

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



# Shendi University



**Faculty of High Graduate Studies and Scientific Research**

**Medical Laboratory Master Degree**

**Hematology Department**

Research About:

**Correlation Between Hematological Parameters and Tumor Markers in  
Breast Cancer Patients Receiving Chemo Therapy**

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الآية

قال الله تعالى:

"اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ مِثْلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ  
الْمِصْبَاحُ فِي زُجَاجَةٍ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ  
مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ  
نَارٌ نُورٌ عَلَى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ  
لِلنَّاسِ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ (35)".

صَدَقَ وَاللَّهُ الْعَظِيمُ

قرآن كريم

## **DEDICATION**

I dedicate my dissertation work to my family and many friends. A special feeling of gratitude to my loving parents, whose words of encouragement and push for tenacity ring in my ears. My sisters have never left my side and are very special. I also dedicate this dissertation to my many friends and who have supported me throughout the process. I will always appreciate all they have done, for helping me develop my technology skills, proofreading, and helping me to master the leader dots. I dedicate this work and give special thanks to my husband for being there for me throughout the entire master program.

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## الخلاصة

**الهدف:** نسبة الإصابة بسرطان الثدي مرتفعة في جميع انحاء العالم. فان معدل الوفيات من سرطان الثدي مرتفع في السودان والدول النامية الأخرى ومع ذلك هناك ندرة في المعلومات المتعلقة بالتغيرات الدموية في مرضي سرطان الثدي في السودان.

تهدف هذه الدراسة الي التحقق من العلاقة بين بعض العوامل الدموية وعلامات الأورام في مرضي سرطان الثدي الذين يتلقون العلاج الكيماوي في شندي. ولاية نهر النيل.

**الطريقة والمواد:** 50 مريضا الذين تم تشخيص سرطان الثدي لديهم وبعد تلقي العلاج الكيماوي تم جمع عينات الدم الوريدي في حاويات K3 EDTA من المرضي الذين تتراوح أعمارهم بين 30 الي 70 سنة وتم تحليل العينات.

تم تقييم العينة الجراحية بعد ذلك علي الانسجة والمضادة كيميائيا لعلامات (ER، HER-، PR، neu2).

**النتيجة:** 50 حالة تم دراستها تراوح عمر المرضي من 30 الي 70 سنة مع متوسط الفئة العمرية 50.28 سنة. شوهد الحد الأقصى لعدد الحالات في الفئة العمرية (40-49) 30%

فقط 2 (4%) وقعت في الذكور. المتبقية 48 (96%) من الحالات كانت في الاناث. نسبة الذكور الي الاناث 2:48.

**الخلاصة:** تشير هذه الدراسة الي أنه لم يتم وصف أي ارتباط بين العوامل الدموية وعلامة الورم في مرضي سرطان الثدي.

## ABSTRACT

**AIM:** The incidence of breast cancer is high in worldwide, the incidence of death from breast cancer is high in Sudan and other developing countries. However, there is paucity of information of hematological changes in breast cancer patients in Sudan. The study is aimed at investigating the correlation between some hematological parameters and tumor marker in breast cancer patients receiving chemo therapy in shendi, river Nile state.

**Material and Methods:** 50 patients who were diagnosed to have carcinoma breast and after receiving chemo therapy. venous blood samples were collected in to K3 EDTA containers from patients aged 30 to 79 years, the samples were analyzed.

The surgical specimen was then evaluated histopathologically and immunohistochemically for ER, PR, HER-2neu markers) dr pawan nikhera).

**Result:** 50 cases were studied, ranging in age from 30 to 70 years with an average age of 50.28 years. Maximum number of cases seen in age group (40-49) 30%

Only 2 (4%) occurred in males. The remaining 48 (96%) cases were in females. Ratio of males to females 48: 2.

**Conclusion:** This study indicates that no association between blood factors and tumor marker has been described in breast cancer patients.

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### List of Abbreviation

No	Subject
CBC	Complete blood count
TWBCs	Total white blood count
HB	Hemoglobin
RBCs	Red blood cells
PLT	Plate let
PR	Progesterone receptor
ER	Estrogen receptor
HER2/neu	Human epidermal growth factor receptor2/neu
IDC	Invasive ductal carcinoma
FNAC	Fine needle Aspiration cytology
MRM	Modified Radical Mastectomy
IHC	Immuno Histo chemistry
SPSS	Statistical package for Social Student

# CHAPTER one

## 1.1 INTRODUCTION

Worldwide breast cancer is the most common invasive malignancy in women [1]. It comprises 22.9% of invasive cancers in women and 16% of all female cancer. The number of cases worldwide has significantly increased since the 1970s, a phenomenon partly attributed to the modern lifestyles. In the year 2010, one million new cases were diagnosed and more than five hundred thousand lives were claimed by breast cancer globally. In India, the average age of breast cancer patients ranges from 44.2 years to 49.6 years. [2]

Over the last few decades there have been outstanding advances in breast cancer management leading to early detection of disease and the development of more effective treatments resulting in significant decline in breast cancer deaths and improved outcome for women living with the disease [3]. Recent attention has been directed singularly at molecular classification of breast cancer. While molecular and genetic testing is very elegant, prognostic and predictive, it is expensive and not yet widely available [4].

The Immunohistochemical (IHC) classification provides both therapeutic and prognostic information, is inexpensive and readily available. IHC-based classification of both ER/PR and HER-2/neu status provides prognostic and therapeutic information not achievable from either alone. Prior classifications separating breast cancer into one of two categories based on ER expression alone is less discriminatory in terms of prognosis, and the additional sub-classification based on Her2 /neu expression provides enhanced and important therapeutic guidance [5].

Thus, this study was carried out with the aim of helping to correlate between some hematological parameters and tumor marker in breast cancer patient receiving chemotherapy.

The complete blood count (CBC)<sup>[6]</sup> is one of the more common laboratory tests ordered during the neonatal period. The CBC may be obtained to evaluate for anemia, infection, and thrombocytopenia.<sup>[7]</sup> The test offers a wealth of clinical information about the hematopoietic system, including erythrocyte, leukocyte, and thrombocyte values. Establishing normal neonatal ranges has been difficult because blood has not been drawn on healthy neonates of similar ages.<sup>[8]</sup> Reference ranges that consist of the 5th to 95th percentile compiled from various studies have been used to approximate normal neonatal values.<sup>[9]</sup> A variety of factors such as sample site, timing of the sample, gestational age, and the neonate's degree of health can affect the CBC.<sup>1</sup> Therefore, the astute practitioner must be able to recognize the clues and nuances of the CBC to guide the diagnostic assessment.<sup>[10]</sup>

Blood cell development begins in the earliest weeks of gestation. Cell differentiation appears to begin from a population of progenitor or stem cells located within the yolk sac, liver, and bone marrow of the developing fetus.<sup>[11]</sup> The microchemical environment of the developing stem cells determines the differentiation of at least two cell lines: the myeloid hematopoietic system and the lymphoid hematopoietic system.<sup>6</sup>

The myeloid hematopoietic cell line leads to the proliferation and differentiation of stem cells into the erythroid, myeloid, and megakaryocyte precursors.<sup>[12]</sup> The erythrocytes, leukocytes, and thrombocytes develop from these precursors.<sup>[13]</sup> The lymphoid hematopoietic cell line produces the lymphocytes. Lymphocytes follow one of two independent pathways to produce the cells that will become either T lymphocytes or B lymphocytes.

## **1.2: RATIONALE**

Breast cancer is a worldwide disease resulting in many [14] 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 stages 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. This 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, while ductal carcinoma in situ was the least prevalent (0.5%) finding.

This study was Aimed to investigating the correlation between hematological parameters and tumor marker in breast cancer patients receiving chemo therapy.

Studies of breast cancer in Sudan are fewer when we compare with other countries, because absent of financial support from government.

## **1.3: Objectives**

### **General objective:**

To evaluate the correlation between some hematological parameters and tumor markers in breast cancer patients receiving chemotherapy.

### **Special objectives:**

1/ To determine the hemoglobin level and RBCs count in breast cancer chemotherapy treatment.

2/ To determine the effect of chemotherapy on white blood cells count and platelets.

3/ To demonstrate the role of tumor markers (hormone receptors) in breast cancer patients in diagnosis and prognosis.

# CHAPTER two



## 2- LITERATURE REVIEW

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

Risk factors for developing breast cancer include being female, obesity, lack of physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation, early age at first menstruation, having children late or not at all, older age, prior history of breast cancer, and family history. About 5–10% of cases are due to genes inherited from a person's parents, including BRCA1 and BRCA2 among others. Breast cancer most commonly develops in cells from the lining of milk duct and the lobules that supply the ducts with milk. Cancers developing from the ducts are known as ductal carcinoma, while those developing from lobules are known as lobular carcinoma. In addition, there are more than 18 other sub-types of breast cancer. Some cancers, such as ductal carcinoma in situ, develop from pre-invasive lesions. The diagnosis of breast cancer is confirmed by taking a biopsy of the concerning lump. Once the diagnosis is made, further tests are done to determine if the cancer has spread beyond the breast and which treatments are most likely to be effective. [16]

The balance of benefits versus harms of breast cancer screening is controversial. A 2013 Cochrane review stated that it is unclear if mammographic screening does more good or harm. [16] A 2009 review for

the US Preventive Services Task Force found evidence of benefit in those 40 to 70 years of age, and the organization recommends screening every two years in women 50 to 74 years old<sup>[ 17]</sup> The medications tamoxifen or raloxifene may be used in an effort to prevent breast cancer in those who are at high risk of developing it Surgical removal of both breasts is another preventative measure in some high risk women. those who have been diagnosed with cancer, a number of treatments may be used, including surgery, radiation therapy, chemotherapy, hormonal therapy and targeted therapy Types of surgery vary from breast-conserving surgery to mastectomy. Breast reconstruction may take place at the time of surgery or at a later date In those in whom the cancer has spread to other parts of the body, treatments are mostly aimed at improving quality of life and comfort. The first noticeable symptom of breast cancer is typically a lump <sup>[18]</sup> that feels different from the rest of the breast tissue. More than 80% of breast cancer cases are discovered when the woman feels a lump 20The earliest breast cancers are detected by a mammogram Lumps found in lymph nodes located in the armpit can also indicate breast cancer <sup>[19]</sup>. Indications of breast cancer other than a lump may include thickening different from the other breast tissue, one breast becoming larger or lower, a nipple changing position or shape or becoming inverted, skin puckering or dimpling, a rash on or around a nipple, discharge from nipple/s, constant pain in part of the breast or armpit, and swelling beneath the armpit or around the collarbone Pain ("mazodynia")is an unreliable tool in determining the presence or absence of breast cancer

[ 2 0 ]

Another reported symptom complex of breast cancer is Paget's disease of the breast. This syndrome presents as skin changes resembling eczema, such as redness, discoloration, or mild flaking of the nipple skin. As Paget's disease of the breast advances, symptoms may include tingling,

itching, increased sensitivity, burning, and pain. There may also be discharge from the nipple. Approximately half of women diagnosed with Paget's disease of the breast also have a lump in the breast [ 21]

In rare cases, what initially appears as a fibroadenoma (hard, movable non-cancerous lump) could in fact be a phyllodes tumor. Phyllodes tumors are formed within the stroma (connective tissue) of the breast and contain glandular as well as stromal tissue. Phyllodes tumors are not staged in the usual sense; they are classified on the basis of their appearance under the microscope as benign, borderline, or malignant.

Occasionally, breast cancer presents as metastatic disease—that is, cancer that has spread beyond the original organ. The symptoms caused by metastatic breast cancer will depend on the location of metastasis. Common sites of metastasis include bone, liver, lung and brain. Unexplained weight loss can occasionally signal breast cancer, as can symptoms of fevers or chills. Bone or joint pains can sometimes be manifestations of metastatic breast cancer, as can jaundice or neurological symptoms. These symptoms are called non-specific, meaning they could be manifestations of many other illnesses .[22] Most symptoms of breast disorders, including most lumps, do not turn out to represent underlying breast cancer. Fewer than 20% of lumps, for example, are cancerous and benign breast diseases such as mastitis and fibroadenoma of the breast are more common causes of breast disorder symptoms [23]

### 2.1.1 Risk factors

Risk factors can be divided into two categories:

- modifiable risk factors (things that people can change themselves, such as consumption of alcoholic beverages), and
- fixed risk factors (things that cannot be changed, such as age and biological sex) [24]

The primary risk factors for breast cancer are being female and older age [25] Other potential risk factors include genetics lack of childbearing or lack of breastfeeding higher levels of certain hormone certain dietary patterns, and obesity. One study indicates that exposure to light pollution is a risk factor for the development of breast cancer Life style [26]

Obesity and drinking alcoholic beverages are among the most common modifiable risk factors, Smoking tobacco appears to increase the risk of breast cancer, with the greater the amount smoked and the earlier in life that smoking began, the higher the risking those who are long-term smokers, the risk is increased 35% to 50%.A lack of physical activity has been linked to about 10% of cases sitting regularly for prolonged periods is associated with higher mortality from breast cancer. The risk is not negated by regular exercise, though it is lowered [27]

There is an association between use of hormonal birth control [28] and the development of premenopausal breast cancer but whether oral contraceptives use may actually cause premenopausal breast cancer is a matter of debate If there is indeed , the absolute effect is small. Additionally, it is not clear if the association exists with newer hormonal birth controls In those with mutations in the breast cancer susceptibility genes BRCA1 or BRCA2, or who have a family history of breast cancer,

use of modern oral contraceptives does not appear to affect the risk of breast cancer .[29]

The association between breast feeding and breast cancer has not been clearly determined; some studies have found support for an association while others have not. In the 1980s, the abortion–breast cancer hypothesis posited that induced abortion increased the risk of developing breast cancer This hypothesis was the subject of extensive scientific inquiry, which concluded that neither miscarriages nor abortions are associated with a heightened risk for breast cancer. .[30]

A number of dietary factors have been linked to the risk for breast cancer. Drinking alcoholic beverages increases the risk of breast cancer, even at relatively low (one to three drinks per week) and moderate levels. The risk is highest among heavy drinkers. Dietary factors which may increase risk include a high-fat diet and obesity-related high cholesterol levels. Dietary iodine deficiency may also play a role. Evidence for fiber is unclear. A 2015 review found that studies trying to link fiber intake with breast cancer produced mixed results. In 2016 a tentative association between low fiber intake during adolescence and breast cancer was observed. [ 31]

Other risk factors include radiation and shift-work. A number of chemicals have also been linked, including polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and solvents Although the radiation from mammography is a low dose, it is estimated that yearly screening from 40 to 80 years of age will cause approximately 225 cases of fatal breast cancer per million women screened.[32]

### **2.1.2 Pathophysiology:**

Breast cancer, like other cancers, occurs because of an interaction between an environmental (external) factor and a genetically susceptible host. Normal cells divide as many times as needed and stop. They attach to other cells and stay in place in tissues. Cells become cancerous when they lose their ability to stop dividing, to attach to other cells, to stay where they belong, and to die at the proper time. Mutations that can lead to breast cancer have been experimentally linked to estrogen. Normal cells will commit cell suicide (programmed cell death) when they are no longer needed. Until then, they are protected from cell suicide by several protein clusters and pathways. One of the protective pathways is the PI3K/AKT pathway; another is the RAS/MEK/ERK pathway. Sometimes the genes along these protective pathways are mutated in a way that turns them permanently "on", rendering the cell incapable of committing suicide when it is no longer needed. This is one of the steps that causes cancer in combination with other mutations. Normally, the PTEN protein turns off the PI3K/AKT pathway when the cell is ready for programmed cell death. In some breast cancers, the gene for the PTEN protein is mutated, so the PI3K/AKT pathway is stuck in the "on" position, and the cancer cell does not commit suicide [33]

Abnormal growth factor signaling in the interaction between stromal cells and epithelial cells can facilitate malignant cell growth. In breast adipose tissue, overexpression of leptin leads to increased cell proliferation and cancer [34]

In the United States, 10 to 20 percent of people with breast cancer and people with ovarian cancer have a first- or second-degree relative with one of these diseases. The familial tendency to develop these cancers is

called hereditary breast–ovarian cancer syndrome. The best known of these, the BRCA mutations, confer a lifetime risk of breast cancer of between 60 and 85 percent and a lifetime risk of ovarian cancer of between 15 and 40 percent. Some mutations associated with cancer, such as p53, BRCA1 and BRCA2, occur in mechanisms to correct errors in DNA. These mutations are either inherited or acquired after birth. Presumably, they allow further mutations, which allow uncontrolled division, lack of attachment, and metastasis to distant organs [35] However, there is strong evidence of residual risk variation that goes well beyond hereditary BRCA gene mutations between carrier families. This is caused by unobserved risk factors. This implicates environmental and other causes as triggers for breast cancers. The inherited mutation in BRCA1 or BRCA2 genes can interfere with repair of DNA cross links and DNA double strand breaks.[36] These carcinogens cause DNA damage such as DNA cross links and double strand breaks that often require repairs by pathways containing BRCA1 and BRCA2.[37] However, mutations in BRCA genes account for only 2 to 3 percent of all breast cancers. Levin et al. say that cancer may not be inevitable for all carriers of BRCA1 and BRCA2 mutations [38] About half of hereditary breast–ovarian cancer syndromes involve unknown genes. [38]

GATA-3 directly controls the expression of estrogen receptor (ER) and other genes associated with epithelial differentiation, and the loss of GATA-3 leads to loss of differentiation and poor prognosis due to cancer cell invasion and metastasis.[39]

### **2.1.3Diagnosis**

Most types of breast cancer are easy to diagnose by microscopic analysis of a sample—or biopsy—of the affected area of the breast. Also, there are

types of breast cancer that require specialized lab exams. The two most commonly used screening methods, physical examination of the breasts by a healthcare provider and mammography, can offer an approximate likelihood that a lump is cancer, and may also detect some other lesions, such as a simple cyst. When these examinations are inconclusive, a healthcare provider can remove a sample of the fluid in the lump for microscopic analysis (a procedure known as fine needle aspiration, or fine needle aspiration and cytology—FNAC) to help establish the diagnosis. The needle aspiration may be performed in a healthcare provider's office or clinic using local anesthetic if required. A finding of clear fluid makes the lump highly unlikely to be cancerous, but bloody fluid may be sent off for inspection under a microscope for cancerous cells. Together, physical examination of the breasts, mammography, and FNAC can be used to diagnose breast cancer with a good degree of accuracy. [40]

Other options for biopsy include a core biopsy or vacuum-assisted breast biopsy which are procedures in which a section of the breast lump is removed; or an excisional biopsy, in which the entire lump is removed. Very often the results of physical examination by a healthcare provider, mammography, and additional tests that may be performed in special circumstances (such as imaging by ultrasound or MRI) are sufficient to warrant excisional biopsy as the definitive diagnostic and primary treatment method. [41]



#### 2.1.4 CLASSIFICATION:

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

- **Histopathology.** Breast cancer is usually classified primarily by its histological appearance. Most breast cancers are derived from the epithelium lining the ducts or lobules, and these cancers are classified as ductal or lobular carcinoma. Carcinoma in situ is growth of low-grade cancerous or precancerous cells within a particular tissue compartment such as the mammary duct without invasion of the surrounding tissue. In contrast, invasive carcinoma does not confine itself to the initial tissue Grade. [42]
- **Grading** compares the appearance of the breast cancer the cells to appearance of normal breast tissue. Normal cells in an organ like the breast become differentiated, meaning that they take on specific shapes and forms that reflect their function as part of that organ. Cancerous cells lose that differentiation. In cancer, the cells that would normally line up in an orderly way to make up the milk ducts become disorganized. Cell division becomes uncontrolled. Cell nuclei become less uniform. Pathologists describe cells as well differentiated (low grade), moderately differentiated (intermediate grade), and poorly differentiated (high grade) as the cells progressively lose the features seen in normal breast cells. Poorly differentiated cancers (the ones whose tissue is least like normal breast tissue) have a worse prognosis.43-84
- **Stage.** Breast cancer staging using the TNM system is based on the size of the tumor (T), whether or not the tumor has spread to the lymph nodes (N) in the armpits, and whether the tumor has

metastasized (M) (i.e. spread to a more distant part of the body).

Larger size, nodal spread, and metastasis have a larger stage number and a worse prognosis.

The main stages are:

- Stage 0 is a pre-cancerous or marker condition, either ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS).
- Stages 1–3 are within the breast or regional lymph nodes.
- Stage 4 is 'metastatic' cancer that has a less favorable prognosis since it has spread beyond the breast and regional lymph nodes...Where available, imaging studies may be employed as part of the staging process in select cases to look for signs of metastatic cancer. However, in cases of breast cancer with low risk for metastasis, the risks associated with PET scans, CT scans, or bone scans outweigh the possible benefits, as these procedures expose the person to a substantial amount of potentially dangerous ionizing radiation.<sup>[43-44]</sup>

Where available, imaging studies may be employed as part of the staging process in select cases to look for signs of metastatic cancer. However, in cases of breast cancer with low risk for metastasis, the risks associated with PET scans, CT scans, or bone scans outweigh the possible benefits, as these procedures expose the person to a substantial amount of potentially dangerous ionizing radiation.

- **2.1.5 Receptor status.** Breast cancer cells have receptors on their surface and in their cytoplasm and nucleus. Chemical messengers such as hormones bind to receptors, and this causes changes in the cell. Breast cancer cells may or may not have three important receptors: estrogen receptor (ER), progesterone receptor (PR), and HER2. ER+ cancer cells (that is, cancer cells that have estrogen receptors)

depend on Estrogen for their growth, so they can be treated with drugs to block estrogen effects (e.g. tamoxifen), and generally have a better prognosis. Untreated, HER2+ breast cancers are generally more aggressive than HER2- breast cancers, but HER2+ cancer cells respond to drugs such as the monoclonal antibody trastuzumab (in combination with conventional chemotherapy), and this has improved the prognosis significantly. Cells that do not have any of these three receptor types (estrogen receptors, progesterone receptors, or HER2) are called triple-negative, although they frequently do express receptors for other hormones, such as androgen receptor and prolactin receptor. [ 45]

lump or other lesion has appeared (a procedure known as prophylactic bilateral mastectomy) may be considered in people with BRCA1 and BRCA2 mutations, which are associated with a substantially heightened risk for an eventual diagnosis of breast cancer. [47] Evidence is not strong enough to support this procedure in anyone but those at the highest risk. BRCA testing is recommended in those with a high family risk after genetic counseling. It is not recommended routinely. This is because there are many forms of changes in BRCA genes, ranging from harmless polymorphisms to obviously dangerous frameshift mutations. The effect of most of the identifiable changes in the genes is uncertain. Testing in an average-risk person is particularly likely to return one of these indeterminate, useless results. It is unclear if removing the second breast in those who have breast cancer in one is beneficial. Removal of both breasts before any cancer has been diagnosed or any suspicious lump or other lesion has appeared (a procedure known as prophylactic bilateral mastectomy) may be considered in people with BRCA1 and BRCA2 mutations, which are associated with a substantially heightened risk for an eventual diagnosis of breast cancer. Evidence is not strong

enough to support this procedure in anyone but those at the highest risk. BRCA testing is recommended in those with a high family risk after genetic counseling. It is not recommended routinely. This is because there are many forms of changes in BRCA genes, ranging from harmless polymorphisms to obviously dangerous frameshift mutations. The effect of most of the identifiable changes in the genes is uncertain. Testing in an average-risk person is particularly likely to return one of these indeterminate, useless results. It is unclear if removing the second breast in those who have breast cancer in one is beneficial. [48]

## **2.2 Chemotherapy for Breast Cancer**

Chemotherapy (chemo) is treatment with cancer-killing drugs that may be given intravenously (injected into your vein) or by mouth. The drugs travel through the bloodstream to reach cancer cells in most parts of the body. Occasionally, chemo may be given directly into the spinal fluid which surrounds the brain and spinal cord.

Not all women with breast cancer will need chemo, but there are several situations in which chemo may be recommended:

- After surgery (adjuvant chemotherapy): Adjuvant chemo is used to try to kill any cancer cells that might have been left behind or have spread but can't be seen, even on imaging tests. If these cells were allowed to grow, they could form new tumors in other places in the body. Adjuvant chemo can lower the risk of breast cancer coming back.
- Before surgery (neoadjuvant chemotherapy): Neoadjuvant chemo can be used to try to shrink the tumor so it can be removed with less extensive surgery. Because of this, neoadjuvant chemo is often

used to treat cancers that are too big to be removed by surgery at the time of diagnosis (called locally advanced cancers). Also, by giving chemo before the tumor is removed, doctors can better see how the cancer responds to it. If the first set of chemo drugs doesn't shrink the tumor, your doctor will know that other drugs are needed. It should also kill any cancer cells that have spread but can't be seen. Just like adjuvant chemo, neoadjuvant chemo can lower the risk of breast cancer coming back. [49]

The most common drugs used for adjuvant and neoadjuvant chemo include:

- Anthracyclines, such as doxorubicin (Adriamycin) and epirubicin (Ellence)
- Taxanes, such as paclitaxel (Taxol) and docetaxel (Taxotere)
- 5-fluorouracil (5-FU)
- Cyclophosphamide (Cytosan)
- Carboplatin (Paraplatin)

Most often, combinations of 2 or 3 of these drugs are used.

Chemotherapy for advanced breast cancer axanes, such as paclitaxel (Taxol), docetaxel (Taxotere), and albumin-bound Chemo drugs useful in treating women with breast cancer that has spread include Chemotherapy for advanced breast cancer

- paclitaxel (Abraxane)
- Anthracyclines (Doxorubicin, pegylated liposomal doxorubicin, and Epirubicin)
- Platinum agents (cisplatin, carboplatin)
- Vinorelbine (Navelbine)

- Capecitabine (Xeloda)
- Gemcitabine (Gemzar)
- Ixabepilone (Ixempra) Albumin-bound paclitaxel (nab-paclitaxel or Abraxane)
- Eribulin (Halaven)

Chemo drugs for breast cancer are typically given into a vein (IV), either as an injection over a few minutes or as an infusion over a longer period of time. This can be done in a doctor's office, chemotherapy clinic, or in a hospital setting. Doctors give chemo in cycles, with each period of treatment followed by a rest period to give you time to recover from the effects of the drugs. Cycles are most often 2 or 3 weeks long. The schedule varies depending on the drugs used. For example, with some drugs, the chemo is given only on the first day of the cycle. With others, it is given for a few days in a row, or once a week. Then, at the end of the cycle, the chemo schedule repeats to start the next cycle.<sup>[50]</sup>

Adjuvant and neoadjuvant chemo is often given for a total of 3 to 6 months, depending on the drugs used. The length of treatment for advanced breast cancer is based on how well it is working and what side affects you have.<sup>[51]</sup>

### Dose-dense chemotherapy

Doctors have found that giving the cycles of certain chemo drugs closer together can lower the chance that the cancer will come back and improve survival for some women. For example, a drug that would normally be given every 3 weeks might be given every 2 weeks. This can be done for both neoadjuvant and adjuvant treatment. It can lead to more problems with low blood cell counts, so it's not an option for all women.<sup>[52]</sup>

## **side effects of chemo for breast cancer**

Chemo drugs can cause side effects. These depend on the type and dose of drugs given, and the length of treatment. Some of the most common possible side effects include:

Hair loss

Nail changes

Mouth sores

Loss of appetite or weight changes

Nausea and vomiting

Diarrhea

Chemo can also affect the blood-forming cells of the bone marrow, which can lead to:

- Increased chance of infections (from low white blood cell counts)
- Easy bruising or bleeding (from low blood platelet counts)
- Fatigue (from low red blood cell counts and other reasons)

## **2.3 CBC**

The cbc is commonly ordered during any period. It not only provides absolute values, but it can also identify changes in the cellular morphology of the erythrocytes, leukocytes, and thrombocytes.

Generally, the CBC is an easy test to obtain in the nursery from a capillary, venous, or arterial sample. The results can be affected by a variety of factors, however, and normal ranges may differ between laboratories. In view of the many benefits and oddities of the CBC, practitioners must assess the maternal history and the individual neonate's perinatal course, state of health, timing of sample, and sample site to interpret the CBC results so that they can guide clinical intervention [53]

### **2.3.1 Hematopoiesis:**

Blood cell development begins in the earliest weeks of gestation. Cell differentiation appears to begin from a population of progenitor or stem cells located within the yolk sac, liver, and bone marrow of the developing fetus.[54] The microchemical environment of the developing stem cells determines the differentiation of at least two cell lines: the myeloid hematopoietic system and the lymphoid hematopoietic system.

The myeloid hematopoietic cell line leads to the proliferation and differentiation of stem cells into the erythroid, myeloid, and megakaryocyte precursors. The erythrocytes, leukocytes, and thrombocytes develop from these precursors. The lymphoid hematopoietic cell line produces the lymphocytes. Lymphocytes follow one of two independent pathways to produce the cells that will become either Lymphocytes [55]

### **2.3.2 ERYTHROCYTE:**

The hematocrit is the proportion of blood volume that consists of the RBCs. It is expressed as a percentage on the CBC. Hemoglobin in blood is measured in grams per one deciliter of whole blood and is expressed as



g/dL (mmol/L) on the CBC. Erythrocytes, red blood Period begins at approximately two weeks gestation and peaks at approximately six weeks gestation. The RBC count measures the number of circulating erythrocytes. A mature RBC is a nonnucleated, biconcave disc, surrounded by a flexible membrane. Fetal (and neonatal) RBCs differ from adult RBCs in that they are larger in size, have a shorter life span, altered shape and deformability, and they contain a high fetal hemoglobin concentration. RBCs transport oxygen to the organs and tissues; it is the protein, hemoglobin, in erythrocytes that carries oxygen. [56]

Two conditions that can be identified by evaluating the RBC count are anemia and polycythemia. Anemia is a deficiency in the concentration of erythrocytes and hemoglobin in the blood. Neonatal anemia can be caused by acute, chronic, or iatrogenic blood loss; decreased erythrocyte production; increased destruction of erythrocytes, as with hemolysis; or shortened erythrocyte survival. Polycythemia is most commonly defined as a venous hematocrit greater than 65 percent. Because RBC concentration directly impacts blood viscosity, neonates with polycythemia may exhibit symptoms as a result of increased viscosity.[57]

There are other indices that can provide estimate of the average size of the erythrocytes and the average concentration and quantity of hemoglobin in the erythrocytes. These indices can be measured directly or calculated electronically using modern hematology analyzers. They can be useful in further classifying anemia according to the hemoglobin quantity in the RBCs or the size of the RBCs or in identifying the pathologic process causing the anemia. The erythrocyte indices include the MCV, the MCHC , and the MCH. The MCH measures the average size of circulating erythrocytes. It can help to quantify anemia as microcytic (small cells) or macrocytic (large cells). An elevated MCV is seen with hyperviscosity/polycythemia and also in anemia caused by

folate or vitamin B12 deficiency. The MCHC measures the hemoglobin concentration in a given volume of red blood cells. The RBCs can be described as normochromic, hypochromic, or hyperchromic, depending on their color, which is determined by the amount of hemoglobin present in the RBC. The MCH measures the average amount of hemoglobin per RBC in a sample of blood, The MCHC can be used to identify anemia due to an acute or chronic blood loss. Many changes in erythrocyte morphology can be identified using the CBC; a few include anisocytosis (variation in cell size), macrocytosis, microcytosis, schistocytes (fragmented cells), and spherocytes (rounded cells). Anisocytosis can be seen on a peripheral blood smear and may indicate a normal variation in the size of the RBCs. Macrocytosis is a condition of abnormally large-sized mature RBCs and may be used in the classification of anemias. Microcytosis describes RBCs of small size and may be seen with anemias caused by chronic blood loss or an iron deficiency. Schistocytes or fragmented red blood cells are indicative of intravascular hemolysis and can also be seen in cases of disseminated intravascular coagulation (DIC) Spherocytes, or rounded red blood cells, may indicate congenital spherocytosis, a condition in which the red blood cell lacks a protein critical to the cell membrane. Without this protein, red blood cells maintain a rounded rather than spherical shape.<sup>[58]</sup>

### **2.3.3 Leukocytes**

Leukocytes, or WBCs, are the body's main defense against invading organisms. Leukocyte formation begins in the liver at approximately 5 weeks gestation. By approximately 20 weeks gestation, the bone marrow becomes the primary site of leukocyte hematopoiesis. Leukocytes may be classified as granulocytes or agranulocytes, depending on the presence of granules in the cytoplasm (. The three types of granulocytes are the

neutrophils, eosinophils, and basophils. These cells are the most active in defending the body, with the neutrophils having the primary role. Neutrophils are phagocytic cells capable of recognizing, ingesting, and digesting foreign particles; they are generally the first to arrive at the infection site the neutrophil progresses through six stages of development before it reaches a mature state. These stages are the myeloblast, promyelocyte, myelocyte, metamyelocyte, band, and finally the polymorphonuclear neutrophil or segmented mature neutrophil.<sup>[59]</sup>

The release of immature neutrophils from the bone marrow storage pool into the bloodstream is not fully understood. It is thought that certain substances regulate the production and movement of the neutrophils

When mature neutrophils leave the storage pool and move into the bloodstream, approximately half circulate freely in the bloodstream, constituting the circulating pool. The remainder adhere to the vessel walls as the marinating pool. The neutrophils move constantly between the circulating pool and the marinating pool. Neutrophils circulate in the bloodstream for about 6–8 hours before they migrate to the tissues, where they can live for an additional 24 hours. A small number of bands, immature neutrophils, are normally released into the bloodstream with the mature neutrophils. If these circulating cells cannot meet the body's demand and the storage pool is depleted, more bands and other immature cells are released from the storage pool into the bloodstream.

Mature eosinophils have a bi-lobed nucleus with distinctive granules in the cytoplasm have immuno-enhancing and immunosuppressive functions and play a role in selective tumor response, helminthic (parasitic) infections, and allergies. <sup>[60]</sup>

Mature basophils have a bi-lobed nucleus with metachromatic granules in the cytoplasm that contain heparin, histamine, and several other proteins.<sup>16</sup> Basophils mature and differentiate in the bone marrow before

they are released into the circulation. They function in chemotaxis; phagocytosis; granule release of histamine, peroxidase, and heparin; and in factor synthesis. Basophils also participate in hypersensitivity reactions.<sup>[61]</sup>

The two types of agranulocytes are lymphocytes and monocytes. Lymphocytes function in the immune response. There are three types of lymphocytes: B cells, T cells, and the natural killer (NK) cells. Lymphocytes are small, round cells with blue-black nuclei after staining; they are not phagocytes, but are migratory cells <sup>[62]</sup>-

Monocytes are large cells with a horseshoe-shaped nucleus. They are specialized phagocytes that are able to release cellular mediators. They can circulate in the bloodstream for approximately eight hours, after which they migrate to the tissues to become macrophages. They defend against intracellular parasites; remove cellular debris; participate in iron metabolism; present antigens to lymphocytes during an immune response; and secrete various enzymes, factors, and interferons.<sup>[63]</sup>

### **Abnormalities of the WBCs**

Morphologic or degenerative changes that may be seen in granulocytes include vacuoles (visible openings), Dohle bodies (cytoplasmic inclusions), and toxic granulation (larger-than-normal granules). These are nonspecific changes that can be found in approximately 63 percent of neonates with confirmed sepsis. <sup>[64]</sup>

Neutrophilia and neutropenia can be identified using the CBC. In 1979, Manroe and colleagues from the University of Texas Southern Medical School published reference values for blood neutrophil concentrations. This landmark study has been used as a baseline to identify and study neutrophil ranges during the first 60 hours after birth. Neutrophilia is an increase in the number of neutrophils in the bloodstream and can result from inflammation, certain malignancies, or the presence of

corticosteroid drugs. Neutropenia is a decrease in the number of neutrophils in the bloodstream and can result from infection, impaired bone marrow production, or abnormal distribution. Neutropenia is more predictive of neonatal sepsis than is neutrophilia, but it can also be associated with PIH, birth asphyxia, intrauterine growth restriction, Rh hemolytic disease, or periventricular hemorrhage. Neutropenia associated with PIH is a result of diminished production and generally resolves in three to five days.<sup>[65]</sup>

**2.3.4 Eosinophilia** is frequently overlooked because its significance and causative factors are not clearly understood. Eosinophilia may be caused by infection, antibiotics, exposure to antigens in parenteral nutrition, catheters, and blood products; it may also be seen in preterm infants experiencing an anabolic growth period.<sup>[66]</sup>

### **2.3.5 Thrombocytes**

Thrombocytes, or platelets, are produced in the bone marrow by polyploidy cells called megakaryocytes. Megakaryocytes become giant cells and undergo a process of fragmentation that creates approximately 1,000 platelets/megakaryocyte. Platelets are tiny, 1–4 microns in size. They are disc-shaped, noncellular, a nuclear, containing cytoplasmic granules and can survive for approximately nine to ten days in the bloodstream. The major function of platelets is to promote primary hemostasis. During a healthy state, platelets circulate in the bloodstream without adhering to the walls of blood vessels or other cells. When the endothelial lining of the blood vessel becomes injured, platelets are activated. In response to injury, they transform their shape, adhering to and aggregating at the injury site to form a primary hemostatic plug.<sup>[67]</sup>

#### **Platelet Abnormalities**

Thrombocytopenia and thrombocytosis can be identified on a CBC. Thrombocytopenia is a condition of reduced platelets. It is one of the

most common hematologic problems in sick neonates. It can be caused by decreased production or by increased destruction, sequestration, or loss as a result of many conditions. The differential diagnosis includes bacterial and viral sepsis, hypoxia, DIC, necrotizing enterocolitis, persistent pulmonary hypertension of the newborn, erythroblastosis fetalis, polycythemia, congenital infections, congenital anomalies/syndromes, neonatal alloimmune thrombocytopenia, maternal immune thrombocytopenic purpura, and preeclampsia. Depending on the severity of the thrombocytopenia, the symptoms may vary, but can include petechiae; purpura; gastrointestinal, cutaneous, and mucosal bleeding; hematuria; and central nervous system hemorrhage [68]

#### **2.4 ER (Estrogen Receptor), PR (Progesterone Receptor) & HER-2/NEU (Human Epidermal Growth Factor Receptor)**

hormone receptor studies such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2/neu) are routinely done in breast carcinoma. It not only helps in the prognosis of the tumor but also helps in deciding its treatment. The goal of doing this receptor status is to provide right treatment to the right patient. The role of the pathologist is to accurately assess these biomarkers, and the role of the oncologist is to treat the patient with one of the several established therapies, depending on the hormone status.

ER and PR are hormone receptors found on breast cells that pick-up hormone signals resulting in cell growth. Cancer is called ER-positive (ER+) if it has receptors for hormone estrogen which receives the signals from estrogen and promotes its growth, just like normal cells. Similarly, the cancer is PR-positive (PR+) if it has receptors for hormone progesterone. Again, this means that the cancer cells may receive signals from progesterone that could promote their growth. Similarly, HER2/neu positive (HER2/neu+) status of the breast carcinoma mean that

HER2/neu gene is making too many HER2/neu proteins. HER2/neu proteins are receptors on breast cells. Normally, HER2/neu receptors control healthy breast cell growth, division, and repair. However, in about 30% of breast cancers, the HER2/neu gene does not work correctly and makes too many copies of it (known as HER2/neu gene amplification). These extra HER2/neu gene copies tell breast cells to make too many HER2/neu receptors (HER2/neu protein overexpression). This ultimately makes breast cells to grow and divide in an uncontrolled way. Biomarkers can be prognostic, predictive, or both. Prognostic biomarkers measure prognosis independently of other factors. The presence or absence of these biomarkers is directly associated with disease recurrence or mortality. Predictive biomarkers, on the other hand, predict whether or not a patient will respond to a given therapy.<sup>[69]</sup>

The presence of the hormone receptors ER, PR in a patient's breast cancer is an example of a weak prognostic but strong predictive biomarker. If a patient's tumor expresses ER and/or PR, we can predict that this patient will positively benefit from endocrine therapy such as tamoxifen. The overexpression of the oncogene HER2/neu in a patient's

breast cancer is an example of both a prognostic and predictive biomarker. HER2/neu expression is associated with poor prognosis (high risk of recurrence [ROR]); however, it also predicts that a patient will more likely benefit from anthracycline and taxane-based chemotherapies and therapies that target HER2/neu (trastuzumab), but not to endocrine-based therapies.<sup>[70]</sup>

**2.4.1 Immunohistochemical Scoring System for Estrogen Receptor, Progesterone Receptor, and Human Epidermal Growth Factor Receptor 2/Neu Allred system of scoring for estrogen receptor and progesterone receptor**

ER and PR are nuclear receptors. In Allred system of scoring, score 0-5 is given to the cells depending on the proportion of cells which are stained (proportion score [PS]) and score 0-3 is given depending on the intensity of staining (intensity score [IS]). By adding the PS and IS, we can calculate the final Allred score ( $PS + IS = AS$ ) Scoring for human epidermal growth factor receptor

2/neu overexpression HER2/neu is a cell membrane receptor and depending on the intensity of staining a score of 0-3 is given to the cells

If HER2/neu score is 2+ (equivocal), and then the second step should be FISH for confirmation, whereas if HER2/neu score is 1+ or 3+ which is negative and positive, respectively, then FISH is usually not recommended.<sup>[69]</sup>

In Allred scoring system, only the invasive tumor cells should be assessed as ER/PR staining is present in normal breast epithelial cells as well. Here, the normal breast epithelial cells act as internal positive control. Any positive test result, whether just for ER, PR, or both means that the breast cancer is considered “hormone-receptor-positive.”

Hormone therapy helps to slow or stop the growth of hormone receptor-positive breast cancers by lowering the body’s estrogen levels or blocking the effects of estrogen. These medications also may reduce the ROR. Likewise, if HER2/neu receptor is present in large amount on the cancer cells; it confers a more aggressive biologic behavior of the

tumor cell. We can use a very effective targeted therapy against HER2/neu such as Herceptin. Herceptin is a biologic treatment, not a chemotherapy drug; rather an antibody that blocks the HER2/neu receptor on the cancer cells and shuts down the signal from HER2/neu, hence stopping the growth of cancer cells. Besides, Allred scoring system, another scoring system in use for ER and PR is “quick score system,” However, there is no worldwide consensus on the scoring system. A few



other histological scoring systems in use for breast cancers are Nottingham Histologic Score (“Elston Grade”) and Bloom–Richardson Grade.<sup>[71]</sup>

#### **2.4.2 Classification of Breast Cancers Depending on Hormone Receptor Status Estrogen Receptor, Progesterone Receptor, and Human Epidermal Growth Factor Receptor-2/Neu**

Based on hormone receptor status and HER2/neu, breast cancers are divided into four different groups. Luminal type A or estrogen receptor positive, progesterone receptor positive, human epidermal growth factor receptor her2/neu negative These types of cancers, as the name describes, are positive for hormone receptors (ER and PR) and negative for HER2/neu and/or low Ki67 index About 80% of breast cancers are ER+, and about 65% of these are also PR+, which means that they grow in response to estrogen and progesterone, respectively. These tumors are more responsive to hormone therapy than hormone receptor negative tumors. They have an overall tumor grade of 1 or 2. Only about 15% of these tumors have p53 gene-mutations, which have a poor prognosis. Luminal Type B or triple positive or estrogen receptor positive, progesterone receptor positive, human epidermal growth factor receptor 2/neu positive, or human epidermal growth factor receptor 2/neu negative with high Ki-67 breast cancers. This term is used to describe cancers that are ER+, PR+, HER2/neu+. They are referred to as luminal because the tumor cells arise from the inner (luminal) cells lining the mammary ducts <sup>[72]</sup>

These cancers can be treated with hormone therapy as well as drugs that target HER2/neu. Estrogen receptor negative, progesterone receptor negative, human epidermal growth factor receptor 2/neu positive, or

simply human epidermal growth factor receptor 2/neu positive breast cancers. About 20% of breast cancers are HER2/neu+ These cancers are very aggressive and fast growing with a high tumor grade. They do not respond to hormone therapies instead they are treated with antibodies against HER2/neu receptors (Herceptin). Women with HER2/neu+ type tumor may be diagnosed at a younger age than luminal A and B tumors. Basal-like or triple negative phenotype, estrogen receptor negative, progesterone receptor negative, and human epidermal growth factor receptor 2/neu negative breast cancers Almost 10-20% of breast cancers are triple negative.[73]

They are referred to as basal-like because the tumor cells have features similar to those of the outer (basal) cells surrounding the mammary ducts. Most breast cancers associated with BRCA-1 gene are triple negative and most of them contain p53 gene mutations. Although these cancers respond fairly well to chemotherapy but they tend to recur. No perfect therapy has been devised so far, but several promising strategies are being aimed at triple negative breast cancers. [74]

## **2.5PREVIOUS STUDIES**

( 1)

Authers: SILAS UFELLE and et al ... some haematological parameters in pre- and post-surgery breast cancer patients in enugu Nigeria Found the Hemoglobin concentration, Hematocrit, Total White Blood Cell count and Platelet count values were significantly decreased in both pre and post surgery breast cancer patients when compared with the control (P<0.05) .[75]

No(2)

AUTHERS: Priyadarshini Biswal and et al

Correlation of Hormonal Receptors Estrogen Receptor, Progesterone Receptor and Her-2/Neu with Tumor

Characteristics in Breast Carcinoma: Study of 100 Consecutive Cases

Significant correlations observed between hormonal receptor status and the grade of the tumor. Inverse relationship is found between Her-2/neu expression and ER, PR receptor status. Her2/neu expression was increased with size and high grade of tumor.<sup>[76]</sup>

(3)

AUTHERS: PAWAN NIKHRA and etal

Study of ER (Estrogen Receptor), PR (Progesterone Receptor) & HER-2/NEU (Human Epidermal Growth Factor Receptor) expression by immunohistochemistry in breast carcinoma

This study was concluded that with incorporation of Immunohistochemistry-based classification of both ER/PR and HER-2/neu status into the histopathology report using the traditional TNM staging and histological grading of breast carcinoma help in better therapeutic management and increases prognostic accuracy and is inexpensive and readily available.<sup>[77]</sup>

# CHAPTER three

### **3. Material and Methodology**

**3.1: Study Design:** Cross sectional study was conducted in Shendi city in oncology center and researches cancer during period from July to September of 2018 that year.

#### **3.2 Study area:**

This study was conducted in shendi city. which located in in northern Sudan, situated on the east bank of the Nile River 150km northeast of Khartoum. shendi is also about 45km southwest of the ancient city of Marawi located in the River Nile Wilayat, shendi is the center of the jaaliin tribe and an important historic trading center. A major traditional trade route across the Bayuda Desert connects Al-matamma to marawi and Napata. 250 km to the northwest.

#### **3.3 Study population samples:**

Fifty patients were diagnosed are invasive ductal carcinoma breast and was receiving chemo therapy after mastectomy.

#### **3.4Data collection tools:**

Three ml of venous blood samples were collected from all the subjects in to K3 –EDETA anti- coagulant containers for the analysis of Total white blood cell count, hemoglobin concentration, red blood cell count and plate lets count using hematology analyzer SYSMIX

Tissue collection:

The tissues of the test population submitted as MRM (Modified Radical Mastectomy) specimen were evaluated by histopathological processing and examination (HPE). The most suitable tissue block was selected for Immunohistochemical evaluation for ER, PR and HER-2/neu markers.

### **3.5 Tissue processing:**

Tissues were fixed in 10% Buffered formalin overnight, for an average period of 16 hrs. The tissue was grossed and blocks were processed in the histokinette with a cycle of 24 hours, after which the processed tissue was embedded into paraffin wax blocks. The wax blocks were trimmed using the rotary microtome. Sections were taken onto slides and stained by the routine H&E stain. During the HPE reporting, the best section representing the tumor was selected for Immunohistochemistry.

The Peroxidase antiperoxidase (PAP) method of Immunohistochemistry was followed. Biogenex reagents were used for the antigen retrieval and IHC staining process. Biogenex Tris- EDTA based antigen retrieval solution with a pH of 9 was used for ER, PR staining and of pH 6 for HER-2/neu staining. The heating cycles followed in the Biogenex temperature-controlled microwave were two cycles of 10 minutes and 5 minutes each at 95°C, with intermittent refilling of the antigen retrieval solution. Thereafter the slides were taken through the steps of wash with TRIS buffer, peroxide block, power block and monoclonal antibodies. After this, slides were again washed in TRIS buffer, the secondary antibody exhibited; thereafter Diamino benzidine chromogen was added. The slides were washed with water, and counterstained with hematoxylin. Then slides were serially dehydrated in alcohol, cleared in xylene and thereafter mounted using Distrain dibutyl phthalate xylene. After drying, the test slides were examined along with the control sections stained simultaneously.

#### **IHC Markers**

The ER/PR assay measures the amount of estrogen receptors (ER) and progesterone receptors (PR) in cancer cells. Human Epidermal growth Factor-2/neu is a receptor found on the surface of breast cancer cells. It

accepts stimulatory growth signals from a substance called epidermal growth factor. The HER-2/neu assay measures the amount of HER-2/neu on the surface of cancer cells. Allred method of ER/PR scoring system and criteria was used in this study.

### **3.6 Statistical Analysis:**

Analysis was done by using statistical package for social students (SPSS ) using students t-test at 95% confidence level with p- value of  $< 0.05$  as significant

# CHAPTER FOUR



#### 4. RESULTS:

Fifty cases were studied, the age of patients ranged from 30 to 79 years, with mean age group of 50.28. maximum numbers of cases were seen in the age group of (40-49) 30%. Only 2 (4) % occurred in a male remaining 48(96%) cases were females.

The male to female ratio 2: 48

Table (4-1):

Distribution of cases according to gender

	Frequency	Percent%
Male	2	4%
Female	48	96%
Total	50	100%

Table (4- 2) : Distribution of cases according to age

Age	Frequency	Percent%
30-39	11	22%
40-49	15	30%
50-59	9	18%
60-69	10	20%
70-79	5	5%

Table (4- 3) : Mean of TWBCs , Hb , RBCs and Platelet count According to HER2/neu result:.

	Positive	Negative	P. value
TWBCs *10 <sup>9</sup> /L	7346	7414	0.893
HB g/dl	11.8	12.1	0.324
RBCs *10 <sup>12</sup> /L	4.1	4.3	0.247
PLT *10 <sup>9</sup> /L	306.5	338.6	0.206

Table (4-4) : Mean of TWBCs , HB , RBCs and Platelet count according to ER result:

	ERPositive	ERNegative	P.value
TWBCs *10 <sup>9</sup> /L	7656	7087	0.517
HB g/dl	11.1	11.1	0.758
RBCs *10 <sup>12</sup> /L	4.2	4.2	0.998
PLT *10 <sup>9</sup> /L	113	289	0.058

Table (4-5) : Mean of TWBCs , HB , RBCs and platelet count according to PR result .

	Positive PR	Negative PR	P.value
TWBCs *10 <sup>9</sup> /L	7977	1	0.235
HB g/dl	12	13	0.452
RBCs *10 <sup>12</sup> /L	4	4	0.534
PLT *10 <sup>9</sup> /L	334	298	0.138

Table (4-6) : Mean of TWBCs , HB , RBCs and Platelet count according to grade result .

	Grade I	Grade II	Grade III	P.value
TWBCs *10 <sup>9</sup> /L	5750	7820	7350	0.528
HB g/dl	11.9	11.8	12.0	0.647
RBCs *10 <sup>12</sup> /L	4.2	4.1	4.2	0.901
PLT *10 <sup>9</sup> /L	325.5	325	397	4.223

# CHAPTER FIVE

## 5-1: DISSCUSSION

Among fifty patient whom were involved in the study, the mean TWBCs, HB, RBCs and plate let in correlation to her2 positive or negative. No significant correlation was found ( p.value  $>0.05$  ) . this result disagrees with SILAS and et al showed that total white blood cell count and platelet count of both pre- and post-surgery breast cancer patients (52), were also decreased significantly when compared with the controls probably due to the ongoing chemotherapy. This study revealed that the hematological parameters studied were decreased among breast cancer patients in Enugu, hence the need for supplementation before and after surgery in breast cancer patients to avoid possible predisposition of the said patients to hematological disturbances such as anemia, leucopenia and thrombocytopenia.

The main of TWBCs, Hb, RBCs and platelet in correlation to ER positive or negative show un significant correlation with p.value more than (0.05).

The main of TWBCs, HB, RBCs and Platelet in correlation to BR positive or negative show un significant correlation with p.value more than(0.05)

The main of TWBCs, HB , RBCs and platelet in correlation to grade positive or negative show un significant correlation with p.value more than (0.05) . This result disagree with Priyadarshini Biswal and et al(December 2015)76, found Significant inverse association between hormonal receptor and histology grade . Greater percentage of grade II tumors had ER, PR positivity as compared to grade III tumors.

## **5-2: CONCLUSION**

This study concludes that no correlation has been described between hematological parameters and tumor marker in breast cancer patients.

### **5-3: RECOMMENDATIONS**

- 1- More research studies about topics in Sudan because breast cancer studies in Sudan are few.
- 2- Early detection in breast cancer can reduce prevalence rate at 90%
- 3- Governmental programmed should focus on identification and assessment of risk factors in order to improve cancer prevention.
- 4- Women self-examination and mammography in pre monopause-s\_phase can decrease death rate

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