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Frequency of p53 Expression among Sudanese Females With Breast Cancer

A thesis Submitted in Partial Fulfillment of the Requirement for the M.Sc. Degree in medical Laboratory Science , (Histopathology & cytology)

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الاستهلال

قال تعالى :

﴿ الَّذِينَ قَالَ لَهُمُ النَّاسُ إِنَّ النَّاسَ قَدْ جَمَعُوا لَكُمْ (172) فَاخْشَوْهُمْ فَزَادَهُمْ إِيمَانًا وَقَالُوا حَسْبُنَا اللَّهُ وَنِعْمَ الْوَكِيلُ (173) ﴾

صــدق الله العــظيم

سورة آل عمران من الآية (172__173)

X

Dedication

I dedicate this research to my

father, my mother , my sisters, and

my brothers .

Acknowledgment

Finally, I am grateful to my family for their constant support and encouragement

List of Abbreviation

DCIS	Ductal carcinoma in situ
BRCA	BReastCAncer
DAB	di amino benzidine tetra hydrochloride
DPX	Dibutyphthalet polystyrene xylene
ERK	Extracellular signal-regulated kinase
FNA	fine needle aspiration
HRT	hormone replacement therapy
HER	Human epidermal growth factor receptor
IBC	Inflammatory breast cancer
IDC	Invasive Ductal Carcinoma
LCIS	lobular carcinoma in situ
MRI	Magnetic resonance imaging
Mdm2	Murine Double Minute 2 Gene
PTEN	Phosphate and tensin
PI3K	phosphatidylinositol-3-kinase
SPSS	Statistical Package for the Social Sciences
TP53	tumor protein

المســـتخلص

أجريت هذه الدراسة التحليلية الوصفية المعملية بأثر رجعي تم إجراؤها في مركز الرحمة الطبي خلال الفترة من يناير 2016 حتى مايو 2017. هدفت الدراسة للكشف عن ظهور البروتين مثبط الورم 53(في سرطان الثدي باستخدام كيمياء الأنسجة المناعية.

تم اختيار عينات **الرقاقة الحيوية** من أربعين كتلة من قالب شمعي. باستخدام المشراح الدوار تم معالجتها بسمك 3مايكروميتر وتم تركيبها على شرائح مشحونة إيجابيا (حرارية). تم جمعها من عينات مرضى كانوا مشخصين مسابقا على أنهم مصابون بسرطان الثدي ، ثم صبغة بواسطة طريقة كيمياء الأنسجة المناعية (الطريقة الجديدة غير مباشرة). واستخدم برنامج الحزم الإحصائية للعلوم الاجتماعية النسخة 5.11 لتحليل البيانات

تراوحت أعمار المرضي بين 40-30 سنة 10 (25.0٪) و 50-41 سنة 13 (32.5٪). كان معظم المرضى أكثر من 50 سنة يمثلون 17 (42.5 ٪)

أظهرت الدراسات أن البروتين مثبط الورم 53 كان موجب الظهور في 40/29 عينة وسالب الظهور في 40/11 أظهرت هذه النتيجة عدم وجود علاقه ذات دلالة إحصائية بين ظهور البروتين مثبط الورم 53 وأورام الثدي(القيمة الاحتمالية= 0.110)

فيما يتعلق بالدرجة النسيجية لورم الثدي تم العثور على ظهور البروتين مثبط الورم 53إيجابي في عينات 40/24 من الدرجة الثالثة ، 40/5 عينات من الدرجة الثانية. وأظهرت هذه النتيجة وجود علاقه ذات دلاله إحصائية بين ظهور البروتين مثبط الورم 53 ودرجات أورام الثدي القيمة الاحتمالية(944. 0)

وخلصت الدراسة إلى أن ظهور البروتين مثبط الورم 53 له ارتبط مع أورام الثدي الخبيثة مع وجود علاقه مع الدرجة النسيجية للأورام الخبيثة.

Abstract

This is a laboratory based analytical and descriptive retrospective study which was conducted At ELrhama medical center during the period from April to August 2018.The study was aimed to detect p53 expression in breast cancer using immunohistochemistry.

Tissue microarry samples were selected from Forty (40) formalin fixed paraffin blocks from patient samples previously diagnosed as breast carcinoma, processed, cut at thickness of 3Mm and mounted on positively charged slides(thermo), then stained by immunohistochemically method (new indirect technique). The data obtained was analyzed using SPSS program.

The patients arranged in group according to age such as 30 to 40 years 10 (25.0%) , 41 to 50years 13 (32.5%) and patients more than 50 years were 17(42.5%).

The expression of the P53 in breast cancer was 72.5% (29/40) while 27.5% (11/40) showed negative expression for p53. this result showed no significant association between p53 and tumors of breast (P.value0.110)

Regarding the histopathological grade of breast tumor p53 expression was found positive in 24/40 samples of grade III , 5/40 samples of grade II, grade I was not reported. This result showed no association between p53 expression and grade of breast cancer(P.value0.944)

The study concluded that the expression of p53 was not significantly association with malignant tumors of breast and histological grade of malignant tumors

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Chapter one
Introduction

Introduction

1.1 Introduction:

1.2   Breast cancer is the most commonly diagnosed cancer in women worldwide, and is second after lung cancer as the leading cause of cancer deaths[1] . Although, the incidence of breast cancer in Sub-Saharan African counties is low compared that in developed countries[2] the cancer picture in Sub-Saharan Africa and especially in Sudan is changing. Lately, breast cancer incidence and mortality has been rising.

Breast cancer in African women is characterized by younger age at onset, advanced stage at diagnosis, and consequently poor prognosis. For example in Nigeria, about two-thirds of women with breast cancer present with advanced stage disease[3],[4] The reason for these unfavorable breast cancer presentations is reported to be the delay by patients presenting to the hospitals, due to ignorance, superstition, a skeptical attitude towards western medicine, and dependency on traditional medicine[5],[6] in contrast to the industrialized world where early detection by mammographic screening and greater awareness by women has resulted in early detection. Early detection plus adjuvant chemotherapy has greatly increased survival rates for breast cancer patients.

This descriptive study was undertaken to shed the light on the type, stage and age distribution of breast cancer at diagnosis in women living in

central Sudan encompassing al-Gezira, Blue Nile, White Nile, and Sennar States.

TP53 geneis located on the short arm of chromosome 17 (17p13.1).The gene spans 20 kb, with a non-coding exon 1 and a very long first intron of 10 kb. The coding sequence contains five regions showing a high degree of conservation in vertebrates, predominantly in exons 2, 5, 6, 7 and 8, but the sequences found in invertebrates show only distant resemblance to mammalian TP53[7]TP53 orthologs[8] have been identified in most mammals for which complete genome data are available.study of Arab women found that proline homozygosity at TP53 codon 72 is associated with a decreased risk for breast cancer[9].

p53 mutation is the most common genetic abnormality found so far in human cancer, and in breast cancer p53 mutation/alteration is seen in up to 50% of primary carcinomas.Together with the increasing knowledge of the characteristics and understanding of the role of p53 over the last two decades, attention in recent years has been focused on how this knowledge can be used in clinical settings for patient care and management in terms of analyzing p53 as a potential marker for studying the relationship between p53 expression and tumour development, progression and outcome; and designing alternative treatment strategies specifically aimed at restoring normal p53 function[10]

1.2 Rationale:

Breast cancer is the most common invasive cancer in woman and second main cause of cancer death in woman P53 mutation are the most frequent genetic alteration in breast tumors. The study directed to fine out the frequency of P53 and breast cancer which can help in tumor diagnoses , prognosis and treatment .

1.3 Objectives:

1.3.1 General objective:

TO study frequency of p53 in breast cancer among Sudanese patients

1.3.2 Specific objective:

1. TO correlate p53 expression and tumor grading in breast cancer
2. TO determine p53 expression in infiltrative breast cancer in relation to age
3. TO find out the frequency of p53 expression in breast cancer

2. Literature Review

2.1 structure of the Breast :

The **breast** is one of two prominences located on the upper ventral region of the torso of primates. In females, it serves as the mammary gland, which produces and secretes milk to feed infants.[11] Both females and males develop breasts from the same embryological tissues. At puberty, estrogens, in conjunction with growth hormone, cause breast development in female humans and to a much lesser extent in other primates. Breast development in other primate females generally only occurs with pregnancy.

Subcutaneous fat covers and envelops a network of ducts that converge on the nipple, and these tissues give the breast its size and shape. At the ends of the ducts are lobules, or clusters of alveoli, where milk is produced and stored in response to hormonal signals[12] During pregnancy, the breast responds to a complex interaction of hormones, including estrogens, progesterone, and prolactin, that mediate the completion of its development, namely lobuloalveolar maturation, in preparation of lactation and breastfeeding.

2.2 pathology of the Breast :

2.2.1 Carcinogenesis

Breast cancer is cancer that develops from breast tissue[.(13)] Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, a newly inverted nipple, or a red or scaly patch of skin[.(14)] In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin[.(15)]

Breast cancer, like other cancers, occurs because of an interaction between an environmental (external) factor and a genetically susceptible host. Normal cells divide as many times as needed and stop. They attach to other cells and stay in place in tissues. Cells become cancerous when they lose their ability to stop dividing, to attach to other cells, to stay where they belong, and to die at the proper time.

Normal cells will commit cell suicide (programmed cell death) when they are no longer needed. Until then, they are protected from cell suicide by several protein clusters and pathways. One of the protective pathways is the PI3K/AKT pathway; another is the RAS/MEK/ERKpathway. Sometimes the genes along these protective pathways are mutated in a way that turns them permanently "on", rendering the cell incapable of committing suicide when it is no longer needed. This is one of the steps that causes cancer in combination with other mutations. Normally, the PTEN protein turns off the

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PI3K/AKT pathway when the cell is ready for programmed cell death. In some breast cancers, the gene for the PTEN protein is mutated, so the PI3K/AKT pathway is stuck in the "on" position, and the cancer cell does not commit suicide[16]

Mutations that can lead to breast cancer have been experimentally linked to estrogen exposure.[17]

Abnormal growth factor signaling in the interaction between stromal cells and epithelial cells can facilitate malignant cell growth.[18][19] In breast adipose tissue, overexpression of bleptin leads to increased cell proliferation and cancer[20]

2.2.2 Inflammatory breast cancer

[21] is one of the most aggressive types of breast cancer that can occur in women of any age (and, extremely rarely, in men). It is called inflammatory because it frequently presents with symptoms resembling an inflammation. Despite the name, whether inflammation contributes to the development of "inflammatory breast cancer" remains an area of ongoing research[22]However it can present with very variable signs and symptoms, frequently without detectable tumors and therefore is often not detected by mammography or ultrasound[23]

Typical presentation is rapid swelling, sometimes associated by skin changes (peaud'orange), and nipple retraction. Other symptoms include rapid increase in breast size, redness, persistent itching, skin hot to touch. IBC often initially resembles mastitis.

Only about 50-75% cases have the typical presentation. Symptoms can be completely atypical such as acute central venous thrombosis as the sole presenting symptom.

IBC makes up only a small percentage of breast cancer cases (1-6% in the USA). IBC is often diagnosed in younger women although average age of presentation does not differ much from other kinds of breast cancer (average age 57 years). African-Americans are usually diagnosed at younger ages than Caucasian women, and also have a higher risk of getting IBC.[24] Recent advances in therapy have improved the prognosis considerably and at least one third of women will survive the diagnosis by 10 years or longer[25]

2.3 Tumors of the breast :

2.3.1 Malignant tumors:

breast cancer is often referred to as one disease, there are many different types of breast cancer.All breast cancers start in the breast. So, they are alike in some ways, but differ in others. They can be non-invasive or invasive. Tumor cells can vary in location (ducts or lobules) and how they look under a microscope. These differences often affect prognosis.[26][27]

2.3.1 Non-invasive and invasive breast cancers:

A pathologist looks at the tissue removed during a biopsy under a microscope to determine whether a tumor is non-invasive (ductal carcinoma in situ) or invasive breast cancer.

2.3.2 Ductal carcinoma in situ (DCIS):

Ductal carcinoma in situ (DCIS) is non-invasive breast cancer. Ductal means that the cancer starts inside the milk ducts, carcinoma refers to any cancer that begins in the skin or other tissues (including breast tissue) that cover or line the internal organs, and in situ means "in its original place." DCIS is called "non-invasive" because it hasn't spread beyond the milk duct into any normal surrounding breast tissue. DCIS isn't life-threatening, but having DCIS can increase the risk of developing an invasive breast cancer later on.

3.3.3 Invasive Ductal Carcinoma (IDC):

Invasive ductal carcinoma (IDC), sometimes called infiltrating ductal carcinoma, is the most common type of breast cancer. About 80% of all breast cancers are invasive ductal carcinomas. *Invasive* means that the cancer has "invaded" or spread to the surrounding breast tissues. *Ductal* means that the cancer began in the milk ducts, which are the "pipes" that carry milk from the milk-producing lobules to the nipple. *Carcinoma* refers to any cancer that begins in the skin or other tissues that cover internal organs — such as breast tissue. All together,

"invasive ductal carcinoma" refers to cancer that has broken through the wall of the milk duct and begun to invade the tissues of the breast. Over time, invasive ductal carcinoma can spread to the lymph nodes and possibly to other areas of the body.

2.4 Breast cancers Classification:

Breast cancers are classified by several grading systems. Each of these influences the prognosis and can affect treatment response. Description of a breast cancer optimally includes all of these factors.

2..4.1 Histopathology. Breast cancer is usually classified primarily by its histological appearance. Most breast cancers are derived from the epithelium lining the ducts or lobules, and these cancers are classified as ductal or lobular carcinoma. *Carcinoma in situ* is growth of low-grade cancerous or precancerous cells within a particular tissue compartment such as the mammary duct without invasion of the surrounding tissue. In contrast, *invasive carcinoma* does not confine itself to the initial tissue compartment.[28]

2.4.2 Grade. Grading compares the appearance of the breast cancer cells to the appearance of normal breast tissue. Normal cells in an organ like the breast become differentiated, meaning that they take on specific shapes and forms that reflect their function as part of that organ. Cancerous cells lose that differentiation. In cancer, the cells that would normally line up in an orderly way to make up the milk

ducts become disorganized. Cell division becomes uncontrolled. Cell nuclei become less uniform. Pathologists describe cells as well differentiated (low grade), moderately differentiated (intermediate grade), and poorly differentiated (high grade) as the cells progressively lose the features seen in normal breast cells. Poorly differentiated cancers (the ones whose tissue is least like normal breast tissue) have a worse prognosis.

2.4.3 Stage. Breast cancer staging using the TNM system is based on the size of the tumor (**T**), whether or not the tumor has spread to the lymph nodes (**N**) in the armpits, and whether the tumor has metastasized (**M**) (i.e. spread to a more distant part of the body). Larger size, nodal spread, and metastasis have a larger stage number and a worse prognosis.

The main stages are:

- Stage 0 is a pre-cancerous or marker condition, either ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS).
- Stages 1–3 are within the breast or regional lymph nodes.
- Stage 4 is 'metastatic' cancer that has a less favorable prognosis since it has spread beyond the breast and regional lymph nodes.

Chapter two
Literature Review

2.5 Breast cancer subtypes:

Breast cancer is a heterogeneous disease composed of a growing number of recognized biological subtypes. The prognostic and etiologic importance of this diversity is complicated by many factors, including the observation that differences in clinical outcomes often correlate with race. Age-adjusted mortality in the United States from breast cancer in white women is 28.3 deaths per 100 000 compared with 36.4 deaths per 100 000 in African American women[29]

There are five main intrinsic or molecular subtypes of breast cancer that are based on the genes a cancer expresses:

2.5.1Luminal A breast cancer is hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive), HER2 negative, and has low levels of the protein Ki-67, which helps control how fast cancer cells grow. Luminal A cancers are low-grade, tend to grow slowly and have the best prognosis.

2.5.2Luminal B breast cancer is hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive), and either HER2 positive or HER2 negative with high levels of Ki-67. Luminal B cancers generally grow slightly faster than luminal A cancers and their prognosis is slightly worse.

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2.5.3 Triple-negative/basal-like breast cancer is hormone-receptor negative (estrogen-receptor and progesterone-receptor negative) and HER2 negative. This type of cancer is more common in women with *BRCA1* gene mutations. Researchers aren't sure why, but this type of cancer also is more common among younger and African-American women.

2.5.4 HER2-enriched breast cancer is hormone-receptor negative (estrogen-receptor and progesterone-receptor negative) and HER2 positive. HER2-enriched cancers tend to grow faster than luminal cancers and can have a worse prognosis, but they are often successfully treated with targeted therapies aimed at the HER2 protein, such as Herceptin (chemical name: trastuzumab), Perjeta (chemical name: pertuzumab), Tykerb (chemical name: lapatinib), and Kadcyla (chemical name: T-DM1 or ado-trastuzumabemtansine).

2.5.2Normal-like breast cancer is similar to luminal A disease: hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive), HER2 negative, and has low levels of the protein Ki-67, which helps control how fast cancer cells grow. Still, while normal-like breast cancer has a good prognosis, its prognosis is slightly worse than luminal A cancer's prognosis.[30][31]

2.6 Epidemiology of breast cancer:

Worldwide, breast cancer is the most common invasive cancer in women It affects about 12% of women worldwide[32] (The most common form of cancer is non-invasive non-melanoma skin cancer; non-invasive cancers are generally easily cured, cause very few deaths, and are routinely excluded from cancer statistics.) Breast cancer comprises 22.9% of invasive cancers in women[33] and 16% of all female cancers[34] In 2012, it comprised 25.2% of cancers diagnosed in women, making it the most common female cancer.[34]

In 2008, breast cancer caused 458,503 deaths worldwide (13.7% of cancer deaths in women and 6.0% of all cancer deaths for men and women together).[35] Lung cancer, the second most common cause of cancer-related death in women, caused 12.8% of cancer deaths in women (18.2% of all cancer deaths for men and women together).[36]

The incidence of breast cancer varies greatly around the world: it is lowest in less-developed countries and greatest in the more-developed countries. In the twelve world regions, the annual age-standardized incidence rates per 100,000 women are as follows: in Eastern Asia, 18; South Central Asia, 22; sub-Saharan Africa, 22; South-Eastern Asia, 26; North Africa and Western Asia, 28; South and Central America, 42; Eastern Europe, 49; Southern Europe, 56; Northern Europe, 73; Oceania, 74; Western Europe, 78; and in North America, 90.[37]

The number of cases worldwide has significantly increased since the 1970s, a phenomenon partly attributed to the modern lifestyles.[38][39]Breast cancer is strongly related to age with only 5% of all breast cancers occurring in women under 40 years old(40) There were more than 41,000 newly diagnosed cases of breast cancer registered in England in 2011, around 80% of these cases were in women age 50 or older [41] Based on U.S. statistics in 2015 there were 2.8 million women affected by breast cancer[42] In the United States, the age-adjusted incidence of breast cancer per 100,000 women rose from around 102 cases per year in the 1970s to around 141 in the late 1990s, and has since fallen, holding steady around 125 since 2003. However age-adjusted deaths from breast cancer per 100,000 women only rose slightly from 31.4 in 1975 to 33.2 in 1989 and have since declined steadily to 20.5 in 2014.[43]

2.7 Risk factors of breast cancer :

The exact cause remains unclear, but some risk factors make it more likely. Some of these are preventable.

2.7.1 Age

The risk increases with age. At 20 years, the chance of developing breast cancer in the next decade is 0.6 percent. By the age of 70 years, this figure goes up to 3.84 percent.[44]

2.7.2 Genetics

If a close relative has or has had, breast cancer, the risk is higher. Woman who carry the BRCA1 and BRCA2 genes have a higher risk of developing breast cancer, ovarian cancer or both. These genes can be inherited. TP53 is another gene that is linked to a greater breast cancer risk.[45]

2.7.3 A history of breast cancer or breast lumps

Women who have had breast cancer before are more likely to have it again, compared with those who have no history of the disease. Having some types of benign, or non-cancerous breast lumps increases the chance of developing cancer later on. Examples include atypical ductal hyperplasia or lobular carcinoma in situ.[46]

2.7.4 Dense breast tissue

Breast cancer is more likely to develop in higher density breast tissue.[47]

2.7.5 Estrogen exposure and breast-feeding

Being exposed to estrogen for a longer period appears to increase the risk of breast cancer.This could be due to starting periods earlier or entering menopause later than average. Between these times, estrogen levels are higher. Breast-feeding, especially for over 1 year, appears

to reduce the chance of developing breast cancer, possibly because pregnancy followed by breastfeeding reduces exposure to estrogen.[47]

2.7.6 Obesity

Women who are overweight or have obesity after menopause may have a higher risk of developing breast cancer, possibly due to higher levels of estrogen. High sugar intake may also be a factor.[47]

2.7.7 Alcohol consumption

A higher rate of regular alcohol consumption appears to play a role. Studies have shown that women who consume more than 3 drinks a day have a 1.5 times higher risk.[47]

2.8.8 Radiation exposure :

Undergoing radiation treatment for a cancer that is not breast cancer increases the risk of breast cancer later in life.[47]

2.7.9Hormone treatments :

The use of hormone replacement therapy (HRT) and oral birth control pills have been linked to breast cancer, due to increased levels of estrogen.[47]

2.7.10 Occupational hazards

In 2012, researchers concluded that exposure to certain carcinogens and endocrine disruptors, for example in the workplace, could be linked to breast cancer. In 2007, scientists suggested that working night shifts could increase the risk of breast cancer, but more recent research concludes this is unlikely.[47]

2.8 methods of diagnosis of breast cancer:

Tests and procedures used to diagnose breast cancer include:

2.8.1Breast exam. Your doctor will check both of your breasts and lymph nodes in your armpit, feeling for any lumps or other abnormalities.[48]

2.8.2 Mammogram. A mammogram is an X-ray of the breast. Mammograms are commonly used to screen for breast cancer. If an abnormality is detected on a screening mammogram, your doctor may recommend a diagnostic mammogram to further evaluate that abnormality.[49]

2.8.3 Breast ultrasound. Ultrasound uses sound waves to produce images of structures deep within the body. Ultrasound may be used to determine whether a new breast lump is a solid mass or a fluid-filled cyst.[50]

2.8.4 biopsy. A biopsy is the only definitive way to make a diagnosis of breast cancer. During a biopsy, your doctor uses a specialized needle device guided by X-ray or another imaging test to extract a core of tissue from the suspicious area. Often, a small metal marker is left at the site within your breast so the area can be easily identified on future imaging tests. Biopsy samples are sent to a laboratory for analysis where experts determine whether the cells are cancerous. A biopsy sample is also analyzed to determine the type of cells involved in the breast cancer, the aggressiveness (grade) of the cancer, and whether the cancer cells have hormone receptors or other receptors that may influence your treatment options.[50].

2.8.5 Breast magnetic resonance imaging (MRI). An MRI machine uses a magnet and radio waves to create pictures of the interior of your breast. Before a breast MRI, you receive an injection of dye. Unlike other types of imaging tests, an MRI doesn't use radiation to create the images.[50].

2.8.6 Fine Needle Aspiration Biopsy:

Your health care providers may refer you for a fine needle aspiration biopsy (FNA) if a lump is discovered in your breast. The FNA biopsy is used to assess the lump. In the past, this required a sometimes painful

surgical procedure that involved a longer waiting period for the results. With FNA, a sample of the lump is obtained using a small, thin needle. The test often allows doctors to make a diagnosis within two to three days of the test.[50]

2.8.7 Immunohistochemistry:

The biological characteristics of the tumors are used to estimate prognosis and select appropriate systemic therapy for patients with (breast) cancer. The advent of molecular technology has incorporated new biomarkers along with immunohistochemical and serum biomarkers. Immunohistochemical markers are often used to guide treatment decisions, to classify breast cancer into subtypes that are biologically distinct and behave differently, and both as prognostic and predictive factors.[51]

2.8.8 Molecular study: The advent of high throughput molecular methods has allowed a systematic characterization of the molecular subtypes of breast cancer, the identification of novel therapeutic targets, and prognostic/predictive 'gene signatures 'These methods are having a profound effect on the understanding of breast cancer, but their use in clinical practice is still rather limited.[52]

2.9 Treatment of breast cancer:

2.9.1 Surgery: Surgery is usually the first line of attack against breast cancer. This section explains the different types of breast cancer surgery.(**Lumpectomy**, **Mastectomy**)[53]

2.9.2 Chemotherapy: Chemotherapy treatment uses medicine to weaken and destroy cancer cells in the body, including cells at the original cancer site and any cancer cells that may have spread to another part of the body.[54]

2.9.3 Radiation Therapy: Radiation therapy — also called radiotherapy — is a highly targeted and highly effective way to destroy cancer cells in the breast that may stick around after surger[54]

2.9.4 Hormonal Therapy: Hormonal therapy medicines treat hormone-receptor-positive breast cancers in two ways:

- by lowering the amount of the hormone estrogen in the body
- by blocking the action of estrogen on breast cancer cells [55]

2.9.5 Targeted Therapy: Targeted cancer therapies are treatments that target specific characteristics of cancer cells, such as a protein that allows the cancer cells to grow in a rapid or abnormal way[55]

2.9.6 Immunotherapy: Immunotherapy medicines use the power of your body's immune system to attack cancer cells.[55]

2.10 p53 : also known as TP53 or tumor protein (EC :2.7.1.37) is a gene that codes for a protein that regulates the cell cycle and hence functions as a tumor suppression. It is very important for cells in multicellular organisms to suppress cancer. P53 has been described as "the guardian of the genome", referring to its role in conserving stability by preventing genome mutation. The name is due to its molecular mass: it is in the 53 kilodalton fraction of cell proteins .The human p53 gene is located on the seventeenth chromosome (17p13.1). It plays an important role in cell cycle control and apoptosis. Defective p53 could allow abnormal cells to proliferate, resulting in cancer. As many as 50% of all human tumors contain p53 mutants. In normal cells, the p53 protein level is low. DNA damage and other stress signals may trigger the increase of p53 proteins, which have three major functions: growth arrest, DNA repair and apoptosis (cell death). The growth arrest stops the progression of cell cycle, preventing replication of damaged DNA. During the growth arrest, p53 may activate the transcription of proteins involved in DNA repair. Apoptosis is the "last resort" to avoid proliferation of cells containing abnormal DNA.

The cellular concentration of p53 must be tightly regulated. While it can suppress tumors, high level of p53 may accelerate the aging process by excessive apoptosis. The major regulator of p53 is **Mdm2**, which can trigger the degradation of p53 by the ubiquitin system. If the p53 gene is damaged, tumor suppression is severely reduced. People who inherit

only one functional copy of p53 will most likely develop tumors in early adulthood, a disease known as Li-Fraumeni syndrome. p53 can also be damaged in cells by mutagens (chemicals, radiation or viruses), increasing the likelihood that the cell will begin uncontrolled division. More than 50 percent of human tumors contain a mutation or deletion of the p53 gene.In health p53 is continually produced and degraded in the cell. The degradation of p53 is, as mentioned, associated with MDM-2 binding. In a negative feedback loop MDM-2 is itself induced by p53. However mutant p53s often don't induce MDM-2, and are thus able to accumulate at very high concentrations. Worse, mutant p53 protein itself can inhibit normal p53 (Blagosklonny, 2002).(55)(56)

2.11 p53 and breast cancer:

espite an obvious central role of p53 in the hallmarks of cancer, TP53 status is not yet used for the management of breast cancer. Recent findings may lead to reconsider the role of p53 in breast cancer. TP53 mutations are the most frequent genetic alterations in breast cancer, observed in 30% of breast carcinoma[57]

In breast cancers, only a minority of patients fully benefit from the different chemotherapy regimens currently in use. Identification of markers that could predict the response to a particular regimen would thus be critically important for patient care. In cell lines or animal models, tumor protein p53 (TP53) plays a critical role in modulating the response to genotoxic drugs. TP53 is activated in response to DNA

damage and triggers either apoptosis or cell-cycle arrest, which have opposite effects on cell fate. Yet, studies linking TP53 status and chemotherapy response have so far failed to unambiguously establish this paradigm in patients. Breast cancers with a TP53 mutation were repeatedly shown to have a poor outcome, but whether this reflects poor response to treatment or greater intrinsic aggressiveness of the tumor is unknown.[57]

Breast cancer, one of the most common malignancies affecting women today (Jemal *et al*., 2003), is associated with different types of genetic alterations such as mutations in oncogenes and tumor suppressor genes. The p53, a putative tumor suppressor gene, is expressed in a variety of malignancies including **breast cancer** (Borresen-Dale, 2003), often showing missense point mutations and less frequently deletions or loss of heterozygosity (Olivier *et al*., 2002). Most p53 alterations found in breast carcinomas lead to the synthesis of a stable and non-degradable protein that accumulates in tumor cells and thus can be detected by Immunohistochemistry[58]

2.12 previous study:

Shahesmaeili, and Armita, et al. (2018) in their study was reported that A total of 2,838 cases of cancer were registered in Kerman province, 45.6 % were women (n=1,293). Age range was 60-64 years represented the largest proportion (11.6%) of the total cancer prevalence, followed by those aged 55-59 years (10.86%) and 65-69 years (8.99%).

Lara-Padilla, et al (2015) study was reported that patients with breast cancer the mean age was 56.2±11.8 years from 31 to 81 years old. The sample size of the study was 64.

Golmohammadi, Rahim et al (2012) who reported that the P53 protein stability in 39 (48.8%) cases were observed. A descriptive analytical study was conducted on 80 patients with breast cancer who were admitted to the hospitals in Sabzevar in 2006 and they were followed up to 2010.

Ostrowski, J. L., et al(1991) were studied 90 breast cancer patients, tumor p53 protein expression was determined by immunohistochemistry who reported that p53 protein expression showed a significant relationship to high tumor grade .

Chapter three
Materials and Methods

3. Materials and Methods

3.1 Materials:

Archived tissue block obtained from breastsamples previously diagnosed as breast carcinoma were selected for this study.

3.2Methods:

3.2.1 Study design:

This is descriptive prospective study aimed to detect the expression p53 in breast carcinoma among Sudanese patients using immunohistochemistry .

3.2.2 Study samples:

TMA samples were selected from Forty [40] formalin fixed paraffin blocks processed cut at thickness of 3Mm and mounted on positively charged slides(thermo)previously diagnosed as breast carcinoma were

selected from Elrahama medical center. Patient identification (age, grad and diagnosis) were obtained from patients records.

3.2.3 Study area:

This study was conducted at ELrhama medical center during the period from April to August 2018

3.2.4 sample size:

This study included 40 samples

3.2.5 Immunohistochemistrystaining:

The immunohistochemical procedure was done as follows: single section (3µm) from formalin-fixed, paraffin-embedded tumors were cut and mounted onto salinized 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 p53 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 (mouse monoclonal antibody (p53Dako), against LMP1 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 di amino benzidine tetra hydrochloride (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 distal water for 3 min Slides were counterstained with haematoxylin (MAYER'S) for one min were washedin running tap water for several minutes 7-10 (bluing), then dehydrate and, cleaned, mount in DBX. Each slide was evaluated with investigator then the results were confirmed by consultant histopathologist.

3.2.6   Result interpretation:

All quality control measures were adopted positive and negative control sections were used during immunohistochemicalstaining .the staining of positive p53 was seen in the nuclei of tumor cells

3.2.6 Data analysis:

The obtained result and variable arranged in standard master sheet then analyzed using statistical package for social science (SPSS) program Frequencies,Crosstabs and chi squire tests were calculated

3.2.7 Ethical consideration:

Specimens were taken fromELrhama medical center ethically after taken Laboratory permission .

Chapter four
Results

4. Results

This study included 40 samples of breast invasive ductal carcinoma The age of study population ranged between(30__40)years 10(25.0%) (41__50) years representing 13(32.5%) and the remain 17(42.5%) were more than 50 years as indicated in table (1)

The histopathological diagnosis of study samples includes 7 (17.5%) breast carcinoma in grade II , 33(82.5%) breast carcinoma in grade III as showed in table (2).

P53 positive expression was found in 29(72.5%) samples while 11 (27.5) samples showed negative expression showed table(3)

The grade of tumors' showed positive expression for p53 in 24(72.7%) samples of grade III. 5(71.4%) samples of grade II.Showed in table (4)

Table(4.1):shows

distribution of breast cancer patients in relation to age

Age groups	Frequency	Percent
30-40 yrs	10	25.0
41-50 yrs	13	32.5
More than 50 yrs	17	42.5
Total	40	100.0

Table(4.2)shows distribution of breast cancer patients in relation to tumor grade

Grade	Frequency	Percent
I	0	0
II	7	17.5
III	33	82.5
Total	40	100.0

Table(4.3) shows p53 expression in breast cancer

P53 expression	Frequency	Percent
Positive	29	72.5
Negative	11	27.5
Total	40	100.0

table (4.4) shows p53 expression in breast cancer in relation to patient age

			Age groups			
			30-40 yrs	41-50 yrs	More than 50 yrs	Total
Result s	Positiv e	Count	7	7	15	29
		% within Age groups	70.0%	53.8%	88.2%	72.5%
	Negati ve	Count	3	6	2	11
		% within Age groups	30.0%	46.2%	11.8%	27.5%
Total		Count	10	13	17	40
		% within Age groups	100.0%	100.0%	100.0%	100.0 %

P value = 0.110

table (4.5) shows p53 expression in breast cancer in relation to tumor grade

			Grade		
			II	III	Total
Results	Positive	Count	5	24	29
		% within Grade	71.4%	72.7%	72.5%
	Negative	Count	2	9	11
		% within Grade	28.6%	27.3%	27.5%
Total		Count	7	33	40
		% within Grade	100.0%	100.0%	100.0%

P value= 0.944

Chapter five

Conclusion

Recommendations

5.Discussion

The present study included 40 samples of breast cancer stained by Immunohistochemistry for p53expression. The expression p53 was compare to patient age and grading of breast cancer. The study revealed that grouping of patientsaccording to age were 30-40 years old. 10(25.0%) , 41-50 years13(32.5%) ,and More than 50 years 17(42.5%).This result was compatible with Shahesmaeili, Armita, et al (2018)study was reported that A total of 2,838 cases of cancer were registered in Kerman province, 2014. Of these 45. 6% involved women (n=1,293). Individuals aged 60-64 years represented the largest proportion (11.6%) of the total cancer prevalence, followed by those aged 55-59 years (10.86%) and 65-69 years (8.99%).

The histopathological diagnosis of study population revealed that more frequent grade of breast cancer was grade III 33 (82.5%) ,grade II 7(17.5%) and grade I was not reported.this result was agreed with Eleazar, Lara-Padilla et al (2015) study was reported that included 64 samples from patients with breast cancer(mean age of 56.2±11.8 years from 31 to 81 years old

The expression of the P53 in breast carcinoma was 72.5% (29/40) while 27.5% (11/40)showed negative expression for p53 ,this result was consistent with Golmohammadi, Graham et al (2012) study ,who reported that the P53 protein stability in 39 (48.8%) cases were observed .The study revealed that the grade of tumors' showed positive

expression for p53 in 72.5% (29/40) samples for grade III and 27.5% (11/40) samples for grade II. This difference in disruptionsof p53 expression between grade III and II showed no significant association (p. value=0.944), so the study revealed there was no significant association between p53 expression and grading of malignant tumors. This result disagreed withOstrowski, J. L., et al(1991) study of 90 breast cancer patients, tumour p53 protein expression was determined by immunohistochemistry who reported that p53 protein expression showed a significant relationship to high tumor grade .and agreed with Golmohammadi, Rahim et al(2012). A descriptive analytical study was conducted on 80 patients with breast cancer who were admitted to the hospitals in Sabzevar in 2006 and they were followed up to 2010.

5.Conclusion and Recommendations

5.1 Conclusion:

On the basis of this study we conclude that :

1. P53 expression is associated with cancer of breast and no association with tumor grading or patients age .
2. Most histological grade of breast cancer is grade III
3. Recalls for calcifications are much more likely to undergo biopsy compared with other findings.
4. Increased biopsy rates for calcifications should be considered when recalling a patient from mammography screening in the context of practice specific positive predictive values and cancer detection rates.

5.2 Recommendations:

On the basis of this study we recommended that:

1.Similar studies should be carried in larger sample size

2. our results suggest that mutation status of p53 can be important factor in treatment of breast cancer cells

3. The findings also provide implications for interventions and practice for increasing mammography screening among medically underserved populations.

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Appendices

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 .

جامعة شندي
جمعية أصدقاء مرضى السكري

استمارة عضوية مريضرقم ....

الإسـم: ....

تاريخ الميلاد ....

المهنة : ....

المستوى التعليمي: جامعي ☐   ثانوي ☐   أساس ☐   دون. ☐

العنوان: ....

السـكن: ....

التلفون: ....

الحالة الاجتماعية: متزوج ☐   غير متزوج ☐

عدد الأولاد ☐   عدد البنات ☐

تاريخ المرض: .... العمر عند المرض : ....

هل سبب لك المرض مشكلة من الآتي:

تصلب الشرايين ☐   النظر ☐   القلب ☐   الكُلى ☐

الأعضاء الطرفية ☐   بترفي الأطراف " حدد الطرف المبتور" ☐

....

أي عمليات جراحية؟ ....

هل تتناول أي عقاقير غير أدوية السـكري ؟ ....

ما نوع علاجك للسـكري؟ ....

هل المرض موجود في العائلة؟ ....

أي معلومات أخرى :

....

....

....

التاريخ: .... / /   التوقيع: ....