 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 procedure 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. Do not use after expiration date stamp has been removed. If reagents are stored under any conditions other than those specified, the reagents must be verified by the user. These 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.

Specimen preparation including materials required but not supplied
The antibody can be used for labeling formalin-fixed, paraffin-embedded tissue sections. Tissue sections should be cut into sections of approximately 4 μm.
Pre-treatment with heat-induced epitope retrieval (HER) is required using Dako PT Link (Code PT100/PT101). For details, please refer to the PT Link User Guide. Optimal results are obtained by pretreating tissues using Envision™ FLEX Target Retrieval Solution, High pH (50X) (Code K8050XKV000).
Paraffin-embedded sections: Pre-treatment of formalin-fixed, paraffin-embedded tissue sections is recommended using the 3×1 epitope retrieval procedure described in the package insert for Envision™ FLEX Target Retrieval Solution, High pH (50X) (Code K8050XKV000). Note: After staining the sections must be dehydrated, cleared and mounted using permanent mounting medium.
Open/Waxed sections: Pre-treatment of deparaffinized formalin-fixed, paraffin-embedded tissue sections is recommended using Dako PT Link and following the same procedure as described for paraffin-embedded sections. After staining the slides should be mounted using aqueous or permanent mounting medium.
The tissue sections should not dry out during the treatment and during the following Immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of FLEX IHC Microscope Slides (Code K8005) is recommended.

Staining procedure including materials required but not supplied
The recommended visualization system is Envision™ FLEX, High pH, (Dako Autostainer/Autostainer Plus) (Code K8010). The staining step and incubation times are pre-programmed into the software of Dako Autostainer/Autostainer Plus instruments, using the following protocols:
Template protocol: FLEXUT20 (200 μL, dispense volume) or FLEXUT30 (300 μL, dispense volume).
Auto program: p53 (without counterstaining) or p53 (with counterstaining).
The Auxiliary step should be set to "Wash buffer" in staining time 10 μL for slides. For staining with >10 slides the Auxiliary step should be set to "none". This sets timer accordingly.
All incubation steps should be performed at room temperature. For details, please refer to the Operator’s Manual for the dedicated instrument.
Optional conditions may vary depending on specimen and preparation methods, and should be determined by each individual laboratory. If the evaluating pathologist should desire a different staining intensity, a Dako Application Specialist/Technical Service Specialist can be contacted for information on reprogramming of the protocol. Verify that the performance of the adjusted protocol is still valid by evaluating the matching staining pattern described in "Performance characteristics".