بسم الله الرحمن الرحيم

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Frequency of Survivin Expression in Breast Cancer using Immunohistochemistry

Thesis submitted for partial fulfillment for the requirement of master degree in medical laboratory science (Histopathology and Cytology)

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الآية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال تعالى:

﴿فَقُلْتُ استَغْفِرُوا رَبّكُم إنّهُ كَانَ غَفَّارَا(10) يُرسِلُ السَّمَاءَ عَلَيْكُم مِدْرَارَا(11) وَيَمِدُّكُمْ بِأمْوَالٍ وَبَنِيْنَ وَيَجْعَلُ لَكُمْ جَنَّاتٍ وَيَجْعَلُ لَكُمْ أنْهَارَا(12)﴾

صدق الله العظيم

سورة نوح من الآية (10 - 12)

Dedication

I dedicate this research to my father, my mother, my husband, my sister, my children and my brothers.

Acknowledgment

First and foremost, I thank Allah for letting me live to see this dissertation through. I am for ever indebted to Allah who support and give me power to do this dissertation.

I would like to thank my supervisor Dr. Ahmed Mohamed Ahmed, for his patience, continuous guidance throughout my dissertation with his knowledge.

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Abstract

This is retrospective a laboratory based descriptive study which was conducted at ELrhama medical center during the period from January 2018 to April 2018. The study was aimed to detect survivin antigen expression in breast carcinoma using immunohistochemistry.

Forty paraffin embedded blocks were collected from patients samples previously diagnosed as invasive ductal carcinoma. Grade I not reported, grade II 11(27.5%), grade III 29 (72.5%).

Tissue microarrays prepared from paraffin blocks, were cut by rotary microtome at 3µm, and then stained by immunohistochemical method (new indirect technique) .The data obtained was analyzed using SPSS program version 22.

The age of patients ranged between 30 to 71 years with mean age of 48.6 years. Most of patients were less than 50 years representing 23(57.5%) and the remaining 17 (42.5%) were more than 50 years.

Survivin expression was positive in all samples of malignant tumor. Regarding distribution of breast carcinoma samples in relation to tumor grading ,grade II the expression was moderate 11 (27.5%) , grade III was high 29(72.5%) ,this result showed significant association between survivin antigen expression and breast carcinoma (p-value = 0.000).

Regarding the relation between age and expression of survivin antigen was found , less than 50 years 7(17.5%) expression was moderate, 16(40%) high expression, more than 50 years 4(10%) expression was moderate, 13(32.5%) high expression .This result showed no association between survivin antigen expression and age (p-value = 0.508).

The study concluded that the expression of survivin antigen was associated with breast carcinoma. Distribution of survivin expression does not affected by age.

المستخلص

أجريت هذه الدراسة الوصفية التحليلية فى مركز الرحمة الطبى شعبة الأنسجة والخلايا المريضة بولاية الخرطوم فى الفترة من يناير إلى ابريل 2018، هدفت الدراسة للكشف عن ظهور مستضد السيرفيفين فى أورام الثدى الخبيثة باستخدام كيمياء الأنسجة المناعية .

جُمع أربعون قالب شمعى من عينات مرضى كانوا مشخصين مسبقاً على أنهم مصابون بأورام الثدى الخبيثة. المرحلة الأولى من المرض لم تسجَل ، المرحلة الثانية من المرض كانت تمثل 11 بنسبة (27.5 %) ، والمرحلة الثالثة من المرض كانت تمثل 29 بنسبة (72.5%) .

أختيرت مصفوفات الانسجة الصغيرة من قوالب الشمع ، وقطعت باستخدام المشراح الدوار بسمك 3 ميكرون ، صبغت بواسطة طريقة كيمياء الانسجة المناعية (الطريقة الجديدة غير المباشرة) ، واستخدم برنامج الحزم الإحصائية للعلوم الاجتماعية ، النسخة 22 لتحليل البيانات.

تراوحت أعمار المرضى بين 30 الى 71 عام بمتوسط عمر 48.6 سنة. أظهرت الدراسة أن معظم المرضى كانت أعمارهم أقل من 50 سنة كان عددهم 23 بنسبة (57.5%) والذين كانت أعمارهم أكثر من 50 سنة 17 بنسبة (42.5%).

أظهرت الدراسة أن مستضد السيرفيفين كان موجب الظهور فى جميع العينات مع وجود علاقة ذات دلالة إحصائية بين ظهور المستضد ومراحل الورم (القيمة الاحتمالية = 0.000) .

فيما يتعلق بالعلاقة بين ظهور مستضد السيرفيفين وأعمار المرضى وجد أن الأعمار أقل من 50 سنة كانوا 7 وبنسبة (17.5%) ظهر مستضد السيرفيفين بشكل متوسط ، 16 و بنسبة (40%) ظهر بشكل قوي ، أما الأعمار اكثر من 50 سنة ظهر مستضد السيرفيفين بشكل متوسط في 4 وبنسبة (10%) ، وظهر بشكل قوي في 13 وبنسبة (32.5%) .مع عدم وجود علاقة ذات دلالة إحصائية بين ظهور مستضد السيرفيفين وأعمار المرضى (القيمة الاحتمالية = 0.508) .

خلصت الدراسة الى أن ظهور مستضد السيرفيفين له ارتباط مع أورام الثدى الخبيثة مع عدم وجود علاقة مع توزيع أعمارالمرضى.

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IAP	Inhibitor of Apoptosis
FNA	Fine Needle Aspiration
DCIS	Ductal Carcinoma In-Situ
ELISA	Enzyme-Linked Immunosorbent Assay
PARP	Poly ADP Ribose Polymerase
MRI	Magnetic Resonance Imaging
DAB	Di Amino Benzedrine Tetrahydrochloride
SPSS	Statistical Package for Social Science
DPX	Distyrene a plasticizer and xylene

Chapter One

Introduction

Objectives

1. Introduction

1.1 Introduction:

Breast cancer is the most common cancer in women, is the cancer that arises from breast cells, and contains many types [1]. Breast cancer is the most common cancer in women worldwide, with nearly 1.7 million new cases diagnosed in (2012). This represents about 12% of all new cancer cases and 25% of all cancers in women. It is the fifth most common cause of death from cancer in women. The four most common cancers occurring worldwide are lung, breast, bowel and prostate cancer these four account for around 4 in 10 of all cancers diagnosed Worldwide there will be 23.6 million new cases of cancer each year by 2030 (estimated). Breast cancer occurs when abnormal cells in the breast multiply uncontrollably to form a tumor. While most cases of breast cancer occur in women it does occur in men too, although this is rare (about 1% of cases). Survival rates for breast cancer vary worldwide. Advanced and metastatic breast cancer is currently incurable, but treatable, with a median survival rate of 2-3 years [2].

In Sudan data revealed that 74% of the women were < 50 years old or premenopausal [3].

Changes or mutations in DNA can cause normal breast cells to become cancer. Certain DNA changes are passed on from parents (inherited) and can greatly increase your risk for breast cancer. Other lifestyle-related risk factors, Hormones seem to play a role in many cases of breast cancer, but just how this happens is not fully understood [4].

Methods of diagnosis of breast cancer are via fine needle aspirate (FNA), mammography, immunohistochemistry, tissue biopsy, and molecular diagnosis [5].

Treatment options include, Surgery, Radiation therapy, Hormone therapy, Chemotherapy, Targeted therapy and may be more than one type of treatment [6].

Survivin is a protein that inhibits apoptosis and regulates cell division [7]. The survivin gene spans 15 kb, and is located on chromosome 17 at band q25 [8].

Survivin is a member of the inhibitor of apoptosis (IAP) family. The survivin protein functions to inhibit caspase activation, there by leading to negative regulation of apoptosis or Programmed cell death [9]. Several studies were carried out to assess the possibility of survivin as a prognostic molecule. For example, in a study of 275 patients with breast cancer (70), survivin mRNA was highly expressed in tissues from younger patients (<50) and in high-grade cancer tissues. High survivin concentrations were most strongly associated with ER- or PR-negative tumors. Survivin demonstrates a strong, independent association with poor prognosis. Survivin might be used as a new marker to stratify breast cancer patients for more optimal treatment modalities, or it could be a promising new target for therapy. In another study, survivin expression was examined in 167 cases of breast cancer and the results suggest that apoptosis inhibition by survivin, is a significant prognostic parameter of worse outcome in breast carcinoma [9].

Rationale:-

Survivin is apoptosis inhibitor, and usually expressed in tissue as indicator for cancer cells, so it's expression in breast cancer may be helpful in diagnosis and prognosis for breast cancer detection and monitoring.

1.2 Objectives:

1.2.1 General objective:

To study the expression of survivin in breast cancer among Sudanese women patients.

1.2.2 Specific objectives:

- To detect the expression of survivin in the samples of breast cancer patients.
- To determine the percentage of survivin expression in breast cancer tissue.
- To detect the correlation between the survivin as a tumor marker and the histological grading of breast cancer.

Chapter Two

Literature review

2. Literature review:

2.1 Breast Anatomy and histology:

The breast is a modified skin gland enveloped in fibrous fascia. The superficial pectoral fascia is located just beneath the skin and in the retro mammary space. The undersurface of the breast lies on the deep pectoral fascia. Although there are fascial layers between the breast proper and the pectoral is major muscle, the breast is not completely separate from the pectoral is major muscle, as there are penetrating lymphatic's and blood vessels .The breast is composed of three major structures: skin, subcutaneous tissue, and breast tissue (parenchyma and stroma). The parenchyma is divided into 15 to 20 lobes or segments that converge at the nipple in a radial arrangement. The ducts from the lobes converge into 6 to 10 major collecting ducts that have openings at the nipple and connect to the outside. Each of these major ducts arborizes back from the nipple and forms a lobe or segment of glandular tissue that is supported by surrounding connective or stromal tissue. The distribution of lobes is not even as there is a preponderance of glandular tissue in the upper outer quadrant of the breast. Beneath the nipple openings, the lactiferous sinus is visible. The lactiferous sinus is a slight dilation of the ampullary portion of the major duct. The major ducts that converge below the nipple and drain each segment are 2mm in diameter. Each duct drains a lobe made up of 20 to 40 lobules. Each lobule contains 10 to 100 alveoli or acini. Each lobule also consists of branching ducts that divide into sub segmental structures and terminate in the terminal duct lobular unit. The terminal duct lobular unit consists of the terminal duct and the acinus. The glandular tissue and ducts are surrounded by fat and supported by Cooper's ligaments, which are connective tissue elements that arise from stromal tissue and attach to the prepectoral fascia and dermis and support and suspend the breast tissue [(10)].

2.2 Breast pathology:

The vast majority of the lesions that occur in the breast are benign. Much concern is given to malignant lesions of the breast because breast cancer is the most common malignancy in women in Western countries; however, benign lesions of the breast are far more frequent than malignant ones [(11)].

2.2.1 Tumors of the breast:

2.2.1.1 Benign Breast Diseases:

2.2.1.1.1 Inflammatory and Related Lesions:

2.2.1.1.1.1 Mastitis:

These changes are a result of infectious agents; others do not have a well-understood etiology and may represent local reaction to a systemic disease, or a localized antigen-antibody reaction [(12)].

2.2.1.1.1.2 Acute Mastitis:

Acute mastitis usually occurs during the first 3 months postpartum as a result of breast feeding .Also known as puerperal or lactation mastitis, this disorder is a cellulitis of the interlobular connective tissue within the mammary gland, which can result in abscess formation and septicemia. It is diagnosed based on clinical symptoms and signs indicating inflammation [(13)].

2.2.1.1.1.3 Granulomatous Mastitis:

Granulomatous reactions resulting from an infectious etiology, foreign material, or systemic autoimmune diseases such as sarcoidosis and Wegener's granulomatosis can involve the breast many different types of organisms can cause granulomatous mastitis [(14)].

2.2.1.1.1.4 Foreign Body Reactions:

Foreign materials such as silicone and paraffin, which are used for both breast augmentation and reconstruction after cancer surgery, may cause a foreign body-type granulomatous reaction in the breast. Silicone granulomas ("siliconomas")

usually occur after direct injection of silicone in to the breast tissue or after extra capsular rupture of an implant [15].

2.2.1.1.1.5 Recurring Subareolar Abscess:

Recurring subareolar abscess (Zuska's disease) is a rare bacterial infection of the breast that is characterized by a triad of draining cutaneous fistula from the subareolar tissue; a chronic thick, pasty discharge from the nipple; and a history of multiple, recurrent mammary abscesses [16]. The disease is caused by squamous metaplasia of one or more lactiferous ducts in their passage through the nipple, probably induced by smoking [17].

2.2.1.1.1.6 Mammary Duct Ectasia:

Also called periductal mastitis It is a disease of primarily middle-aged to elderly parous women who usually present with nipple discharge, a palpable subareolar mass, noncyclical mastalgia, or nipple inversion or retraction .Smoking has been implicated as an etiologic factor in mammary duct ectasia [18].

2.2.1.1.1.7 Fat Necrosis:

Fat necrosis of the breast is a benign nonsuppurative inflammatory process of adipose tissue. It can occur secondary to accidental or surgical trauma, or it may be associated with carcinoma or any lesion that provokes suppurative or necrotic degeneration, such as mammary duct ectasia and, to a lesser extent, fibrocystic disease with large cyst formation [19]. It is characterized by anuclear fat cells often surrounded by histiocytic giant cells and foamy phagocytic histiocytes [20].

2.2.1.1.1.8 Fibrocystic changes (FCCs):

Constitute the most frequent benign disorder of the breast. Such changes generally affect premenopausal women between 20 and 50 years of age [21]. Although many other names have been used to describe this entity over the years, (including fibrocystic disease, cystic mastopathy, chronic cystic disease, mazoplasia, Reclus's

disease), the term “fibrocystic changes” is now preferred, because this process is observed clinically in up to 50% and histologically in 90% of women [22].

2.2. 1.1.1.9 Cysts:

Are fluid-filled, round or ovoid structures that are found in as many as one third of women between 35 and 50 years old. Although most are subclinical “microcysts,” in about 20% – 25% of cases, palpable (gross) cystic change, which generally presents as a simple cyst. Cysts cannot reliably be distinguished from solid masses by clinical breast examination or mammography; in these cases, ultrasonography and fine needle aspiration (FNA) cytology, which are highly accurate, are used [23].

2.2.1.1.1.10 Adenosis:

Adenosis of the breast is a proliferative lesion that is characterized by an increased number or size of glandular components, mostly involving the lobular units. Various types of adenosis have been described, of which sclerosing adenosis and microglandular adenosis [24].

2.2.1.1.1.11 Metaplasia:

Apocrine metaplasia is characterized by the presence of columnar cells with abundant granular, eosinophilic cytoplasm and luminal cytoplasmic projections or apical snouts. These cells line dilated ducts or can be seen in papillary proliferations. They are more frequently found in younger women. All normal and metaplastic apocrine cells can be stained with gross cystic disease fluid protein. Atypical apocrine metaplasia should be diagnosed only when the nuclei of the apocrine cells display significant cytologic atypia [25].

2.2.1.1.1.12 Epithelial Hyperplasia:

Epithelial hyperplasia is the most common form of proliferative breast disease, divided to ductal and lobular type [26].

2.2.1.1.1.12.1 Ductal lesions:

The most important cytologic features of mild, moderate, or florid epithelial hyperplasia are an admixture of cell types (epithelial cells, myoepithelial cells, and metaplastic apocrine cells) and variation in the appearances of epithelial cells and their nuclei [(26)].

2.2.1.1.1.12.2 Lobular Lesions:

Lobular neoplasia is most prevalent in perimenopausal women. It is a multifocal lesion, and many patients have lesions involving multiple quadrants of the breast, and increase the risk for the subsequent development of invasive carcinoma [(27)].

2.2.1.1.1.13 Columnar Cell Lesions:

Columnar cell lesions of the breast represent a spectrum of lesions that have been encountered with increasing frequency in needle core breast biopsies because these lesions are commonly associated with microcalcifications and detected by mammographic screening [(28)].

2.2.1.1.1.14 Intraductal Papilloma:

Intraductal papilloma is a discrete benign tumor of the epithelium of mammary ducts. It can arise at any point in the ductal system and shows a predilection for the extreme ends of the ductal system: the lactiferous sinuses and the terminal ductules [(29)].

2.2.1.2 Proliferative Stromal Lesions:

2.2.1.3 Neoplasms:

2.2.1.3.1 Fibroadenoma:

Is the most common lesion of the breast; it occurs in 25% of asymptomatic women [(30)]. It is usually a disease of early reproductive life; the peak incidence is between the ages of 15 and 35 years. Conventionally regarded as a benign tumor of the breast, fibroadenoma is also thought to represent a group of hyperplastic breast lobules called “aberrations of normal development and involution. The lesion is a

hormone-dependent neoplasm that lactates during pregnancy and involutes along with the rest of the breast in perimenopause [31].

2.2.1.3.2 Adenoma:

An adenoma is pure epithelial neoplasm of the breast. This lesion is divided into tubular, lactating, apocrine, ductal, and so-called pleomorphic (i.e., benign mixed tumor) adenoma [32].

2.2.2 Malignant Tumor of the Breast:

2.2.2.1 Noninvasive:

2.2.2.1.1 Intraductal carcinoma:

Carcinoma limited to the ducts (Ductal Carcinoma in situ DCIS) is reported in different age groups with increasing frequency, mainly attributable to the benefits of screening mammography [33].

2.2.2.1.2 Lobular carcinoma in situ:

It is generally a non-palpable lesion diagnosed by mammography which may be located adjacent to fibrocystic changes or occurs concomitantly with infiltrative lobular carcinoma [33].

2.2.2.2 Invasive:

2.2.2.2.1 Invasive ductal carcinoma:

This is the most common type exhibiting marked increase in dense fibrous stroma or desmoplastic response giving the tumour a hard consistency (Scirrhous). This type of cancer is usually associated with DCIS. The tumor margins are usually irregular [33].

2.2.2.2. 2 Invasive lobular carcinoma:

Arises from the terminal ductules of the breast lobule. This type tends to be bilateral and multi centric [33].

2.2.2.2. 3 Mutinous carcinoma:

Tends to occur in older patients and often produces large masses which give the tumor its soft consistency on palpation [33].

2.2.2.2. 4 Medullary carcinoma:

This defined by (WHO) as a well-circumscribed carcinoma composed of poorly differentiated cells with scanty stroma and prominent lymphoid infiltration [33].

2.2.2.2. 5 Papillary carcinoma:

A rare carcinoma in which invasive pattern is predominantly in the form of papillary structures. It may be adjacent to the nipple causing bloody or serosanguinous discharge [33].

2.2.2.2. 6 Tubular carcinoma:

These tumors occur as small, firm, discrete masses. The (WHO) describes it as well differentiated carcinoma whose cells are arranged in regular well defined tubules typically lined by one epithelial layer and accompanied by abundant fibrostroma. Uncommon tumors, having characteristic cribri form pattern and are of the type seen more typically in the salivary gland [33].

2.2.2.2. 2.7 Paget's disease of the nipple:

It is a specialized form of ductal carcinoma arising in the main secretory ducts and extend to involve the skin of the nipple and areola, which exhibit eczematous changes [33].

2.2.3 Mixed Connective Tissue And Epithelial Tumors:

Fibro adenoma, Phyllodes Tumor (cystosarcoma phyllodes), Carcinosarcoma [34].

2.3 Epidemiology of breast cancer:

Breast cancer is the most common malignancy in women [34]. Breast cancer is the most common cancer in women worldwide, with nearly 1.7 million new cases diagnosed in 2012 (second most common cancer overall). This represents about

12% of all new cancer cases and 25% of all cancers in women. It is the fifth most common cause of death from cancer in women [2].

In Sudan Data revealed that 74% of the women were < 50 years old or premenopausal. Invasive ductal carcinoma was the most common pathology (82%) and women presenting with stage III or higher tumors that had already metastasized, while ductal carcinoma in situ was the least prevalent (0.5%) finding [3].

2.4 Risk factors of breast cancer:

2.4.1 Sex:

Women are 100 times more likely to develop breast cancer than men [35].

2.4.2 Age:

Increasing age is one of the strongest risk factors for breast cancer. Age is considered to be a likely surrogate for DNA damage accumulated during life [36].

2.4.3 Affluence and lifestyle:

Breast cancer occurs more frequently in affluent and western populations [37].

2.4.4 Family history:

Family history is an important and well-established breast cancer risk factor. Women with a mother, sister or daughter with breast cancer are, on average, at twice the risk of those with no affected first-degree relative [38].

2.4.5 Endogenous oestrogens:

Postmenopausal women with high levels of circulating oestrogens (women with levels in the top 20%) have a two-fold increased risk of breast cancer compared with women with low levels of circulating oestrogens (women with levels in the bottom 20%) [39].

2.4.6 Hormonal factors:

Factors such as reproductive history, menstrual history, menopausal status and exogenous hormone use are associated with breast cancer risk [40].

2.5 Method of diagnosis of breast cancer:

2.5.1 Imaging Tests to Find Breast Cancer:

Different tests can be used to look for and diagnose breast cancer. If your doctor finds an area of concern on a screening test (a mammogram), or if you have symptoms that could mean breast cancer, you will need more tests to know for sure if it's cancer. Mammograms, Breast Ultrasound, Breast MRI Scans, Newer and Experimental Breast Imaging Tests [(41)].

2.5.2 Biopsy:

A biopsy is done when mammograms, other imaging tests, or a physical exam shows a breast change that may be cancer. A biopsy is the only way to know for sure if it's cancer [(42)].

2.5.3 Fine needle aspiration (FNA) biopsy:

In an FNA biopsy, a very thin, hollow needle attached to a syringe is used to withdraw (aspirate) a small amount of tissue from a suspicious area. The needle used for an FNA biopsy is thinner than the one used for blood tests [(43)].

2.5.4 Core needle biopsy:

A core biopsy uses a larger needle to sample breast changes felt by the doctor or seen on an ultrasound, mammogram, or MRI [(44)].

2.5.5 Surgical (open) biopsy:

In rare cases, surgery is needed to remove all or part of the lump for testing. This is called a surgical or open biopsy. Most often, the surgeon removes the entire mass or abnormal area as well as a surrounding margin of normal breast tissue [(45)].

2.5.6 Lymph node biopsy:

The doctor may also need to biopsy the lymph nodes under the arm to check them for cancer spread. This might be done at the same time as biopsy of the breast tumor [(46)].

2.5.7 Biomarker-based methods:

Such as radioimmunoassay, immunohistochemistry, enzyme-linked immunosorbent assay (ELISA) and fluoroimmunoassay also cater to the diagnostic requirements for breast cancer [47].

2.6 Breast Cancer treatment:

2.6.1Chemotherapy:

It is known that chemotherapy can be helpful for many breast cancer patients [48].

2.6.2 Oncoplastic surgery:

Breast-conserving surgery (lumpectomy or partial mastectomy) can often be used for early-stage breast cancers [49].

2.6.3 Triple-negative breast cancer:

Since triple-negative breast cancers cannot be treated with hormone therapy or targeted therapy such as HER2 drugs, the treatment options are limited to chemotherapy [50].

2.6.4 Targeted therapy drugs:

Targeted therapies are a group of drugs that specifically target gene changes in can cells that help the cells grow or spread. New targeted therapies are being studied for use against breast cancer, including PARP inhibitors [51].

2.6.5 Supportive care:

There are trials looking at different medicines to try and improve memory and brain symptoms after chemotherapy [52].

2.7 Survivin:

Survivin is a protein that inhibits apoptosis and regulates cell division [53]. Survivin plays an important role in the suppression of apoptosis by either directly or indirectly inhibits the activity of caspases. Survivin expression was not found in most normal adult human tissues when examined for reports investigating the role of survivin in cancer, human survivin expression has been reported in some normal

adult human tissues, including colonic mucosa [54], placenta [55], bone marrow and keratinocytes of the basal layer of the skin [56]. Over expression of survivin has been reported in almost all human malignancies including bladder cancer, lung cancer, breast cancer, stomach, esophagus, liver, ovarian cancers and hematological cancers [57].

2.8 Survivin and breast cancer:

Boidot *et al.* (2000) showed that survivin expression might induce breast tumour proliferation by promoting genetic instability, the expression in breast cancer 70.7%-90.2% [58].

Ambrosi *et* al.(1999) Survivin is expressed in most human cancers including that of colon, breast, lung, pancreas, prostate, stomach, esophagus, and in highgrade non-Hodgkin's lymphoma (59) .

Yamashita *et al.* (2002) showed that there was a significant differences between the high and low expression groups ($p<0.000$), Patients with low survivin expression showed better disease-free survival than patients with high survivin expression (60).

Shin-chi Yamashita *et al.* (2007) reported that patients with low survivin expression showed significantly better disease-free survival than patients with high survivin expression in stage I and II breast cancer ($p<0.0001$) [61].

Yong-Gang *et al.* (2010) assessed the possibility of survivin as a prognostic molecule. For example, in a study of 275 patients with breast cancer (70), survivin mRNA was highly expressed in tissues from younger patients (<50) and in high-grade cancer tissues. Survivin demonstrates a strong, independent association with poor prognosis [9].

Chapter Three

Materials and Method

3.1 Material and Methods

3.1 Materials:

Archived tissue block obtained from breast samples previously diagnosed as breast cancer were selected randomly for this study.

3.2 Methods:

3.2.1 Study design:

This is a descriptive, retrospective laboratory based study aimed to detect the expression of survivin in breast cancer among Sudanese women patients using immunohistochemistry.

3.2.2 Study sampling and sample size:

(40) Paraffin blocks previously diagnosed as breast cancer and positive control for survivin were selected from Elrahama medical center according to samples availability. Patient identification (age, grad and diagnosis) were obtained from patients records.

3.2.3 Study area:

This study was conducted at Elrahama medical center during the period from January to April 2018.

3.2.4 Immunohistochemistry Staining:

The immunohistochemical procedure was done as follows: one section (3μm) from formalin-fixed, paraffin-embedded tumors using tissue micro array technique, were cut and mounted onto Stalinized slides (Thermo). Following deparaffinization in xylene, slides were rehydrated through a graded series of alcohol and were placed in distilled water. Samples were steamed for antigen retrieval for Survivin using high PH (9) by water bath at 95C for 40 min. After washing with PBS for 3 min Endogenous peroxides activity were blocked with 3% hydrogen peroxide and methanol for 10 min, and After washing with PBS for 3 min then Slides were incubated with (100 μ L) of (rabbit monoclonal antibody (Survivin) Quartett),

against Survivin antigen for 30 min at room temperature in a moisture chamber. After washing with PBS for 3 min, binding of antibodies will be detected by incubating for 20 min with dextrin labeled polymer (Dako). Finally, the sections washed in three changes of PBS, followed by adding 3, 3 diaminobenzidinetetrahydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. After washing with distilled water for 3 min. Slides were counterstained with haematoxylin (MAYER'S) for one min and washed in running tap water for several minutes 7-10 (bluing), then dehydrate, cleaned, and mounting DBX. Each slide was evaluated with investigator then the results were confirmed by consultant histopathologist.

3.2.5 Data analysis:

The obtained results and variables arranged in standard master sheet, then analyzed using statistical package for social (SPSS) program. Frequencies, means and chi squire tests were calculated.

3.2.6 Result interpretation:

All quality control measures were adopted; positive and negative control sections were used during immunohistochemical staining. Detection of more than 5 cells with cytoplasmic and nuclear reaction per one field considered as positive result.

3.2.7 Ethical consideration:

The Samples were collected after permission according to the laboratory guidelines and regulation.

Chapter Four

Results

4. Result

The study included forty samples previously diagnosed as breast cancer. The age of patients ranged between 30 to 71 years with mean age of 50 years, and standard deviation was 10.7.

Most of patients were less than 50 years old representing 23/40 (57.5%) and the remaining 17/40 (42.5%) were more than 50 years, as indicated in table (4.1).

The histological grade of the study samples includes 11/40 (27.5%) of grade II, and 29/40 (72.5%) of grade III, grade I was not present, as indicated in table (4.2).

Survivin expression was positive in all samples of malignant tumor 40/40 (100%), the expression of survivin was moderately expressed in grade II 11/40 (27.5%) and highly expressed in grade III 29/40 (72.5%).This result showed a significant association between survivin antigen expression and histological grade (p-value = 0.000) as indicated in table (4.3).

Regarding the age of patients, the age group that were less than 50 years, Survivin was moderately expressed in 7/23 (30.4%) and highly expressed in 16/23 (69.6%), more than 50 years group, Survivin was moderately expressed in 4/17 (23.5%) and highly expressed in 13/17 (76.5%).This result showed no association between survivin antigen expression and age (p-value = 0.508) as indicated in table (4.4).

Table (4.1): Distribution of age groups among study patients:

Age	Frequency	Percent
≤ 50 years	23	57.5
≥50 years	17	42.5
Total	40	100

Table (4.2): Distribution of histological grade among study samples:

Grade	Frequency	Percent
Grade I	11	27.5
Grade II	29	72.5
Total	40	100

Table (4. 3): Correlation between the expression of survivin in breast carcinoma and histological grading:

Grade	**Expression**		**Total**	**P.Value**
	Moderate	High		
Grade II	11 (27.5%)	0 (0.0%)	11 (27.5%)	0.000
Grade III	0 (0.0%)	29 (72.5%)	29 (72.5%)	
Total	11 (27.5%)	29 (72.5%)	40 (100.0%)	

Table (4. 4): Correlation between expression of survivin and patients age:

Age	Expression		Total	P.Value
	Moderate	High		
≤ 50 years	7 (17 .5%)	16 (40%	23 (57.5%)	0.508
≥50 years	4 (10%)	13 (32.5)	17 (42.5%)	
Total	11 (27.5%)	29 (72.5%)	40 (100.0%)	

Chapter Five

Discussion

5. Discussion

The present study included 40 samples of breast carcinoma stained by immunohistochemistry for survivin antigen expression .The study revealed that patient age less than 50 years old are more affected with breast carcinoma 23(57.5%) .This result was compatible with Elgaili, et al. (2010), who reported that common involved age by breast carcinoma was the age group of 30-50 years .

The expression of survivin antigen was positive in all study samples of breast carcinoma 40/40 (100%), this agree with Ambrosi *et* al.(1999) is reported Survivin is expressed in most human cancers including that the breast.

Survivin antigen expression in study group was increase with grading of breast carcinoma (p-value = 0.000). This was agreed with Shin-chi Yamashita, et al. (2007) reported the survivin expression increased with breast tumor grading highest in grade III.

The present study revealed there was no significant association between survivin expression antigen and patient age (p-value 0.508).This result is disagreed with Alkhala, et al. (2013) who reported that tumor Survivin expression increases with age.

Chapter Six

Conclusion

Recommendations

6. Conclusion and Recommendations

6.1Conclusion:

This study concluded that:

- The expression of survivin antigen was positive in all breast carcinoma.
- Survivin expression is associated with tumor grading, but no association with patient age.
- Most breast carcinoma patients in this study appear less than 50 years old.

6.2 Recommendation:

- Similar studies should be carried in larger sample size.
- Survivin recommended to be used as diagnostic and prognostic marker with other breast cancer marker panels.

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Appendices

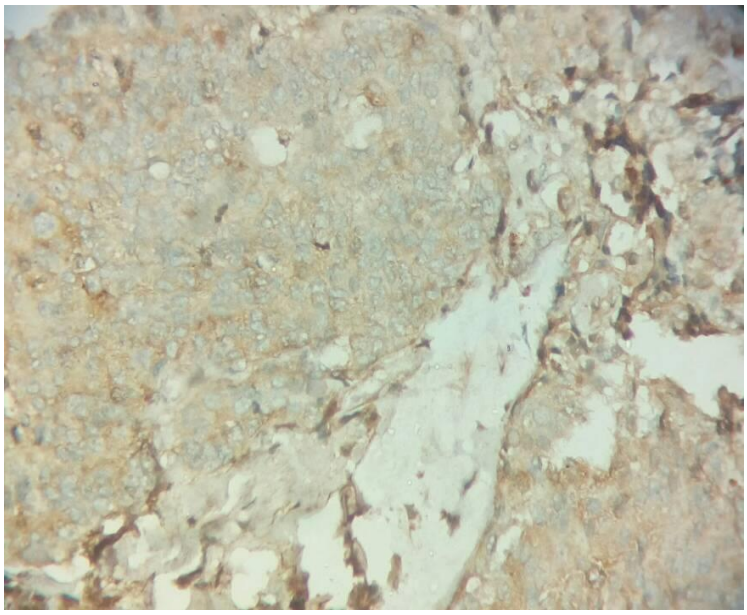
Appendix 1:

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

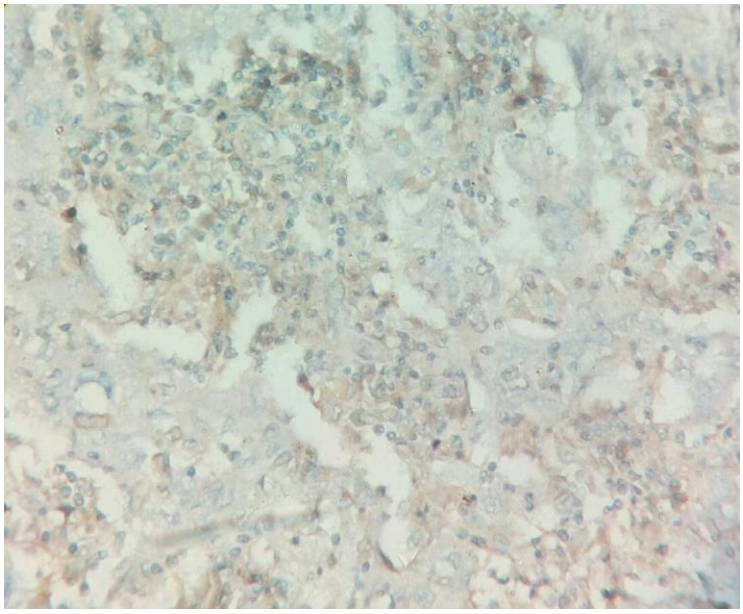
- Disposable gloves
- Microtome knife
- Positively charged slide
- Cover glass
- Dry oven
- Water bath
- Embedding center
- Coplin jar
- Humidity chamber
- Ethanol (100%, 90%, 70%, 50%)
- Mayer s haematoxylin (haematoxylin , DW,K or ammonium alum ,sodium iodated ,citric acid ,chororal hydrate)
- Reaction buffer
- Primary antibody (EBV)
- Tris EDTA buffer (PH9)
- Phosphate buffer saline (PH7.4)
- Peroxides blocker(3% hydrogen peroxide in methanol)
- Secondary anti body (dextran polymer conjugated secondary – HRP)
- DAB (3,3 di amino benzidin tetra hydrochloride)substrate solution
- Bluing Reagent (0.1MLi2 CO3 , 0.5 M Na2CO3)

- Xylene
- DPX mounting media

Microphotograph (4.1): high expression of Survivin in grade III



Microphotograph (4.2): moderate expression of Survivin in grade II



quartett

Cat. No.	2-SU044-10
Lot No.	351430
Quantity	3 ml

Antibody to Survivin

Host:	**Rabbit**
Immunogen:	Synthetic peptide corresponding to residues residues on the N terminus of human Survivin.
Subclass:	IgG
Presentation:	Prediluted antibody in TRIS, pH 7.4, containing < 0.1 % sodium azide.
Titre:	Used in a corresponding system, vial is sufficient for 30 slides using 100 µl per slide. Incubate primary antibody for at least 60 min.
Assay system:	IHC(p,f)
Fixation:	1) NOTOXhisto 2)Formalin
Treatments:	Staining of formalin/paraffin tissues is enhanced by deparaffinazation, rehydration and antigen retrieval. The recommended method is HIER (Heat Induced Epitope retrieval), including boiling of tissue sections in ProTaqs Antigen Enhancer I, Cat# 401602092, for 20 min, followed by cooling at RT for 20 min. The optimal method of antigen retrieval must be determined in user's own system.
Reactivity:	Human, others not tested.
Cell. Local.:	Nuclear/Cytoplasmic
Control:	Colon and Colon cancer
Storage:	Aliquot and store at -20 °C. Do not use after expiration date indicated on the vial.

FOR IN VITRO USE, NOT FOR DIAGNOSTIC USE

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