Assessment of P63 Expression among Sudanese Females with Breast Tumor

A Thesis Submitted in Partial fulfillment of the Requirement for the M.Sc. Degree in Medical Laboratory Science, (Histopathology & Cytology)

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بسم الله الرحمن الرحيم

وصُنِّد مفاتيح الغيب لا يعلمها إلا هو، ويعلم ما في البر والبحر وما تستفعته من ورقة إلا يعلمها ولا حبكة في ظلمات الأرض ولا رطب ولا يابس إلا في كتاب مبين (95)

سورة الانعام
DEDICATION

To my Mother... Fatima

To my Father... Prof. Dr. Eltayieb Elgadi

To my Husband... Asst. Prof. Dr. Abubaker Elrazi

To my Family... Mohammed, MinaAllah, Ljain...

To my Brothers and Sisters
ACKNOWLEDGEMENTS

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Abstract:

This descriptive, prospective study conducted at Alrahma center at Khartoum and collage of medical laboratory during the period (April 2018 - August 2018). The study was aimed to detect P63 expression in breast cancer in Sudanese female.

55 sample of tissue microarray of breast tumors were included in this study (45) malignant, (10) benign. A tissue block obtained from breast samples previously diagnosed as breast carcinoma were selected for this study, tissue microarray from the defined areas. And stain with immune histochemistry (IHC) for detection of P63. Data analysis done by using SPSS (Version.22) computer Program; person's correlation method was used to evaluate the correlation between the protein expressions in the studied population P <0.05 was considered as statistically significant.

The study consists of (81.8%) from malignant and the remaining (18.2%) were benign. From malignant 4/45 samples were positive expression for P63 and 41/45 samples were negative expression while the begin breast tumor 10/10 were positive expression (P. value was 0.00) was considered as statistically significant. The age of patients ranged between 30 to 70 years old with mean age of 49.5 years SD. The study revealed that most patients were more than 50 years representing (41.8%) and the remaining (40%) were less than 50 years. Regarding the histological types includes (72.7%) invasive ductal carcinoma, (5.5%) Invasive lobular carcinoma, (3.6%) paget disease and 10 (18.2%) fibro adenoma, the study revealed that most patients were diagnosed as invasive ductal carcinoma. The grade of tumor include (23.6%) sample grade II and (58.6%) grade III representing, most patients were found in grade III. P63 showed positive result among benign breast tumors in (18.2%) samples. While in malignant breast tumors give negative expression in (81.8%) samples.
المخلص:
أجريت هذه الدراسة التحليلية الاسترجاعية الحالة والحالة الضابطة في مركز الرحمة وكلية علوم المختبرات الطبية في الفترة من أبريل - أغسطس 2018م. هدفت الدراسة للكشف عن ظهور بروتين 63 في أورام الثدي في الإناث السودانيات باستخدام كيمياء الأنسجة المناعية.

جُمعت 55 قالب شمي في مصفوفة الأنسجة الصغيرة لعينات مرضى كانوا مشخصين مسبقاً على أنهم مصابون بأورام الثدى (45) منهم كانوا مشخصين بأورام الثدي الخبيثة، و(10) كانوا مشخصين بأورام الثدي الحميدة. وقُطعت المقاطع قالب شمعي لمصفوفات الأنسجة الصغيرة وصُبِّغت بواسطة طريقة كيمياء الأنسجة المناعية للكشف عن بروتين 63. وخللت البيانات باستخدام الحزم الإحصائية للعلوم الاجتماعية (الإصدارة 22) لتحليل البيانات. ثم أُستخدمت طريقة الارتباط لتقييم العلاقة في عينة الدراسة حيث كانت (القيمة الاحتمالية تساوي 0.05) والتي تعتبر ذات دلالة إحصائية.

احتوت مجموعة الدراسة على (81.8%) أورام خبيثة و(18.2%) أوراماً حميدة (المجموعة الضابطة). أوضحت الدراسة أن العينات 45/4 كانت تعبيراً إيجابياً وأن العينات 41/45 كانت تعبيرا سلبيا في حين أن ورم الثدي كان 10/10 وكان التعبير إيجابي (القيمة الاحتمالية تساوي 0.00) والتي تعتبر ذات دلالة إحصائية. تراوحت أعمر المرضى بين 30 و 70 سنة بمتوسط عمر 49.5 سنة. وكشفت الدراسة أن معظم المرضى كانوا أكثر من 50 سنة يمثلون (41.8 %) وأن النسبة المتبقية (40%) كانت أقل من 50 سنة. وفيما يتعلق بالأنواع النسيجية والتي تشمل سرطان الألواح الغازية (72.7 %)، وسرطان مفصص الغازية (5.5 %)، مرض باجيت (6.3 %)، والورم الحميد الليفي (18.2 %)، وكشفت الدراسة أن معظم المرضى الذين تم تشخيصهم من سرطان الألواح الغازية. وشملت درجة الورم (23.6 %) عينة من الصف الثاني و (58.6 %).
من الدرجة الثالثة، وتم العثور على معظم المرضى في الدرجة الثالثة. أظهر بروتين 63 نتيجة إيجابية بين أورام الثدي الحميدة في عينات (18.2٪) بينما في سرطان الثدي الحبيث نتيجة سلبية في عينات (81.8٪).
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LIST of ABBREVIATIONS:

DAB : Di amino Benzidin tetra hydrochloride
DCIS : Ductal Carcinoma In Situ
FNA : Fine Needle Aspiration
HER2 : Human Epidermal Growthfactor Receptor 2
IDC : Invasive Ductal Carcinoma
IHC : Immuno Histo Chemistry
ILC : Invasive Lobular Carcinoma
M : Micron
ml : Melee Liter
mm : Melee Meter
MRI : Magnetic Resonance Imaging
SPSS : Statistical Package for the Social Sciences
V : Violet
SD : Standard Deviation
CHAPTER ONE

INTRODUCTION
1.1. Introduction:

Breast cancer is the most common cancer affecting women worldwide around 1.1 million cases were recorded 2004 (Shah R, 2014). Approximately 1.38 million new breast cancer cases were diagnosed in 2008 with almost half of all breast cancer cases and nearly 60% of deaths occurring in lower income countries (Ferlay J, 2010). Most women diagnosed with breast cancer are over 50 years but younger woman can also get breast cancer. Breast cancer mortality is high in Sudan and most patients are detected at later staging of the disease due to the lack of awareness and absence of screening programs (Ahmed HG, 2010).

Breast cancer can have a number of symptoms but the first noticeable symptoms are usually a lump or area of thickened breast tissue. Should also see if notice any of the following: change in the size or shape of one or both breast, discharge from either of the nipples which may streaked with blood, a lump or swelling in either of the armpits, dimpling on the skin of breast, A rash on or around nipple, and A change in the appearance of nipple such as becoming sunken into breast (Reproductive Health, 2006).

The exact risk factors of breast cancer aren’t fully understood these include: age, family history of breast cancer, a previous benign breast lump, a being tall overweight or obese, excessive use of alcohol (Siegel R, 2013).

There are several different types of breast cancer, which can develop in different parts of the breast: Noninvasive breast cancer carcinoma in situ, invasive breast cancer, inflammatory breast cancer, and Paget’s disease of the breast(Centers).
Diagnosis of breast cancer after examines the breast general population that patient may refer to a specialist breast cancer clinic for further tests. This might screen (mammography) or biopsy may also need test on lymph nodes in the arm pit maxilla to see whether these are also affected(Society).

Immunohistochemistry (IHC) involves the process of selectively imaging antigens (protein) in cells of a tissue section by exploiting the principle of antibodies bindings specifically to antigens in biological tissue. Immunohistochemical staining is widely used in the diagnosis of abnormal cells such as those found in cancerous tumors. Specific molecular markers are characteristic of particular cellular events such as proliferation or cell death(Ramos, 2005).

P63 a member of the p53 gene family is involved in cellular different and is expressed in the nuclei of myoepithelial cells of normal breast ducts and lobules and a highly sensitive and specific marker of metaplastic carcinoma, myoepithelial cells constitute the basal cell layer of normal mammary epithelia, and their identification is of particular diagnostic value because they are retained in most benign lesions while being lost in malignancy. In breast carcinomas p63 is a very useful marker for myoepithelial differentiation(Hu Min, 2008).
2.1. Rational:

Carcinoma of the breast is common in Sudan. Usually women present late with disease due to absence of screening program, and also due to lack of sufficient health care system.

P63 is a novel diagnostic and prognostic bio marker, detection of this marker and its correlation with breast cancer may play an important role in management of breast cancer and thus may lead to reduce cancer mortality rate.
1.3. **General Objective:**

To assess the expression of p63 among Sudanese females with breast cancer by IHC.

1.4. **Specific objective:**

- To correlate p63 expression biomarker in breast cancer among benign fibro adenoma.
- To correlate p63 expression with different histological types of breast carcinoma.
- To compare between p63 expression and tumor grading.
CHAPTER TWO

LITERATURE REVIEW
2. Review of Literature:

2.1 Breast cancer Definition:

Breast cancer is disease in which cells in the breast grow out of control (Sudhakar, 2009). Breast cancer can begin in different parts of the breast (Perou Charles M, 1999). A breast is made up of three main parts: lobules, ducts, and connective tissue. The lobules are the glands that produce milk. The ducts are tubes that carry milk to the nipple. The connective tissue (which consists of fibrous and fatty tissue) surrounds and holds everything together. Most breast cancer benign in the ducts or lobules. Breast cancer can spread outside the breast through blood vessels and lymph vessels. When breast cancer spreads to other parts of the body, it is said to have metastasized (Du Zhijian, 2010).

Breast cancer is typically detected either during a screening examination, before symptoms have developed, or after a woman notices a lump. Most masses seen on a mammogram and most breast lumps turn out to be benign, that is they are not cancerous, do not grow uncontrollably or spread, and are not life-threatening. When cancer is suspected, microscopic analysis of breast tissue is necessary for a definitive diagnosis and to determine the extent of spread (in situ or invasive) and characterize the type of the disease. The tissue for microscopic analysis can be obtained via a needle or surgical biopsy is based on individual patient clinical factors, availability of particular biopsy devices (Society).

2.2. Epidemiology of breast cancer:

Breast cancer is a worldwide disease resulting in many deaths. Although breast cancer incidence is lower in Sub-Saharan African countries than in developed countries, African women are more likely
than women in the developed world to be diagnosed at later staging of the disease and, thus, are more likely to die from it. This is due to the lack of awareness by women, accessibility to screening methods, and availability of African-based research findings that would influence decision making at the governmental level (Alero F, 2005). The descriptive study was undertaken to shed 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. Cases comprised 1255 women from central Sudan diagnosed with breast cancer and referred to and treated at Institute of Nuclear Medicine, Molecular Biology, and Oncology, from January 1999 to December 2006. 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 (Elgaili EM, 2010). Other study demonstrates a woman’s risk of breast cancer is increased if she has a family history of the disease. In the Nurses’ Health Study follow-up, women with a mother diagnosed before age 50 had an adjusted relative risk of 1.69 and women with a mother diagnosed at 50 or older had a relative risk of 1.37 compared to women without a family history of breast cancer (Shah R, 2014).

A history of a sister with breast cancer also demonstrated an increased relative risk of 1.66 if the diagnosis was made prior to age 50 and a relative risk of 1.52 if diagnosed after age 50 compared to patients without a family history. The highest risk is associated with increasing number of first degree relatives diagnosed with breast cancer at a young age (under age 50). Compared with women who had no affected relative, women who had one, two or three or more affected first degree relatives had risk ratios of 1.80, 2.93 and 3.90, respectively. (Shah R, 2014)
In this literature review, the average age at presentation of breast cancer in Arab women is shown to be a decade earlier than western countries. Across 18 articles, the average and median averages were 48 and 48.5 years old, respectively. Moreover, 65.6% of the patients were younger than 50 years old in 11 studies. The explanation could be related to the fact that Arab nations have younger population compared to western countries; the median age is almost one decade younger according to WHO statistic. However, these findings do support the hypothesis that breast cancer may present at an earlier age in Arab women (Najjar H, 2010).

2.3. Risk factor of breast cancer:

Age as get older, the risk of breast cancer increases—for most women, getting older is their biggest risk factor for breast cancer. At least four out of five of all breast cancer is uncommon in women under the age of 40 years (Siegel R, 2013).

For women being taller slightly increases the risk of developing breast cancer, while being shorter slightly decreases the risk. Height is determined by the combination of our genes, nutrition and hormone levels during our developing years (Society).

Women who started their periods at an early age have a slightly increased risk of breast cancer, because these women are exposed to the naturally occurring female hormone oestrogen for longer than women who started their periods later (Hsieh CC, 1990).

Most cases of breast cancer are not related to an increased risk due to family history for example BRCA1 or BRCA2 (Sharif S, 2007) (Colditz GA, 2012).
The amount of fat and breast tissue woman have in their breasts can differ greatly from woman to woman the amount of breast tissue compared to breast fat is known as (breast density) having high breast density is one of the biggest risk factors for breast cancer(Lahmann PH, 2004).

Women who go through the menopause later than average have a slightly increased risk of breast cancer .the increase in risk of breast cancer seen in women who have a late menopause is probably because these woman are exposed to the naturally occurring female hormone oestrogen for longer than women who go through the menopause earlier(Hsieh CC, 1990) (Kelsey JL, 1993).

Other breast conditions proliferative benign breast disease. A few but not all benign breast conditions have been linked to an increased risk of breast cancer. There are many different types of benign (non- cancerous) Breast conditions and most don’t have any effect on the risk of developing breast cancer(Society). Bigger size at birth it is possible that women who were longer when born have a slightly greater risk of developing breast cancer than women who were smaller or lighter at birth.

Our life styles and life choices that alcohol intake, hormone replacement therapy, contraceptive pill, in vitro fertilization treatment, to exposure ionizing radiation(Danaei G, 2005).

Radiation exposure from various sources including medical treatment and nuclear explosion increases the risk of breast cancer. Radiation to the chest wall for treatment of childhood cancer increases the risk of breast cancer linearly with chest radiation dose(Henderson TO, 2010).
2.4. Signs and symptoms of breast cancer:

It is important that any symptoms or breast changes are properly investigated, this may include change in the size or shape of breasts, change to the nipple such as crusting and redness, nipple discharge (liquid) that occurs without squeezing, new lump or lumpiness, especially if it's only in one breast, change in the skin, such as puckering or dimpling (like orange peel), Nipple becoming inverted (or pulled in), and unusual pain that does not go away (Reproductive Health, 2006).

More specific Symptoms to invasive breast cancer are as irritated or itchy breasts, change in breast color, increase in breast size or shape (over a short period of time), and change in touch (may feel hard, tender, or warm).

2.5. Type of breast cancer:

Breast cancer occurs in two broad categories include non invasive and invasive (Centers). In non invasive (in situ) breast cancer cancerous cells remain in a particular location of the breast, without spreading to surrounding tissue, lobules or ducts. Where in invasive (infiltrating) breast cancer cancerous cells break through normal breast tissue barriers and spread to other parts of the body through the blood stream and lymph nodes. There are two main type of breast carcinoma that include invasive breast cancer include Invasive ductal carcinoma (IDC) and Invasive lobular carcinoma (ILC). Invasive ductal carcinoma (IDC), also known as infiltrating ductal carcinoma, is cancer that began growing in a milk duct and has invaded the fibrous or fatty tissue of the breast outside of the ducts. IDC is the most common form of breast cancer, representing 80 percent of all breast cancer diagnosis. The other type is invasive lobular carcinoma, sometimes called infiltrating lobular carcinoma is the second
most common type of breast cancer after invasive ductal carcinoma (cancer that begins in the milk carrying ducts and spreads beyond it). According to the American cancer society, about 10 percent of all invasive breast cancers are ILC.

The other type of breast carcinoma include Inflammatory breast cancer, Metastatic breast cancer, Papillary carcinoma and Triple – negative breast cancer. The Inflammatory breast cancer is a unique type of breast cancer that often starts and causes the lymph vessels in the skin of the breast to become blocked as result, the breast can become firm, tender, itchy, red and warm due to increased blood flow and a build –up of white blood cells. Metastatic breast cancer occurs when cancer cells spread to another part of the body breast cancer can be metastatic at the time of diagnosis, or following treatment .cancer cells can travel through the blood stream, lymph nodes and spread to other organs parts of the body. The papillary carcinoma is a rare type of breast cancer, accounting for about three percent of all breast cancers papillary carcinoma typically has a better prognosis than other, more common breast cancers. The triple –negative breast cancer is where the cancer cells do not contain receptors for estrogen, progesterone, or her2 about 10-20 percent of all breast cancers are triple-negative .this type of breast cancer is usually invasive and usually begin in the breast ducts.

There are other less common type of breast cancers include Paget disease of the nipple, Phyllodes tumor and Angiosarcoma. Paget disease of the nipple starts in the breast ducts and spread s to the skin of the nipple and then to the areola (the dark circle around the nipple). It is rare, accounting for only about 1-3 percent of all cases of breast cancer. Where Phyllodes tumor are rare breast tumors .they develop in the connective tissue (stroma) of the breast. And angiosarcoma; sarcoma of the breast is
rare making up less than 1 percent of all breast cancer; start in cells that line blood vessels or lymph vessels.

2.6. Staging of breast cancer:

Cancer is staged according to the size of the tumor and whether it has spread to lymph nodes or other parts of the body. There are different ways of staging breast cancer. One way is from stage 0 to 4, but these may be broken down into smaller staging (Wikipedia).

Stage 0 known as ductal carcinoma in situ (dcis) the cells are limited to within a duct and have not invaded surrounding tissues. Stage I at the beginning of this stage, the tumor is up to 2 centimeters (cm) across and it has not affected any lymph nodes. Stage II the tumor is 2 cm across and it has started to spread to nearby nodes. Stage III the tumor is up to 5 cm across and it may have spread to some lymph nodes. Stage IV the cancer has spread to distant organs, especially the bones, liver, brain, or lungs.

2.7. Diagnosis:

A diagnosis often occurs as the result of routine screening, or when a woman approaches her doctor after detecting symptoms (Society).

2.7.1. Clinical breast examination:

Mammography is one of the most important advances in the treatment of breast cancer is early detection of non-palpable masses. Also Magnetic resonance imaging mammography remains the gold standard for breast imaging but magnetic resonance imaging (MRI) has become an important modality in the detection, assessment, staging, and management of breast cancer in selected patients. Screening (MIR) is more sensitive but less specific for the detection of cancer in high risk woman. And Ultra sound; there are several studies supporting the use of adjunctive screening ultra
sound in high risk patient s with dense breast tissue, which imparts a substantial but accepted number of false positives.

2.7.2. History and physical examination:

The clinical history is directed at assessing cancer risk and establishing the presence or absence of symptoms indicative of breast disease it should include age at menarche, menopausal status, previous pregnancies and use of oral contraceptives or post–menopausal hormone replacements, nipple discharge or change, malaise, bony pain weight loss.

Most biopsy result is not cancer. But a biopsy is the only way to find out. During a biopsy, doctor will remove cells from the suspicious area so they can be looked at in the lab to see if cancer cells are present. In a Fine needle aspiration (FNA) biopsy, a very thin hollow needle attached to a syringe is used to withdraw, 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.

Core needle biopsy uses a large needle to sample breast changes felt by the doctor or seen on an ultrasound, mammogram or MRI. In rare cases, surgery is needle to remove all or part of the lump for testing; there are two type of surgical biopsies, incisional biopsy, excisional biopsy. The lymph nodes biopsy under arm to check them for cancer spread. This done by needle biopsy or with a sentinel lymph nodes biopsy and/or an axillary lymph node dissection.

2.7.3. Immunohistochemistry (IHC):

Involve the process of selectively imaging antigens (protein) in cells of a tissue section by exploiting the principle of antibodies bindings specifically to antigens in biological tissue(Ramos, 2014).
Immunohistochemical staining is widely used in the diagnosis of abnormal cells such as those found in cancerous tumors. Specific molecular markers are characteristic of particular cellular events such as proliferation or cell death (GR, 1998). Immunohistochemical is also widely used in basic research to understand the distribution and localization of biomarkers and differentially expressed protein in different parts of biological tissue.

In immunostaining method, an antibody is used to detect a specific protein epitope. These antibodies can be monoclonal or polyclonal. Detection of this first or primary antibody can be accomplished in multiple ways. The primary antibody can be directly labeled using an enzyme or fluorophore, the primary antibody can be labeled using a small molecule which interacts with a high affinity binding partner that can be linked to an enzyme or fluorophore (Ramos, 2005). The biotin-streptavidin is one commonly used high affinity interaction, the primary antibody can be probed for using a broader species-specific secondary antibody that is labeled using an enzyme, or fluorophore, in the case of electron microscopy antibodies are linked to a heavy metal atom, as previously described, enzymes such as horseradish peroxidase or alkaline phosphatase are commonly used to catalyse reactions that give a coloured or chemiluminescent product, fluorescent molecules can be visualized using fluorescence microscopy or confocal microscopy (Ramos, 2005).

2.7.4. P63 Protein:

P63 is a member of the P53 gene family. The gene is located on chromosome 3q27-29 (Di Como Charles J, 2002). It encodes at least six different transcripts with transactivation (TAp63) or negative effects (Np63) on the p53 reporter genes, resulting in tumour suppressor and oncogenic effects respectively (Shih Ie, 2004). The Np63 isoforms lack
the N-terminal transactivation domain and inhibit p53 (Shah R, 2002). During embryogenesis, it is essential for the development of several epithelia. Human germline mutations result in limb mammary syndrome (ectrodactyly, ectodermal dysplasia and facial clefts) with hypoplasia/aplasia of the breasts. P63 mice do not develop a prostate (Signoretti S, 2000). It appears to be a sensitive and specific marker of myoepithelial cells (Hu Min, 2008). It is superior to SMA and calponin, in that myofibroblastic cells are negative.

2.7.4.1. Pathphysiology:

The Pathological and pathological in P63 involve encodes at least 6 different proteins with different biologic functions, appears to regulate growth and development of epithelial organs, as germline mutations cause ectrodactyly (missing or irregular fingers or toes), ectodermal dysplasia and facial clefts syndrome, may be molecular switch for initiation of "epithelial stratification program", regulates human keratinocyte proliferation, olfactory stem cell self renewal and differentiation, gene and protein expression may not correlate due to presence of isoforms and post translation modification and TAp63 is highly expressed in most benign tumors; negative / weak in most carcinomas, but deltaNp63 is negative / weak in most benign tumors and highly expressed in adenoid cystic, mucoepidermoid and myoepithelial carcinomas (Maruya S, 2005).

2.7.4.2. Application:

In carcinomas p63 is a very useful marker for squamous, urothelial and myoepithelial differentiation. The same tumours are usually expressing cytokeratin. However, p63 often shows a more extended reaction.
2.7.4.3. Interpretation:
Typically a nuclear stain and cytoplasmic staining of Z-bands of benign and malignant skeletal muscle tumors.

2.7.4.4. Positive staining – normal:
In breast myoepithelium and Toker cells, where in gynecologic tract basal and parabasal cells of mature cervical, vaginal and vulval squamous epithelium; cervical reserve cells at transformation zone, immature metaplastic and atrophic cervical squamous epithelium and in Lung bronchial reserve cells; metaplastic squamous bronchial epithelium (lower strata). Also in prostate (basal cells), skin (basal cells), thymus (epithelial cells), urothelium.

2.7.4.5. Negative staining
In anus anal gland carcinoma, but anal squamous cell carcinoma is usually p63+. Where in breast normal epithelium, stromal cells, myofibroblasts; may be reduced or occasionally absent in benign apocrine lesions, benign sclerosing lesions or microglandularadenosis; invasive ductal adenocarcinoma (usually). Also in cervix glassy cell carcinoma and melanoma.

2.8. Treatment:
The main treatments for breast cancer are: surgery, radiotherapy, chemotherapy, hormone therapy, and biological therapy (NHS).

2.8.1. Surgery:
There are two main types of breast cancer surgery include breast-conserving surgery and Mastectomy. In the breast-conserving surgery the cancerous lump (tumor) is removed. Breast-conserving surgery ranges from a lumpectomy or wide local excision, where just the tumor and a
little surrounding breast tissue is removed, to a partial mastectomy or quadrantectomy, where up to a quarter of the breast is removed. Where A mastectomy is the removal of all the breast tissue, including the nipple. If there are no obvious signs that the cancer has spread to lymph nodes, may have a mastectomy, where breast is removed, along with a sentinel lymph node biopsy. If the cancer has spread to lymph nodes, will probably need more extensive removal (clearance) of lymph nodes from the axilla under the arm. In many cases, a mastectomy can be followed by reconstructive surgery to try to recreate a bulge to replace the breast that was removed.

To find out if the cancer has spread, a procedure called a sentinel lymph node biopsy may be carried out. The sentinel lymph nodes are the first lymph nodes that the cancer cells reach if they spread. They're part of the lymph nodes under the arm (axillary lymph nodes). The position of the sentinel lymph nodes varies, so they're identified using a combination of a radioisotope and a blue dye.

2.8.2. Radiotherapy:

Radiotherapy uses controlled doses of radiation to kill cancer cells. It's usually given after surgery and chemotherapy to kill any remaining cancer cells. If the need radiotherapy, treatment will begin about a month after surgery or chemotherapy to give the body a chance to recover. Probably have radiotherapy sessions three to five days a week, for three to six weeks. Each session will only last a few minutes.

The type of radiotherapy has will depend on cancer type and the type of surgery has. Some women may not need to have radiotherapy at all. The types available are breast radiotherapy, chest wall radiotherapy, breast boost and Radiotherapy to the lymph nodes. Breast radiotherapy; after breast-conserving surgery, radiation is applied to the whole of the
remaining breast tissue. Chest wall radiotherapy; after a mastectomy, radiotherapy is applied to the chest wall. Breast boost; Some women may be offered a boost of high-dose radiotherapy in the area where the cancer was removed; however, the boost may affect the appearance of the breast, particularly if have large breasts, and can sometimes have other side effects, including hardening of the breast tissue (fibrosis). And radiotherapy to the lymph nodes; where radiotherapy is aimed at the armpit (axilla) and the surrounding area to kill any cancer that may be present in the lymph nodes.

2.8.3. Chemotherapy:

Chemotherapy involves using anti-cancer (cytotoxic) medication to kill the cancer cells. It's usually used after surgery to destroy any cancer cells that haven't been removed. This is called adjuvant chemotherapy. In some cases, may have chemotherapy before surgery, which is often used to shrink a large tumor. This is called neo-adjuvant chemotherapy. Several different medications are used for chemotherapy, and three are often given at once.

The choice of medication and the combination will depend on the type of breast cancer and how much it's spread. If the breast cancer has spread beyond the breast and lymph nodes to other parts of the body, chemotherapy won't cure the cancer, but it may shrink the tumor, relieve symptoms and help lengthen the life.

2.8.4. Hormone treatment:

Some breast cancers are stimulated to grow by the hormones oestrogen or progesterone, which are found naturally in the body. These types of cancer are known as hormone receptor-positive cancers.
Hormone therapy works by lowering the levels of hormones in the body or by stopping their effects.

The type of hormone therapy will have depend on the stage and grading of cancer, which hormone it's sensitive to, age, whether have experienced the menopause, and what other type of treatment are having.

2.8.5. Biological therapy (Targeted Therapy):

Some breast cancers are stimulated to grow by a protein called human epidermal growth factor receptor 2 (HER2). These cancers are called HER2-positive. Biological therapy works by stopping the effects of HER2 and helping the immune system to fight off cancer cells. If they have high levels of the HER2 protein and are able to have biological therapy, probably be prescribed a medicine called trastuzumab. Trastuzumab, also known by the brand name Herceptin, is usually used after chemotherapy.

2.8.6. Complementary therapies

Complementary therapies are holistic therapies that can promote physical and emotional wellbeing.

2.9. Pervious study:

Pervious study found that P63 was exclusively expressed in the myoepithelial cells of normal breast, partially expressed in ductal hyperplasia, rarely expressed in carcinoma in situ and not expressed in invasive carcinomas. The results suggest an association between loss of P63 expression and progression of breast ductal carcinoma. P63 immunostaining might be of assistance for distinguishing invasive ductal carcinoma from ductal carcinoma in situ or rare questionable ductal hyperplastic lesions, leading to correct therapy clinically (Wang Xiaojuan, 2002).
Other previous study found that P63 expression showed positive result among benign breast tumors, while in malignant breast tumors gave positive expression; this result showed significant statistic association. The study concluded that the P63 expression is associated with benign breast tumors (Mohamed, 2016).

In another study done by Koker and his colleagues, they examined 189 invasive breast carcinomas, including 15 metaplastic carcinomas, as well as 10 Phyllodes tumors, and 5 pure sarcomas of the breast for pattern and intensity of p63 staining using an anti-P63 antibody (clone 4A4, Neomarkers). P63 was strongly expressed in 13 of 15 metaplastic carcinomas (86.7%). p63 was positive in all the metaplastic carcinomas with spindle cell and/or squamous differentiation (12 of 12), and in 1 of 3 metaplastic carcinomas with cartilage foci. In stark contrast, only 1 of 174 (0.6%) nonmetaplastic invasive carcinomas was positive for p63 (Koker MM, 2004).

Other study conducted by Di Como and his colleagues, they found that p63 expression was restricted to the nucleus, with a nucleoplasmic pattern. We also observed that the expression was restricted to epithelial cells of stratified epithelia, such as skin, esophagus, exocervix, tonsil, and bladder, and to certain subpopulations of basal cells in glandular structures of prostate and breast, as well as in bronchi. Consistent with the phenotype observed in normal tissues, we found that p63 is expressed predominantly in basal cell and squamous cell carcinomas, as well as transitional cell carcinomas (Di Como Charles J, 2002).
CHAPTER THREE

MATERIALS AND METHODS
3. Materials and Methods

3.1. Materials:

Archived blocks obtained from breast tissue biopsy.

3.2. Methods:

3.2.1. Study design:

This is internal control study aimed to detect expression of P63 in breast tissue using immunohistochemical method

3.2.2. Study samples:

Tissue microarray blocks were obtained from breast tissue forty five samples were previously diagnosed as malignant and ten samples were diagnosed as benign.

3.2.3. Study area:

This study held in Alraham Center at Khartoum during period from April to August 2018.

3.2.4. Sample processing:

Archived tissue block obtained from breast samples previously diagnosed as breast carcinoma were selected for this study, tissue microarray from the defined areas, tissue cores with a diameter of 0.6 mm were punched from each specimen and arrayed on a recipient paraffin block, 5 M section s of these tissue array blocks were cut and placed on charged poly–lysine coded slides. These sections were used for immune histochemical analysis.

Immunohistochemical staining: Deparaffinize and rehydrate tissue section, to reduce nonspecific background staining due to endogenous peroxidase, incubate slide in hydrogen peroxide block for 10—15
minutes, wash 2 times in buffer. If required, incubate tissue in digestive enzyme. Wash 4 times in buffer. (Optional) apply ultra V block and incubate for 5 minutes at room temperature to block non specific background staining. Rinse (optional) Apply primary anti body and incubate according to manufactures protocol. Wash 4 times in buffer, apply Biotiny lated Goat Anti –Polyvalent and incubate for 10 minutes at room temperature, wash 4 times in buffer. Apply streptavidin peroxidase and incubate for 10 minutes at room temperature, rinse 4 times in buffer. Add 1 drop(4) ul DAB plus chromogen to 2 ml of DAB plus substrate, mix by swirling and apply to tissue incubate for 5 –15 minutes depending on the desired stain intensity, counter stain and cover slip using a permanent mounting media.

3.2.5. Result interpretation:

All quality control measures were adopted; positive slides were used during staining.

3.2.6. Data analysis:

Data analysis done by using SPSS (Ver.22) computer Program; frequencies, mean, and chi-square test values were calculated. Person's correlation method was used to evaluate the correlation between the protein expressions in the studied population P <0.05 was considered as statistically significant.

3.3 Ethical Consideration:

The samples were collected after permission according to the laboratory guide lines and regulations.
Chapter Four

RESULT
Result:

The study included 55 samples, 45 (81.8%) were malignant tumors and 10 (18.2%) were benign tumors as indicated in table 4.1. Table 4.2 demonstrate the correlation between study group and the control group in the expression of P63, the P63 is more expressed among fibro adenoma in compare to breast carcinoma as the P. value was 0.00.

The age of study population ranged between 30 and 70 years with mean age of 49.5 years SD. Most patients were more than 50 years 23/ 45 (41.8%) and remain 22/45 (40%) were less than 50 years as indicated in table 4.3. Table 4.4 illustrates the study group numbers and the control group. Most patients were post menopause (23) and remain (22) were pre menopause.

The histological diagnosis of study samples includes 40(72.7%) invasive ductal carcinoma, 3(5.5%) Invasive lobular carcinoma, 2 (3.6%) Paget disease and 10 (18.2%) fibro adenoma as showed in table 4.5. Table 4.6 demonstrate the correlation between histological type of breast cancer and the P63 gene expression, the P63 is more expressed among invasive ductal carcinoma in compare to invasive lobular carcinoma and paget disease. The P. value was 0.7 that indicate it insignificant.

Regarding the tumor grading, most of them were grade III representing 32 (58.6%), grade II 13 (23.6%) as indicated in table 4.7. Table 4.8 demonstrate the correlation between cancer grading and the P63 gene expression, the P63 is more expressed among grade III in compare to grade II and grade I. The P. value was 0.05 that indicate it significant.
Table (4.1): Shows Distribution of P63 Expression in Breast tumor:

<table>
<thead>
<tr>
<th>Breast tumor</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma</td>
<td>45</td>
<td>81.8</td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>10</td>
<td>18.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>55</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

Table (4.2): Expression of P63 among Breast Carcinoma and Fibroadenoma:

<table>
<thead>
<tr>
<th>Breast tumor</th>
<th>P63 Expressions</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Negative</strong></td>
<td><strong>Positive</strong></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>41</td>
<td>4</td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>41</strong></td>
<td><strong>14</strong></td>
</tr>
</tbody>
</table>
**Table (4.3):** Show Distribution of Age in Study Population:

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 50</td>
<td>22</td>
<td>40.0</td>
</tr>
<tr>
<td>More than 50</td>
<td>23</td>
<td>41.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>45</strong></td>
<td><strong>81.8</strong></td>
</tr>
</tbody>
</table>

**Table (4.3):** Show Distribution of Breast Carcinoma in Relation to Menstrual Cycle:

<table>
<thead>
<tr>
<th>Menstrual cycle</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre menopause</td>
<td>22</td>
</tr>
<tr>
<td>Post menopause</td>
<td>23</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>45</strong></td>
</tr>
</tbody>
</table>
Table (4.5): Shows Distribution of P63 Expressions in breast tumor:

<table>
<thead>
<tr>
<th>P63 Expressions</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>41</td>
<td>74.5</td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>25.5</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table (4.6): Shows Expression of p63 among Breast Cancer Type:

<table>
<thead>
<tr>
<th>Breast Cancer Type</th>
<th>P63 Expressions</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Ductal Carcinoma</td>
<td>36</td>
<td>4</td>
</tr>
<tr>
<td>Lobular Carcinoma</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Paget</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>4</td>
</tr>
</tbody>
</table>
Table (4.7): Shows Distribution of breast Carcinoma according to Grading:

<table>
<thead>
<tr>
<th>Cancer Grading</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade II</td>
<td>13</td>
<td>23.6</td>
</tr>
<tr>
<td>Grade III</td>
<td>32</td>
<td>58.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>45</strong></td>
<td><strong>81.8</strong></td>
</tr>
</tbody>
</table>

Table (4.8): Shows Expression of P63 in Breast Carcinoma According to Grading:

<table>
<thead>
<tr>
<th>Cancer Grading</th>
<th>P 63 Expressions</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Grade I</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Grade II</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Grade III</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>41</strong></td>
<td><strong>4</strong></td>
</tr>
</tbody>
</table>
Chapter Five

DISCUSSION
Discussion:

The present study included (55) samples were investigated by Immunohistochemical (IHC) stain for p63 antigen. Concerning the age group of study population, the study revealed that most patients were more than 50 years 23/45 (41.8%); indicating that patients more than 50 years are more affected with breast cancer. This result was incompatible with the studies (Elgaili EM, 2010). The study reported that 74% of the women were <50 years old or premenopausal.

The histological diagnosis of the study population revealed that more frequent type of breast cancer was invasive ductal carcinoma 40(72.7%). This compatible with (Elgaili EM, 2010), invasive ductal carcinoma was the most common pathology (82%). The P63 is more expressed among invasive ductal carcinoma in compare to invasive lobular carcinoma and paget disease, but these different of no statistically significant P. value was 0.7) these result was agree reported was (Mohamed, 2016) the study revealed the most patient were diagnosed as invasive ductal carcinoma.

The tumor grading of study population most of them were gradeIII 32 (58.6%). These agrees with (Elgaili EM, 2010) the women presenting with stage III or higher tumors that had already metastasized.

P63 immuno stain expression was detected in benign breast tumors 10/10 (100%) and few expression in malignancy 4/45 (8.69%); this result is agree with(Wang Xiaojuan, 2002), this study found that P63 was exclusively expressed in the myoepithelial cells of normal breast, and disagree with (Koker MM, 2004) the nonmetaplastic invasive carcinomas was positive for P63 using anti P63. Immuno stain strong association with negative expression with malignancy compare with benign (p. value
0.000) ; this result is agree with (Mohamed, 2016) the significant statistic association was (P.value 0.000).

P63 negative in invasive ductal carcinoma, weakly positive in grading II 3/45(6.67%) due to residual begin cells in invasive area; this result agree with (Mohamed, 2016) where reported 7 (23.3%) samples grade I , 11 (36.7%) samples grade II, 10 (33.3%) samples grade III and 2 (6.7%) samples not graded, most patients were found in grade II and III.
CHAPTER SIX

CONCLUSION AND RECOMMENDATION
6.1. Conclusion:

On the basis of this study we concluded:

- P63 was expressed in all begin breast tumor and few in breast carcinoma showed expression.
- P63 was a very useful marker for myoepithelial differentiation.
- Most female's patient with breast carcinoma were more than 50 years old.
- Most of histological type of breast cancer was invasive ductal carcinoma.
- The majority of breast carcinoma grading was type III.

6.2. Recommendation:

On the basis of this study we recommended that:

- Similar studies should be carried in large sample size.
- Use in panels diagnosis.
References:


Yao Jun Hu Min, Carroll Danielle K, Weremowicz Stanisława, Chen Haiyan, Carrasco Daniel, Richardson Andrea, Violette Shelia, Nikolskaya


# APPENDIX:

## Appendix (1): Materials:

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
Appendix (2): Procedure Sheet:

**Thermo Scientific**

**c-erbB-2 / HER-2 / neu Ab-17**
Catalog # MS-730-P0, -P1, or -P (0.1ml, 0.5ml, or 1.0ml)
Catalog # MS-730-R7 (7.0ml)

Please note this data sheet has been changed effective July 8, 2015

**STORAGE and STABILITY:**
This product contains sodium azide and is stable for 24 months when stored at 2-8°C. Do not use after expiration date. If the label of the product is not stored as recommended, performance claims are not guaranteed.

**REFERENCES:**

---

**UltraVision Detection System**
Anti-Polyvalent, HRP/DAB (Ready-To-Use)

**INTENDED USE**
For In Vitro Diagnostic Use

**AVAILABILITY:**
Catalog #: 20040100

**SPECIFICITY:**
Enzyme: Peroxidase

**CHROMOGEN/SUBSTRATE:**
Diaminobenzidine (DAB)

**REAGENTS**

<table>
<thead>
<tr>
<th>Qty</th>
<th>Component</th>
<th>TP Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hydrogen Peroxide Block</td>
<td>TA0055B</td>
</tr>
<tr>
<td>1</td>
<td>Ultra T Block</td>
<td>TA005UB</td>
</tr>
<tr>
<td>1</td>
<td>Biotinylated Goat Anti-Polyvalent</td>
<td>TR015BN</td>
</tr>
<tr>
<td>1</td>
<td>Streptavidin Peroxidase</td>
<td>T5055 HR</td>
</tr>
<tr>
<td>1</td>
<td>DAB Plus Substrate</td>
<td>TA0095X</td>
</tr>
<tr>
<td>1</td>
<td>DAB Plus Chromogen</td>
<td>TA0013X</td>
</tr>
</tbody>
</table>

(The three-digit number in the middle of each Catalog is designated the reagent volume in mL or number of tablets)

**DESCRIPTION**
The reagents in this kit constitute a labeled streptavidin-biotin immunoperoxidase antigen detection system. This technique involves the sequential incubation of the specimen with an unlabeled primary antibody specific to the target antigen, a biotinylated secondary antibody that reacts with the primary antibody, enzyme-labeled streptavidin, and substrate-chromogen.

**PRINCIPLE OF THE PROCEDURE**
This UltraVision detection system detects a specific antibody bound to an antigen in tissue sections. The specific antibody is located by a biotinylated secondary antibody. This step is followed by the addition of a streptavidin-enzyme conjugate that binds to the biotin present on the secondary antibody. The specific antibody-secondary antibody-streptavidin-enzyme complex is then visualized with an appropriate substrate-chromogen.

**WARNINGS & PRECAUTIONS**
Refer to MSDS.

**STORAGE & SHELF LIFE**
Store at 2-8°C. Each component is stable for 18 months.

**MICROBIOLOGICAL STATE**
Product(s) not sterile.

**MATERIALS REQUIRED BUT NOT PROVIDED**
Datasheet for Breast Cancer

1. Name: .................................................................

2. Age of Patient: .............

3. Type of Breast Cancer: ............................................

4. Grading of Breast Cancer
   A. Grade I       B. Grade II       C. Grade III

5. P63 Expression Result
   A. Positive       B. Negative
Appendix (4): Photography:

Recipient Block
Recipient Block Samples
Positive Result Control
Negative Result Test Sample