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DECLARATION

This thesis is a presentation of my original research work, wherever contributions of others are involved, and every effort is made to indicate this clearly, with due to reference to the literature, and acknowledgment of collaborative research and discussion. The work was done under the guidance of Dr. AbdALrheem Ali Babepker and Dr. Elhadi Abd allah. at University of Gezira, Faculty of Medical Laboratory sciences.

Eltayeb Elzain Mohamad Elzain

Place: Wad Madani
Dedication

This work is dedicated to

my father, mother, family

Thanks for your support throughout my life and for giving me strength to reach for the stars and chase my dreams.

my mother

my father

my brothers

my wife
Acknowledgements

First and foremost Praise be to Allah give me strength and patience to finish my research and praise be to Allah for the blessing of family and friends thanks all I appreciated.

I offer my sincerest gratitude to my supervisor, Dr. AbdAlrheem Ali Babker, who has supported me, with his patience, encouragement and effort one simply could not wish for a better or friendlier supervisor.

To my second supervisor Dr. Elhadi Abdallah for his support and help to overcome my problem.

I would like to thank all the people who contributed in some way to the work described this.
Eltayeb Elzain Mohamad Elzain

Abstract

Breast cancer is the most commonly diagnosed cancer in women worldwide, and the second cause of cancer death. Progesterone receptor is one of the successful tumor markers in breast cancer that effectively predict the hormonal responsiveness. The study was conducted at Alrahmah, Khartoum state. This retrospective study was carried out to evaluate the expression of progesterone receptor by using tissue microarray technique in the diagnosis of breast cancer. Thirty five were examined using immune-histo-chemistry technique. The data analyzed by SPSS version twenty. In the results patient age ranged from 30 to 70 years, grade two breast cancer patients was equal 34.3% (n=12/35) while grade three was equal 65.7% (n=23/35). 77.1% (n=27/35) of examined tissues gave positive result for progesterone antigen while negative results 22.9% (n=8/35). There was significance relation between TMA result and progesterone receptor scoring and its predictive value (0.000) while was no relation between scoring and grade, no relation between TMA and grade. The results showed progesterone receptor has good prognostic value in breast cancer management.
تقييم مستشعر البروجسترون كبروتين مبديء في سرطان الثدي باستخدام تقنية الرقاقي المصفوفة مركز الرحمة، ولاية الخرطوم، السودان (2018)

الطبيب الزين محمد الزين

ملخص الدراسة

سرطان الثدي هو السرطان الأكثر شيوعا بين النساء في جميع أنحاء العالم، ويحتل المرتبة الثانية بعد سرطان الرئة حيث أنه السبب الرئيسي للوفاة بين النساء. مستقبلات البروجسترون هو أحد علامات الورم الناجمة في سرطان الثدي والتي تتنبأ بفعالية الاستجابة الهرمونية. أجريت الدراسة بمركز الرحمة ولاية الخرطوم السودان وقد أجريت هذه الدراسة بتأثر رجعي تقييم ظهور مستقبلات البروجسترون باستخدام تقنية الرقاقي المصفوفة في تشخيص سرطان الثدي. وقد تم استخدام خمسة وثلاثون عينة تم تشخيصها سابقا بأنها عينات موجبة لسرطان الثدي (سرطان الأقنية الغاوية) خمسة وثلاثون مريضا كانت اعمارهم تتراوح بين ثلاثون وسبعون عاما، ومستوى درجة السرطان هي الثانية والثالثة. تم الكشف عن النتائج بواسطة المجهر الضوئي الذي تم جمعها وتحليلها باستخدام الحزمة الإحصائية لعلوم الاجتماعية. أظهرت النتائج أن 34.3% (23/5) كانت إيجابية، و9.2% (2/5) كانت سلبية. وكانت هناك علاقة ذات دلالة إحصائية بين نتيجة الرقاقي المصفوفة ونتائج مستقبلات البروجسترون. وقد أظهرت النتائج أن مستقبلات البروجسترون ذات قيمة جيدة في إدارة معالجة السرطان.

(43/4) %. (43/4) % %. 11.2 % (8/43) %. 5 (2/43) %. 23/5. 2/5. 34.3 % (23/5). 9.2 % (2/5). (0.000) .
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Abbreviations

ADH: atypical ductal hyperplasia
ALH: atypical lobular hyperplasia
DCIS: ductal carcinoma in situ
ER: estrogen receptor
FNA: fine needle aspiration
HE: haematoxylineosin
IDC: Invasive Ductal Carcinoma
IHC: immunohistochemistry
LCIS: lobular carcinoma in situ
NST: no specific type
PEFF: paraffinembedded, Formalinfixed
PR: progesterone receptor
SPSS: statistical package for social science
TMA: Tissue Micro Array
Chapter one

1-1 Introduction

Breast cancer starts when cells in the breast begin to grow out of control. These cells usually form a tumor that can often be seen on an x-ray or felt as a lump. The tumor is malignant (cancer) if cells can grow into (invade) surrounding tissues or spread (metastasized) to distant areas of the body. Breast cancer occurs almost entirely in women, but men can get breast cancer too.

Cancer is becoming a global health problem and the number of cancer cases in sub-Saharan Africa is rising. Being an African country, Sudan has its share of cancer burden. However, population-based data in cancer incidence, prevalence, and mortality in Sudan were available and most published cancer cases were based on estimates from hospital-based information sources. The National Health Laboratories is the main diagnostic laboratory in Khartoum and its cancer data captures histopathologically confirmed cases (Awadelkarim et al., 2012). Recently in 2009, Sudan established the first National Cancer Registry (NCR). NCR is to develop a system that will facilitate creation and maintenance of local and regional data and assemble these data into a single centrally accessible system.

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 (Ad ebamowo et al., 2000) (Anyanmu SNC et al., 2000). 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 (Smith RA, et al., 2006). In contrast to the industrialized world where early detection by mammographic screening and greater awareness by women has resulted in early detection (Jacopsen BM et al., 2012). Early detection plus adjuvant chemotherapy has greatly increased survival rates for breast cancer patients in the developed world. Assessment of hormonal receptors expression status is required to determine patient eligibility for hormonal therapy. However, in the developing countries clinicians administer hormonal therapy without any knowledge of their patient’s receptors status. Estrogen receptors (ER) and progesterone receptors (PR) expression status is not routinely determined in the developing countries because of limited resources and the relatively high cost of testing.
However, it should be noted that in a few studies conducted in Africa that examined ER and PR expression status, most African women were observed to have estrogen and progesterone negative tumors (McCormaco et al., 2007).

1.2. Immunohistochemistry:

Involves the process of selectively imaging antigens (proteins) in cells of tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues (Ramos-vara, 2014). It takes its name "immuno" reference to antibodies used in the procedure, and "histo" meaning tissue (compare to immunocytochemistry). Albert Coons conceptualized and first implemented the procedure in 1941 (Coons et al., 1941). Immunohistochemical staining is widely used in the diagnosis of abnormal cells such as those found in cancerous. Specific molecular markers are characteristic of particular cellular events such as proliferation or cell death (apoptosis) (Witeside, et al 1998). Immunohistochemistry is also widely used in basic research to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue. Visualizing an antibody-antigen interaction can be accomplished in a number of ways. In the most common instance, an antibody is conjugated to an enzyme, such as peroxidase, that catalyze a color-producing reaction (Ramos-vara JA, 2005). Alternatively, the antibody can be tagged to a fluorophore, such as fluorescin or rhodamine.

3. Justification

Increase incidence of breast cancer and increase mortality rate due to miss-diagnose and poor plan for treatment of patients with breast cancer

1.4 Objectives

1.4.1 General objectives

To evaluate the expression of progesterone receptor in breast cancer as prognostic marker

1.4.2 Specific objectives

- To compare between conventional technique and microarray in the diagnosis of breast cancer

- To correlate the existence of PR with the grade of breast carcinoma.
Chapter Two

Literature Review

2.1. The normal breast:
The breasts consist of a group of modified sweat glands, which develop from 15–25 down
growths of the epidermis. At first solid cords, they develop a lumen and become the major
(segmental) ducts, each of which opens separately at the nipple. Each segmental duct gives rise
to the branching duct system of a segment of breast tissue. Before puberty the structure of male
and female breast tissue is identical. In the female, under the hormonal stimulation of puberty,
the duct system proliferates. Lobules, composed of acini and intralobular stroma, bud from
subsegmental ducts to form physiologically functional terminal duct lobular units. Apart from
duct ectasia and duct papilloma, most lesions in the breast are believed to arise from the
terminal duct lobular unit. The connective tissue between the terminal duct lobular units is less
cellular and more densely collagenous, and during puberty becomes infiltrated with fatty tissue;
this accounts for most of the enlargement of the female breast at this time. Apart from a stratified
squamous epithelial lining close to the nipple, the ducts and ductules are lined by a two-layered
epithelium, an inner layer of cuboidal or columnar epithelial cells, and an outer discontinuous
layer of smaller, contractile, myoepithelial cells. These two layers are invested in a continuous
basement membrane and the duct system is ensheathed in a layer of loose connective tissue that
is rich in lymphatics. There is little or no elastic tissue in the lobules, but an elastic layer
surrounds the larger ducts. The ductal epithelium of the mature female breast has some minor
secretory activity, but the secretion is normally reabsorbed. During pregnancy, proliferation
increases and secretory acini develop from the terminal ductular alveoli. After the menopause,
the breast epithelium atrophies and the lobular connective tissue changes to less cellular hyaline
collagen; the terminal ductules may virtually disappear, but sometimes become dilated, forming
microcysts lined by flattened, attenuated epithelium(C.Simon Herington,2014)
2.2. Breast histology:
Normal breast tissue varies considerably between individuals in its ratio of epithelial and stromal elements, and typically becomes less dense with age. Similarly it varies from region to region throughout an individual breast and this imparts patchy variations in radiodensity that can be interpreted as abnormalities. Normal breast consists of a system of branching modified sweat ducts that originate as down-growths of the epidermis of the nipple region. Each duct begins as a lactiferous duct and the branches of this duct give rise to a mammary lobe. The size of the lobes varies greatly and the margins of the breast lobes are not circumscribed or contained within a capsule. The distal branches of the ducts are the terminal ducts that end in a grape-like cluster of blind sac-like dilatations termed acini. This structure constitutes the terminal duct-lobular unit. A dual layer of cells – an inner luminal cell layer and an outer myoepithelial cell layer, which are separated from the periductal stroma by a basement membrane, lines the ducts and lobular acini. The stroma of the breast consists of the specialized periductal or lobular stroma, vascularized loosely woven delicate collagen fibres and stromal fibroblasts. The non-specialized interstitial stroma is composed of adipose tissue and dense collagenous fibrous tissue that is hypocellular and contains coarse closely packed collagen bundles. Other mesenchymal elements including lymphatics, blood vessels, nerves, fibroblasts and myofibroblasts are found in the stroma. Occasionally, these mesenchymal components give rise to neoplasms of the breast similar to those encountered in soft tissues elsewhere in the body (Malcolm MM, et al 2008).

2.3. Breast cytology:
Normal breast cells and minute fragments of breast tissue may be seen in some fine needle aspirates. These elements are easily identified. Ductal epithelial cells are columnar in shape and show benign, oval nuclei and granular, defined cytoplasm. They are commonly seen in small cohesive sheets with a honeycomb pattern or in small clusters. Myoepithelial cells appear singly, have oval hyperchromatic nuclei and are either bipolar or show no visible cytoplasm. Acinar cells are polygonal in shape and display oval nuclei, inconspicuous nucleoli and clear or granular cytoplasm. The acinar cells appear singly, in small clusters or groups. Lobules of a cinar cells partially surrounded by a thin basement membrane are observed occasionally (Malcolm MM, et al 2008).
2.4. Carcinoma

With the exception of skin cancers, breast cancer is the most common of human female cancers throughout the world. During the mid-1980s, mortality from cancer of the breast overtook that of every other female cancer to become the most common cause of cancer death. Mortality from lung cancer is now overtaking that from breast cancer in some countries but, importantly, nearly twice as many individuals develop breast cancer as die of it. Of note, the incidence and mortality of breast cancer are high and remarkably constant in most developed countries; the incidence is increasing, especially in younger females, and this is not entirely due to an increase in the ‘at-risk’ population. It is more than 200 times more common in women than in men. Carcinoma of the breast may occur at any age, but is rare before age 25 years and most common in the developed world in those aged between 40 and 70 years. About 50% of invasive carcinomas occur in the upper outer quadrant of the breast (where there is the greatest proportion of breast parenchymal tissue), the remainder being distributed equally throughout the rest of the breast. The main presenting symptom is a palpable mass and for this reason all lumps in the breast, whatever the age of the patient, must be regarded clinically as possibly malignant until proved otherwise. A cancer arising in the axillary tail may be mistaken clinically for an enlarged lymph node. In practice, all breast masses should be investigated and a definitive diagnosis made by fine-needle aspiration cytology or, now more commonly, needle-core biopsy. In this way women with breast cancer can have preoperative counselling, discussions about appropriate treatment options can take place, and surgeons can plan operations and operating time. Perioperative frozen section should be avoided (except in exceptional circumstances), particularly in small impalpable (screen-detected) lesions that may then be unavailable for full paraffin histology and assessment of prognostic factors. In the UK, women aged 50–70 years are routinely invited for 3-yearly mammography as part of the UK National Health Service Breast Screening Programme (NHS BSP); in this service, lesions are most commonly detected as microcalcifications, masses, distortions, or parenchymal deformities on mammography and are often impalpable(Malcolm MM, et al 2008).
2.5. Ductal Carcinoma in situ:

Ductal carcinoma in situ (DCIS) of the breast is a complex pathologic entity in which malignant cells arise and proliferate within the breast ducts without invasion of the basement membrane. The increased use of screening mammography has led to a significant increase in the diagnosis of earlier stage breast cancers, including ductal carcinoma in situ. According to the Surveillance Epidemiologic and End Results program (SEER) from 1975–2008, in situ breast cancers represented approximately 15% of all new breast cancer diagnoses in the United States (N. HowLader, et al. 2011). DCIS consists of approximately 84% of all in situ disease, with lobular carcinoma in situ forming the bulk of the remainder. DCIS will account for approximately 27% of all newly diagnosed breast cancers or 77,795 new cases estimated in 2011 (C. Desantis, et al. 2011).

The age adjusted DCIS incidence had increased an average of 3.9% annually from 1973 to 1983 and approximately 15% annually from 1983 to 2008 (V. L. Ernster, et al. 1996). Since 2003, the incidence of DCIS has declined in women aged 50 years and older, while the incidence continues to increase in women younger than age 50 (K. Kerlikowske, 2010). Overall, the rate of increase in incidence has been higher for DCIS than for any other type of breast cancer. As the incidence of DCIS increases, the treatment options continue to evolve.

In the past, DCIS was an uncommon disease that was routinely treated with mastectomy. However, with the increasing acceptance of breast conservation therapy for invasive breast cancers, initial attempts at breast-conserving surgery have also indicated a potentially acceptable treatment modality for DCIS (L. G. Arneston, et al. 1989). Currently, several studies have shown breast conservation therapy to be effective for the management of DCIS. In 1983, 71% of cases were treated by mastectomy in contrast to only 33% in 2007 (K. P. McGuire, et al. 2009). Today, mastectomy, lumpectomy followed by radiation therapy, and lumpectomy alone have all been advocated as management strategies for DCIS. Treatment selection for the individual patient with DCIS requires a clinical, mammographic, and pathological evaluation. A large proportion of women diagnosed with DCIS today are candidates for breast conservation, with relatively few absolute or relative contraindications.
due to toxicity concerns. With improvements in modern breast reconstructive techniques, mastectomy may be a more appealing alternative for individuals with anticipated poor cosmetic outcome as a result of breast-conserving surgery and radiation therapy. One factor affecting cosmesis may include a large surgical defect required to attain negative margins. Prior to the determination of a patient’s suitability for breast-conserving therapy, a thorough evaluation to determine the extent and characteristics of the patient’s disease is necessary. Patient preference will also play a role in the final treatment decision. We present this paper as an update to our previous review in 2009 (J.L. Peterson, et al. 2009).

2.6. Invasive Ductal Carcinoma

Invasive ductal carcinoma (IDC) is the most common malignant tumor of the breast, accounting for 40% to 75% of all breast cancers (Tavassoli FA, et al. 2003). IDC is almost invariably solid and can be detected by palpation or mammography. Many IDCs have a characteristically gritty consistency, appreciable during FNA. Although most are pure ductal carcinomas, limited foci of tubular, papillary, mucinous, or medullary differentiation can be present. IDC ranges from well to poorly differentiated, and is usually graded by a combination of nuclear and architectural features; thus FNA is of limited use on grading breast carcinomas. (Ductman BS, et al. 1993). A subtype of IDC known as scirrhous carcinoma is characterized by abundant, dense fibrosis. Because of fibrosis, scirrhous carcinomas may result in nondiagnostic FNA specimens despite multiple passes (Park IA, et al. 1997). In this circumstance a tissue biopsy is needed for diagnosis.

Phyllodes tumor (PT). A, Epithelial clusters in a phyllodes tumor resemble those of fibroadenoma (FA) but may be more crowded (Diff-Quik stain). B, Stromal clusters may be cellular (Papanicolaou stain). A B Phyllodes tumor (PT). Atypical stromal and epithelial cells are noted. The background of this tumor is necrotic. Although cytologically more alarming than the previous case, this tumor was a low-grade PT and the one was a high-grade tumor of ductal carcinoma is hypercellularity. Even at lower power, the nuclear atypia is also prominent (Diff-Quik stain).

The differential diagnosis includes DCIS, the presumed precursor lesion to IDC. Not surprisingly, IDC and DCIS appear identical on cytologic examination (Wang HH, et al. 1989). The significance of malignant cells embedded in fat or stroma is controversial (Mckee GT, et al.
Some authors believe that invasion can be suggested if strict criteria (e.g., identification of “true infiltration” of fibrofatty tissue) are applied to smears (Klijianenko J., et al. 2004). Others have found this finding unreliable because it is seen in the majority of cases of DCIS (Maygarden SJ, 1997). In fact, benign ductal cells are commonly seen in association with fatty tissue. This finding should not be taken as a sign of malignancy; rather, it is a mechanical artifact of aspiration and smear preparation. The cohesiveness of some invasive tumor cells, and the lack of tubular structures can suggest in situ carcinoma (Bonzanini M., et al. 2011). Important clues to the presence of invasion are cell Ductal carcinoma. The specimen is cellular and the cells are present both singly and in loosely cohesive clusters (Papanicolaou stain).

2.7. Routes of Spread:

Breast carcinomas may spread by direct infiltration of skin, skeletal muscle, and chest wall via the lymphatic system to axillary and internal mammary lymph nodes via the vascular system (haematogenous spread) particularly to lungs, bone, and liver. Unfortunately, at the time of diagnosis a breast cancer may already be widely disseminated. There are three main ways in which breast cancer may spread from the primary site: by local infiltration, via the lymphatic system, and via the blood. Locally, if a tumour remains undetected and continues to grow it will eventually invade the overlying skin, and the deep fascia and chest wall. This is termed a ‘locally advanced primary’. Careful histological studies have shown that lymphatic permeation can be observed at the periphery of many breast carcinomas, and axillary lymph nodes may be involved by metastatic carcinoma in up to 40% of females with apparently ‘operable’ tumors. Metastatic carcinoma may also be found in internal mammary lymph nodes, especially if the primary tumor is located in an inner quadrant of the breast. Distant metastasis occurs via the bloodstream; many organs may be involved but the most common are lungs, bone and liver.

2.8. Predictive Markers in Invasive Breast Carcinoma:

Prognostic factors, are applied to identify how a tumor is likely to behave in terms of tumor recurrence and patient survival, and thus which patients require subsequent systemic therapies. Superimposed on these prognostic factors, predictive factors are analyzed to determine which treatments are most likely to benefit the individual, e.g. in many females with breast cancer the course of the disease may be influenced by alterations in the hormonal background of the patient. This was first demonstrated by Beattie in Glasgow in 1896 when he carried out bilateral oophorectomy in females with advanced breast cancer. The estrogen receptor (ER) competitor
tamoxifen or, more recently, the aromatase inhibitors have been used successfully in the treatment of hormone receptor-positive metastatic disease and high-risk operable disease. Assessment of ER protein in tumor samples provides a good prediction of likely response to endocrine therapy; a favorable response is unlikely if an ER cannot be detected. ER status is routinely examined in tissue sections from all invasive breast cancers using immunochemistry and about 80% are positive. Thus the likelihood of response to hormone therapy can be predicted and the most appropriate therapy.

2.9. Progesterone receptor PR

is one of the successful tumor markers in breast cancer that effectively predict the hormonal responsiveness. (Shah Ret a 2014) It is a member of the family promoters of nuclear hormone receptors that specifically binds to progesterone. PR is encoded by single gene PGR presenting on chromosome 1 Iq22. Human PR proteins are of two isoforms, termed PR-A and PR-B, that are transcribed from a certain gene under the control of separate (Banegas M ,2012). The PR has an amino and a carboxyl terminal, and between the regulatory domains, a DNA binding domain, the hinge section and activation function domains (AFs). Detailed molecular dissection has identified two distinct functional domains (AFs) within both isoforms of PRs. AF-1 is located in the N-terminal region and is ligand independent. AF-2, which is ligand dependent, is contained in the ligand-binding domain that is located near the C-terminal region. Furthermore, a unique activation function domain 3, is contained in the upstream segment of PR-B, at the amino acid fraction that is not present in PR-A cancer cells, although some genes are regulated by progesterone through both PR isoforms, most genes are regulated through one or the other isoform, predominantly through PR-B (Marco P.et al 2011). The mechanisms by which PR regulates hormone-response genes are complex. Progesterone binds PR, inducing a conformational change in PR causing its nuclear translocation, dimerisation and interaction with specific DNA progesterone response elements (PREs) present in the promoter regions of target genes. PR can also mediate its effect independently of PREs, through the protein-protein interactions of PR with other specific transcription factors. Protein products from PR target genes are involved in a variety of cellular activities, including transcription, steroid and lipid metabolism, cell growth and apoptosis. Some of these proteins are associated with mammary gland breast cancer development. (Marco P.et al 2011). Clinically, PR are important therapeutic
targets. Progestational agents are widely used for oral contraception, menopausal hormone replacement therapy (HRT), and to treat breast cancer and endometrial hyperplasia. Antiprogestins are used for contraception, induction of labor, treatment of meningiomas, endometriosis, and endometrial carcinoma. PR should be analyzed in every invasive breast cancer as well as metastatic lesions if the results would influence treatment plan. In both pre- and postmenopausal patients, steroid hormone status should be used to identify patients most likely to benefit from endocrine therapy in both early breast cancer and metastatic disease. (Lanari C et al 2012) It was recognized that transcription of the progesterone receptor (PR) gene was regulated by estrogen in breast and reproductive tissues and that estrogen receptor-positive (ER+) breast tumors that lacked PR expression were less responsive to endocrine therapy than those that express high levels of PR. During tamoxifen therapy, levels of both PR and ER decrease but PR levels decrease more dramatically than ER levels, with up to half of the tumors completely losing PR expression as they develop tamoxifen resistance. In patients with such tumors, the loss of PR translates into a more aggressive disease and worse overall survival, suggesting that other alterations in the molecular machinery driving tumor growth accompany the loss of PR receptor expression. Loss of PR in ER+ tumors may be a marker of aberrant growth factor signaling that could contribute to the tamoxifen resistance found in the tumors leading to a poorer survival in women treated with NeoMarkers Rabbit monoclonal anti-human progesterone receptor antibody is an immunohistochemical (IHC) assay intended for laboratory use for the qualitative detection of progesterone receptor (PR) antigen by light microscopy in sections of formalin fixed, paraffin embedded normal and neoplastic tissues on a lab vision automated slide stainer.

2.10. Etiology of Breast Carcinoma:

The incidence of breast cancer, similar to carcinomas in general, increases with age, but the increase occurs earlier than for most cancers, being most rapid between the ages of 30 and 50 years, after which it rises more slowly to a maximum in old age. The strongest aetiological factor is positive family history; there is a definite increased risk if a female relative, i.e. mother, maternal grandmother, or sister, has had breast cancer. Occasional families exist in which there is a very high incidence of breast cancer.
2.11. Clinical breast exam and breast self-exam:

Research has not shown a clear benefit of regular physical breast exams done by either a health professional (clinical breast exams) or by yourself (breast self-exams). There is very little evidence that these tests help find breast cancer early when women also get screening mammograms. Most often when breast cancer is detected because of symptoms (such as a lump), a woman discovers the symptom during usual activities such as bathing or dressing. Women should be familiar with how their breasts normally look and feel and report any changes to a health care provider right way.

2.12. Mammograms

Regular mammograms can help find breast cancer at an early stage, when treatment is most successful. A mammogram can find breast changes that could be cancer years before physical symptoms develop. Results from many decades of research clearly show that women who have regular mammograms are more likely to have breast cancer found early, are less likely to need aggressive treatment like surgery to remove the breast (mastectomy) and chemotherapy, and are more likely to be cured. Mammograms are not perfect. They miss some cancers. And sometimes a woman will need more tests to find out if something found on a mammogram is or is not cancer. There’s also a small possibility of being diagnosed with a cancer that never would have caused any problems had it not been found during screening. It’s important that women getting mammograms know what to expect and understand the benefits and limitations of screening.

2.13. FNA

FNA is widely used for evaluating palpable breast masses, breast cysts, and even nonpalpable mammographic abnormalities. The use of FNA or core biopsies significantly decreases health care costs by decreasing the number of open surgical biopsies per breast cancer identified, without sacrificing early detection(Logan-Yong W, et al 1998). When the diagnosis is benign, such as a lactating adenoma in a patient who is pregnant, FNA spares a patient with a solid and palpable lesion an open biopsy. A diagnosis of malignancy allows preoperative discussion of available therapeutic options (lumpectomy with radiation versus mastectomy), or it might persuade a reluctant patient to undergo surgical biopsy. Ultrasound-guided FNA of axillary lymph nodes is advocated as a way to triage patients for appropriate management(Kuenen-BoumeesterV, et al 2003). Patients with positive aspirates proceed directly to axillary dissection.
or neoadjuvant chemotherapy, whereas those with negative aspirates have sentinel lymph node mapping. The technique for performing an FNA of a palpable breast mass is the same as for any other superficial organ. Twenty-three-gauge or 25-gauge needles with a 10mL syringe are standard. A local anesthetic is usually not used because the swelling that results can obscure the nodule. A syringe holder is often used to stabilize the syringe or needle set-up. Because many breast lesions are densely fibrous, a larger (22-gauge needle) is preferred, and in this circumstance local anesthetic may be advisable. Because many cancers have a stellate configuration, one should aspirate the center and not the periphery of a mass. After placing the needle in the center of the mass, suction is applied via the syringe. One should release suction when blood or material is seen in the hub in the needle; the exception to this is with fluid-filled cysts that should be drained in their entirety with reaspiration of any residual mass. The needle is withdrawn from the mass without any vacuum suction; otherwise, the cells end up in the syringe (rather than staying in the needle) and are dried and difficult to expel onto slides. To prepare smears, the needle is removed from the syringe and the syringe is filled with air. By reattaching the needle and pushing this air with the plunger of the syringe, a small drop of aspirated material is expressed onto each slide and smears prepared. To harvest as much of the sample as possible, the needle should be washed with a preservative solution for cell block or liquid-based preparations. Although the cytologic appearance is slightly different because clusters are more three-dimensional, nuclei are smaller and darker, and less background material is seen, the accuracy of thin-layer preparations is comparable to that obtained with direct smears (Florentine BD et al 1999).

Complications of FNA are rare. The most common is bleeding. Occasionally, FNA causes infarction of the lesion, particularly fibroadenomas, which can make subsequent confirmation of the diagnosis more difficult (Pinto RP et al 1996). Whether patients with breast cysts need cytologic analysis is controversial. Aspiration of cysts is certainly therapeutic and collapses them. The great majority of cyst fluids are benign; only about 2% prove to be carcinoma (Roesn PP, et al 1993). Even those complex cystic lesions noted on ultrasound are benign; in one study only 0.3% proved to be malignant (Vental A et al 1999). Furthermore, atypical cells can be seen in a cyst fluid, resulting in overtreatment when conservative follow-up would have been adequate (Smith D, et al 1997). On the other hand, a small number of carcinomas are cystic and yield fluid that looks grossly much like that of benign cysts (Howell LP, et al 1990). If the fluid
is not submitted for cytologic evaluation, these carcinomas will remain undiagnosed and untreated. It has been suggested that symptomatic complicated cysts, cystic lesions with thick indistinct walls or thick septations, intracystic masses, and predominantly solid masses with cystic degeneration are more likely to be malignant and thus need further evaluation such as FNA, CNB, or excisional biopsy (Bergw et al 1991). The accuracy of FNA of the breast is highly operator dependent: Sensitivity for malignancy is high, but ranges from 65% to 98%, and specificity ranges from 34% to 100% in a variety of clinical environments (Lieske B . et al 2006). False-positive results are uncommon, occurring in 0% to 2% of cases. False-suspicious results are higher, ranging from 1% to 13%. In general, the sensitivity of FNA for palpable and nonpalpable malignant lesions (i.e., those sampled with mammographic or ultrasound guidance) is comparable (Azavedo E , et al 1991). False-negative results occur because of errors in sampling, interpretation, or both (Zarbo RJ , et al 1991). Some studies show that satisfactory specimens are more likely when pathologists rather than physicians perform the aspiration (Barrows G, et al 1996). Whether physician or pathologist, however, practice makes perfect, and the one with more FNA experience obtains the more accurate result (Mayall F, et al 1998). The use of p63 immunostaining has recently been proposed as an adjunct to increase the accuracy of FNA by differentiating the well-differentiated carcinomas from benign lesions (Harton AM, et al 2007). FNA of the breast has its limitations. Although generally quite sensitive in detecting ductal carcinomas, it cannot

2.14. 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

2.15. TMA:

Tissue microarray technology (TMA) involves core needle biopsies of multiple pre-existing paraffin-embedded tissue blocks and re-embedding them in the form of an arrayed master block. Thus it means biopsy of a biopsy. Construction of multiple tissue arrayed block is also possible by fresh tissues and frozen section, as well (Fejzo MS, et al 2001).

The first reports concerning TMA appeared through 1998–2001 by Kannonen, Kallioniemi et al from National Human Genome Research Institute,
Bethesda, USA2–8 They worked on many different tumor types, about the technique and different markers. After that so many papers about this subject has become appearing in literature. Tissue microarray technology is a new method used to analyze several tissues especially tumor samples on a single slide (Fejzo MS, et al. 2001). The recent development of tissue microarray technology has potentiated largescale retrospective cohort studies using archival formalin-fixed, paraffin-embedded tissues (Camp RL, et al. 2008). It is shown that many proteins retain their antigenicity for more than 60 years (Dhansekaran SM, 2001). A major obstacle to broad acceptance of microarrays is that they reduce the amount of tissue analyzed from a whole tissue section to a disk, 0.6 mm in diameter (Benbendorf L, 2001). As many as 1000 cylindrical tissue biopsies from individual tumors can be distributed in a single tissue microarray. Sections of the microarray provide targets for parallel in situ detection of DNA, RNA and protein targets in each specimen on the array, and consecutive sections allow the rapid analysis of hundreds of molecular markers in the same set of specimens (Kononen J, et al. 1998). One difficulty with paraffin embedded tissue relates to antigenic changes in proteins and mRNA degradation induced by the fixation and embedding process. But there are technical reports to improve preservation of genome DNA and proteins in paraffin blocks, such as zinc based fixation, buffered formalin fixation (Wester K, et al. 2003). There are reports describing construction and use of frozen arrays. Analysis of hundreds of specimens from patients in different s diagnostic, prognostic and therapeutic importance of each of the emerging cancer gene candidates. Most of the applications of the TMA technology have come from the field of cancer research. Examples include analysis of the frequency of molecular alterations in large tumor materials, exploration of tumor progression, and identification of predictive or prognostic factors and validation of newly discovered genes as diagnostic and therapeutic targets.3 It can be used to correlate lymph node positive and negative tumors, it helps for molecular classification of tumors. It provides rapid linking of molecular changes to clinical endpoints. Predicting the response of chemotherapeutics or hormonotherapy (Hu YC, et al. 2003), comparison of methods, testing interlabolatory and interobserver reproducibility of methods is easier and faster with TMA. Since it provides studying a parameter for 100-1000 samples on a single slide, community based retrospective cohort studies could be available (Kluger HM, et al. 2004).21 By this way the tissue microarray
data exchange specification: a community based open source tool for sharing tissue microarray data collection is needed.

2.16. Tumor marker:
A biomarker that is found in blood, urine or body tissues that can be elevated by the presence of one or more types of cancer. It is produced either by the tumor itself or by the host in the response to a tumor (Kelpatri, et al 2009). The ideal tumor marker should be both specific and sensitive to detect small tumors to allow early diagnosis or help in screening. Few markers are specific for a single tumor. Most markers are produced by different tumors of the same tissue type. They are present in higher quantities in cancer tissue in blood from cancer patients more than in the blood of normal subjects. Tumor markers are mostly useful in evaluating the progression of the disease status after initial chemotherapy and radiotherapy to monitor subsequent treatment strategies (Amayo AA.2009).

Breast cancer is the second most common type of cancer after lung cancer (10.4% of all cancer incidence, both sexes counted) and the fifth most common cause of cancer death (Kabel et al 2015). It is a disease caused by a combination of genetic and environmental factors. Numerous risk factors that may be associated with breast cancer have been recognized. Not all breast cancer patients have the same clinical picture. Some factors increase a woman's risk of breast cancer more than others (Kkabel AM et al 2016).

Early detection of breast cancer both primary and recurrent, is of considerable clinical importance, and it can be used to make treatment decisions while tumor burden is low, and when patients are most likely to respond to adjuvant therapy (Shah R, et al 2014). In recent decades, the serum concentration of tumor markers has been used to detect tumor activity. Tumor markers provide a minimally invasive cost-effective source of data valuable for monitoring disease course, determining prognosis, and helping in treatment planning. An understanding of the individual test characteristics and limitations is important for optimal use and accurate interpretation of results (Banegas MP, et al 2012). The real usefulness of tumor markers in the management of breast cancer has been questioned because of the low diagnostic sensitivity for early disease.
3.1 Material

3.1.1 Study design:
Retrospective cross sectional laboratory base study to evaluate the expression of progesterone receptor in breast cancer at Alrahamah center-Khartoum state in period from August to October 2018.

3.1.2 Study area and duration:
This study was conducted at ELrahma, Khartoum state, during the period from August to October 2018.

3.1.3 Study material:
Formalin Fixed Paraffin Embedded (FFPE) tissue blocks of breast cancer previously diagnosed as Invasive Ductal Carcinoma (IDC) by using haematoxyline and eosin staining method were used for tissue microarray technique using immunohistochemistry staining method.

3.1.4 Study samples and samples size:
Thirty five (35) formalin fixed paraffin embedded tissue blocks previously diagnosed as IDC.

3.1.5 Inclusion criteria:
Paraffin embedded tissue blocks diagnostic with breast cancer.

3.1.6 Exclusion criteria:
Other paraffin embedded tissue block.

3.1.7 Ethical consideration:
Ethical approval from the Ethics Committee of Elgezira university obtained, sample were collected after taking ethical acceptance from ELrahma Medical Lab administration.
3.1.8. Data analysis:

The obtained results and variables arranged in standard master sheet, then analyzed using statistical package for social science (SPSS) version 20 computer program. Frequencies, Means and Chi squire tests were calculated and presented in form figure and table.

3.2. Methods

3.2.1. TMA:

First we prepared block to sectioned by using special mold and plastic cassette. We filled the mold with paraffin wax, there was special wax when we want to demonstrate DNA, and we put the plastic cassette with in the mold and place it on cold surface, when become solid we separate the cassette from the mold, and use special needle (0.06 mm) take the specimen from tissue and put it into cassette tray and labeled the tray A, B, C, ..., to J, and every one of them had four Colum, the incubate over night in an an oven so as to be soft, by microtome we sectioned and put into water path and take it special slide and prepared for IHC stains.

3.2.2. ImmunoHistology Techniques:

The immunohistochemical procedure was done as follows: one section (3μm) from formalin-fixed, paraffin-embedded tumors were cut and mounted onto salinized slides (Thermo - USA). Following de paraffinization in xylene (Thermo Fisher L TD), slides were rehydrated through a graded series of ethyl alcohol 100% 4 min, 90% 2 min, 70% 2 min, and water 2 min) and were placed in distilled water. Samples were steamed for antigen retrieval for PR using high PH (9) by water bath (histoline- TEC 2601-Italy) 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 (Dako -USA), and After washing with PBS for 3 min then Slides were incubated with (100 μ L) of mouse monoclonal antibody (PR), against LMP-1 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 - USA). 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 Mayer ’s haematoxylin (RAL- faranca) for one min were washed in
running tap water for several minutes 7-10 (bluing), then dehydrate and, cleaned, mount in DBX (Ramos -vara J.A. 2005 ). Each slide was evaluated with using LMP1 (Latent membrane protein 1 (LMP1) of PR) Antibody immuno histochemistry. The diagnosis of breast cancer was established depending on clinical examination and histopathological features of the biopsy.
Chapter four

Result and discussion

4.1. Result:

A total of 35 tissue samples diagnosed previously as invasive ductal carcinoma were used in this study to evaluate the expression of progesterone receptor as a prognostic factor in breast cancer by using TMA technique. The patients age ranged between 30 to 70 years. The age 30 to 45 years was the most frequent age group 48.5% (17/35) followed by (46 to 60 years) 40% (14/35), then (61 to 70 years) 11.5% (4/35) see table 4-1. The study cleared all patient were diagnosed IDC 35 (100%) see table (4-2). The study showed the majority of sample were grade three 65.7% (23/35) and grade two 34.3% (12/35) (Table 4-3). The study showed the majority of TMA result were positive 77.1% (27/35) and negative result were 22.9% (8/35) see table (4-4). The study showed the majority of TMA score were (+) 31.4% (11/35) followed by (++) 25.7% (9/35), then (+++) 20% (7/35), and negative result were 22.9% (8/35) see table (4-5). There was significance relation between TMA result and progesterone receptor scoring, and no relation between score and grade, and no relation between grade and TMA.
Table (4-1): distribution of according to their Age

This table shows the majority (48.6%) of the study sample where (“30-45”).

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-45</td>
<td>17</td>
<td>48.6%</td>
</tr>
<tr>
<td>46-60</td>
<td>14</td>
<td>40%</td>
</tr>
<tr>
<td>61-75</td>
<td>4</td>
<td>11.4%</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table (4-2): distribution of according to their Type of Cancer

This table shows the majority (100%) of the study all sample used where IDC.

<table>
<thead>
<tr>
<th>H&amp;E</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDC</td>
<td>35</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table (4-3): distribution of according to their Graded

This table shows the majority (65.7%) of the study sample where (there).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two</td>
<td>12</td>
<td>3.34%</td>
</tr>
<tr>
<td>There</td>
<td>23</td>
<td>65.7%</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table (4-4): distribution of according to the TMA results
This table shows the majority (77.1%) of the study sample where (Positive).

<table>
<thead>
<tr>
<th>TMA</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>27</td>
<td>77.1%</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>22.9%</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table (4.5): distribution of according to their Score

This table shows the majority (31.4%) of the study sample where (Score +).

<table>
<thead>
<tr>
<th>Score</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>11</td>
<td>31.4%</td>
</tr>
<tr>
<td>++</td>
<td>9</td>
<td>25.7%</td>
</tr>
<tr>
<td>+++</td>
<td>6</td>
<td>17.1%</td>
</tr>
<tr>
<td>++++</td>
<td>1</td>
<td>2.9%</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>22.9%</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table (4-6): distribution of Relation between Score and TMA.

This table shows the Relation between Score and TMA because P.value (.000) less than Significance level (.05) and Strong correlate
Table (4-7): distribution of Relation between Score and Grade

This table shows the No Relation between Score and Grade because P.value (.410) greater than Significance level (.05) and medium correlated.

<table>
<thead>
<tr>
<th>Chi-Square</th>
<th>Correlation</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.970</td>
<td>.055</td>
<td>.410</td>
</tr>
</tbody>
</table>

Table (4-8): distribution of Relation between grade and TMA

This table shows the No Relation between grade and TMA because P.value (.827) greater than Significance level (.05) and very weak correlate.

<table>
<thead>
<tr>
<th>Chi-Square</th>
<th>Correlation</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>.048</td>
<td>-.037</td>
<td>.827</td>
</tr>
</tbody>
</table>
Figure 4.1: The frequency of H&E 100% (35/35).
Figure 4-2: frequency of TMA 77.1% (28/35)

Figure 4-3: the relation between grade and age
Figure 4-4: The relation between age and TMA
Figure 4-5: The relation between age and score

Figure 4-6: The relation between TMA and score
Figure 4-7: the relation between grade and score

Figure 4-8: the relation between grade TMA
4-2 Discussions

The incidence of breast cancer in Sub-Saharan African counties is low compared to that in developed countries (Parkin DM et al., 1999), 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. In this study we found that TMA technique was a good tool for PR expression and when PR positive in breast cancer that mean there was good prognosis and response to hormonal therapy, in other side positive result were deferential diagnosis for IDC, and negative may be lobular carcinoma or other types of breast carcinoma. In this study we found the expression of progesterone receptor equal 77.1% that means it was good prognostic factor in breast carcinoma by using TMA technique using immunohistochemistry staining method.

Previous studies agree with this study, the first one said: (The showed that progesterone receptor expression using immunohistochemistry was important prognostic factor in breast cancer, which suggest the utility of progesterone receptor as a marker for differentiation between breast cancer types.) (A Part, M.C, et al 2014) .The second one (the study reports revealed that progesterone receptor expression was a prognostic factor in HR+ breast cancer) (C Davies, et al 2011). The third one (the study showed that, the expression of progesterone receptor can hinder estrogen proliferation and estrogen receptor transcriptional activity in ER+ breast cancer) (Zheng ZY, et al 2005)
Chapter five

Conclusion & Recommendation

5-1 Conclusion:
TMA technique more specific in differential diagnosis of breast cancer, and it is reliable, cheap, and saving time, money, and effort.

5-2 Recommendations:
TMA technique must be used routinely as a diagnostic tool in histopathological center, and a lot of studies must be carried out on this technique.
Chapter six

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cyto book


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