Chapter one

1. Introduction

1.1 Introduction:

Nearly about 796,000 new cases of breast cancer were diagnosed all over the world, and about 314,000 deaths due to breast cancer (Sharma, *et al*. 2016). Although breast cancer is thought to be a disease of the developed world, almost 50% of breast cancer cases and nearly 60% of deaths occur in lower income countries (Globocan, 2012). There is a large variation in breast cancer survival rates around the world, with an estimated 5- year survival of 80% in high income countries to below 40% for low income countries (WHO, 2017). Low and middle income countries face resource and infrastructure constraints that challenge the goal of improving breast cancer outcomes by early detection, diagnosis and treatment (WHO, 2017).

It is found in high rates in developing countries as well as low middle income countries. 1 in 9 women is diagnosed with breast cancer worldwide (Elgaili *et al*., 2010). In Sudanese hospitals in 2000, cancer was the third leading cause of death after malaria and viral pneumonia, accounting for 5% of all deaths (Hussein, 2006) In Sudan it is more common at a young age contrary to the West where it is more common in old age (after 60 years) (Mahmood *et al*., 2006).

Studies on breast cancer in Sudan have been limited. The reasons for this include the lack of population-based cancer registry as well as lack of research resources (manpower and financial) (Elgaili, *et al*. 2010).

Methods of breast cancer diagnosis include mammography, magnetic resonance imaging (MRI), molecular breast imaging (MBI), breast biopsy,

immunohistochemistry (IHC), Fluorescence in situ hybridization test (FISH), blood based assay (Bakhet,2016).

E-Cadherin (EC) is a calcium-regulated adhesion molecule expressed in most normal epithelial tissues (Takeichi,1990). The EC gene is located on chromosome 16q22.1 (Berx, *et al.*1995). EC knockouts have been associated with nonviability and abnormal epithelial morphogenesis. Selective loss of EC can cause dedifferentiation and invasiveness in human carcinomas. In various cell lines, a reciprocal relationship has been shown between levels of EC expression and invasiveness (Qureshi, *et al.*2006).

Ki67 antigen is a nuclear protein expressed in proliferating cells throughout all phases of the cell cycle, and is a marker of cell proliferation (Kos and Dabbs, 2016). Ki-67 is one of the proliferative markers strongly linked to evaluate cell cycle. It was found to be universally expressed by proliferating cells (G0 and, S, G2, M) and absent in quiescent cells or G0 phase , making it ripe for evaluation as a tumor proliferation biomarker. It represents easy and reliable method of assessing the cell cycle pathways particularly in breast cancer. In addition to the predictive value of Ki-67 , the prognostic significance of this protein in breast cancer of the Western women has been reported. Moreover, the proliferation has a major impact on calculating the risk of recurrence (Nday, *et al.*2018).

The reports of previous studies showed that the expression of ki 67 and E.cadherin have importance role in prognosis of breast cancer.

1.2 Objectives

General objective:

To assess the expression of ki 67 and E.cadherin in breast cancer in Sudanese patients.

Specific objectives :

To determine ki 67 and E.cadherin results in breast cancer using immunohistochemistry technique.

To correlate between ki67 and E.cadherin expression in triple negative and non triple negative of Breasr cancer .

To correlate between ki67 and E.cadherin expression and cancer grade.

Chapter tow

2 . Literature review

2.1 Histology of the breast:

Each breast consists of 15 to 25 independent units called breast lobes, each consisting of a compound tubulo-acinar gland. The size of the lobes is quite variable and the bulk of the breast is made up of a few large lobes that connect to the surface. Immediately before opening onto the surface, the duct forms a dilatation called the lactiferous sinus. Smaller lobes end in blind ending ducts that do not reach the nipple surface. The lobes are embedded in a mass of adipose tissue, subdivided by collagenous septa (Woodford, *et al.*2014).

2.2 Breast cancer:

Breast cancer is a malignant tumor that starts in the cells of the breast. A malignant tumor is a group of cancer cells that can grow into surrounding tissues or spread (metastasize) to distant areas of the body. The disease occurs almost entirely women, but men can get it, too (American Cancer Society, 2016).

There are several types of breast cancer, but some of them are quite rare Currently, majority of all breast cancers worldwide are the ductal and lobular subtypes .However, the ductal subtype accounting acounts for the majority of the diagnosed cases ,constituting for about 40–75% (Rakha, *et al*.2006). In addition, several linear models of breast cancer initiation, transformation and progression, as depicted in Fig. 1.5 have been formulated. There are two models for the ductal subtype The first 'ductal' model, reported by Lerwill (Lerwill, 2008) are as follow: First of all, it recognizes flat epithelial atypia (FEA), to atypical ductal hyperplasia (ADH) and then ductal carcinoma *in situ* (DCIS) as the non-obligate precursors of the advanced invasive and metastatic ductal carcinoma. In the second model, usual epithelial

ductal hyperplasia (UDH) was proposed as an intermediate stage of progression between FEA and DCIS (Page *et al.,* 1985). In the case of lobular subtype, atypical lobular hyperplasia (ALH) and lobular carcinoma *in situ* (LCIS) was also proposed as the non- obligate precursor lesions to invasive lobular carcinoma (Boecker, *et al*. 2002).

2.2.1 Classification of breast cancer

Breast cancer can be classified using histological findings (morphology) and intrinsic genetic characteristics (molecular) using gene expression profiling and immunohistochemical markers for hormone receptors and HER2 receptor as surrogates. Molecular classification of breast cancer is very useful in therapeutic decision making, prognostication, assessing survival rates and clinical outcome of patient managed for breast cancer. It is also useful for selection of targeted treatment (patient-tailored treatment strategies) and prediction of response to therapy, hence almost all current studies in breast cancer is concentrated more on molecular classification rather than morphological classification (Shawarby, *et al*. 2013).

a classification system based on gene expression analysis was proposed by Perou, et al(2000). in this system of classification, breast cancer consist of four major molecular classes. These are luminal-like, normal-like and HER2 positive subtype (non triple negative), basal like (triple negative). immunohistochemical markers (oestrogen Receptor(ER), Progesterone Receptor(PR), Human Epidermal factor receptor 2 (HER2) are used in this classification (Perou, et al. 2000).

2.3 Risk factors of breast cancer:

2.3.1 Age:

The risk of developing breast cancer increases with age. The probability of a woman in the United States developing breast cancer in a lifetime is 1 in 8: 1 in 202 from birth to age 39 years of age, 1 in 26 from 40-59 years, and 1 in 28 from 60-69 years

(Siegel et al., 2013). In the UK in 2012-2014, on average each year almost half (48%) of cases were diagnosed in people aged 65 and over (Cancer Research UK, 2014).

2.3.2 Genetic and familial factors:

A family history of breast cancer in a first-degree relative is the most widely recognized breast cancer risk factor, but only 5-10% of women diagnosed with breast cancer have a known genetic predisposition. Women with a family history of breast cancer in a mother or sister have a 1.5-3 fold increase in the risk of developing breast cancer. Even in the absence of a known genetic risk factor, the presence of a family history may suggest the presence of an unknown genetic risk, or a shared environmental risk. A family history of ovarian cancer in a first-degree relative, especially if the disease occurred at an early age (<50 y), has been associated with an increased risk of breast cancer risk (Salehi, *et al.* 2008).

2.3.3 Hormone replacement therapy:

Hormone replacement therapy (HRT) is used to increase levels of estrogens around menopause when women naturally have lower endogenous estrogen content. HRT enhances risk of breast cancer among women who use it for at least 5 yr, with the risk rising by about 2.3% per year of use (Collaborative Group on Hormonal Factors in Breast, 1997). Breast cancer risk varies by type of HRT, and was reported to be considerably higher among those using estrogen–progestin combinations compared to estrogen alone (Salehi, *et al.*2008).

2.3.4 Oral contraceptive (OC) hormones:

Compared to never-users of OC, breast cancer risk is 24% higher among current users, and 16% higher among women who ceased use within the past 10 yr. However, breast cancer risk returns to normal 10 yr or more after cessation of OC use (Salehi, *et al.* 2008).

2.3.5 Height:

A pooled analysis of seven cohort studies showed positive associations between height and breast cancer risk among postmenopausal women (Salehi, *et al.* 2008). The association observed may be related to energy intake and nutritional status in childhood and adolescence and hormone profile during puberty (Salehi, *et al.* 2008)

2.3.6 Lifestyle factors:

2.3.6.1 Alcohol consumption:

A study by Chen et al found that low levels of alcohol consumption were associated with a small increase in breast cancer risk; cumulative alcohol intake throughout adult life was the most consistent measure (Chen, *et al.*2011). Alcohol intake occurred early and late in adult life was independently associated with risk (Chen, *et al*. 2011). The mechanism, though unclear, likely is mediated via increasing estrogen levels.

2.3.6.2 Physical activity:

Consistent physical activity has been shown to reduce the risk of breast cancer in a dose dependent manner, with modest activity conferring a 2% decrease in risk and vigorous activity a 5% decrease in risk (Shah, *et al*. 2014).

2.3.4.3 Tobacco smoking:

Tobacco abuse portends a 24% higher risk of developing invasive breast cancer. Former smokers carry a 13% increased risk. Starting smoking at an earlier age has a profound impact. Compared with never smoking, beginning tobacco use prior to menarche increases breast cancer risk by 61%, and beginning tobacco use 11 or more years prior to parity carries a 45% increased risk(Gaudet, *et al*. 2013).

2.3.6.4 Obesity:

Obesity, specifically in postmenopausal women, has also been shown to increase a woman‟s risk of breast cancer. Postmenopausal women who did

not use HRT had elevated breast cancer risk with increasing weight, body mass index (BMI) and hip circumference. Studies show there is a relative risk of 1.28 for overweight women (BMI 25.0-29.9) and obese women (BMI > 30.0) compared to women in the normal weight range (Shah, et al. 2014).

2.3.7 Irradiation:

Radiation, particularly to the chest or in the first decade of life, profoundly increases the risk of developing breast cancer. Radiation to the chest wall for treatment of childhood cancer increases the risk of breast cancer linearly with chest radiation dose. Survivors of childhood cancers who received therapeutic radiation are at a dose dependent risk for the development of breast cancer, and those treated for Hodgkin‟s disease are at highest risk (RR = 7)(Shah, *et al.* 2014).

2.4 Diagnosis of breast cancer:

2.4.1 Mammography:

A mammogram is an X-ray picture of the breast. Digital mammography has replaced conventional (film screen) mammography in some breast screening services. Potential advantages of DM include the use of computer-aided detection, algorithm-based computer programs that alert the radiologist to possible abnormalities on the mammogram and allowing centralized film reading. Mammography frequent use, however, warrants diligent analysis of potential radiation risk. Moreover, false-positive calls lead to additional imaging or histopathological assessment, mainly percutaneous breast biopsy (Nounou, *et al.* 2015).

2.4.2 Magnetic resonance imaging (MRI):

MRI is a powerful imaging tool that produces high-resolution images without requiring the application of harmful radiation. This technique is similar to nuclear

magnetic resonance where a proton density image of the tissue is studied to generate an MRI image. MRI of breast depends on the enhancement of lesions after intravenous injection of contrast agent. The neovascularization of the tumor tissues is characterized by high permeability and thus the contrast material extravasates in the tumor tissue (Nounou, *et al.*2015).

2.4.3 Molecular breast imaging (MBI):

MBI uses a radioactive tracer that lights up cancer tissues of the breast, visualized by a nuclear medicine scanner. MBI has comparable sensitivity to MRI and rather a higher specificity that can detect small breast lesions (Nounou, *et al.*2015).

2.4.4 Ultrasound:

There are several studies supporting the use of adjunctive screening ultrasound in high risk patients with dense breast tissue, which imparts substantial but accepted number of false positives (Nounou, *et al*. 2015).

2.4.5 Breast biopsy:

The only definitive method for diagnosing breast cancer is with a breast biopsy. There are several different types of breast biopsies. To increase diagnostic accuracy and eliminate as many false negative results as possible, clinical breast examination, breast imaging, and biopsy are performed simultaneously (triple test). Two types of needle biopsies are used to diagnose breast cancer: fine needle aspiration cytology (FNAC) and core needle biopsy (CNB) (Nounou, *et al.* 2015).

2.4.6 Immunohistochemistry (IHC):

IHC is a technique that uses antibodies as a tool to detect protein expression. 40 Monoclonal or polyclonal antibodies complementary to the antigen of interest are labeled with a marker (either visible by light microscopy or fluorescence), allowing detection of the antibodies bound to regions of protein expression in a tissue sample. Diagnostic IHC is widely used, for example, to detect tissue markers associated with specific cancer (Nounou, et al. 2015).

2.4.7 Fluorescence in-situ hybridization (FISH):

FISH is a technique used to identify the presence of specific chromosomes or chromosomal regions through hybridization (attachment) of fluorescently labeled DNA probes to denatured chromosomal DNA. Examination under fluorescent lighting detects the presence of the hybridized fluorescent signal (and hence presence of the chromosome material) (Nounou, *et al.*2015).

2.5 Molecular biomarkers in breast cancer:

2.5.1 Estrogen receptor alpha (ER-α):

The estrogen receptors comprise of two isoforms, encoded by different genes. The ER-α is a high affinity estrogen receptor that is responsible for the estrogen-related cellular effects in breast cancer while ER-β, which is expressed in more tissues and for which the data are limited, appears to exert an antiproliferative function in the breast. I will be using ER to denote ER-α from now onwards in this paper.

ER expression is identified in approximately 75-80% of breast cancers (Kos and Dabbs, 2016). ER expression is the major predictive marker for response to endocrine therapies which significantly have altered the natural history of breast cancer. Adjuvant endocrine therapy is the standard of care for all women diagnosed with hormone-positive breast cancer as it has been shown to reduce breast cancer–specific mortality and the risk of recurrence and of contralateral breast cancer and to increase overall survival (OS)(Burstein, et al. 2014). In ER-positive tumors, primary or acquired resistance may occur and is more often the result of ER loss, although non-genomic mechanisms such as activation of membrane bound, receptor tyrosine kinase (RTK) signaling pathways, including the AKT and MAPK kinase pathways are also involved (Giuliano, *et al.* 2011).

2.5.2 Progesterone receptor:

Progesterone Receptor **(**PR) expression is regulated by the genomic transcriptional activity of ER; thus, PR positivity is usually observed in ER-positive tumors. In ER-positive tumors the status of PR expression appears of lesser importance; however, in ER-positive/PR-negative tumors there is a 28% higher relative risk for recurrence compared to the ER-positive/PRpositive tumors. Thus, PR appears to have an intrinsic positive effect on prognosis (Prat, *et al.* 2013). The ER-negative and PR-positive breast carcinomas are rare; they comprise approximately 1-3% of the tumors and are regarded as being either falsely negative for ER or falsely positive for PR and the recommendation is to repeat the ER examination on another tissue sample to exclude a false-negative ER result that would preclude the patient from the endocrine treatment 16 benefit (Patani, *et al*. 2013). Early reports have described a modest benefit from tamoxifen or aromatase inhibitors in ER-negative/PR-positive tumors; recent studies, however, failed to confirm this (Patani, *et al.* 2013). Despite the questions on the predictive role of PR, its modest prognostic effect still justifies its evaluation.

2.5.3 Human epidermal growth factor receptor 2:

Human Epidermal Growth Factor Receptor 2 (HER2), also known as ERBB2 and HER2/neu, is a transmembrane receptor with tyrosine kinase function (RTK) and a member of the HER family, also including HER1 (also known as EGFR), HER3 and HER4. HER2 is an orphan receptor. Ligand binding on the extracellular portion of the other family member receptors, results in formation of homodimers or heterodimers among the four members of the family, transphosphorylation of the intracellular domains of the receptors, enhancement of the kinase activity, recruitment

of signaling effector proteins and activation of intracellular signaling pathways. The type of ligand, the heterodimers formed and the autophosphorylation pattern, all influence the signaling effector to be recruited, and the intracellular pathway activated. HER2 has the most potent kinase activity, activates the PI3K/Akt and MAPK pathways, and induces cell proliferation and survival, while disrupting epithelial cell organization, polarity and adhesion, thereby facilitating the formation of metastases (Rexer and Arteaga, 2012).

2.5.4 Ki67 antigen:

The Ki67 antigen is a nuclear protein expressed in proliferating cells throughout all phases of the cell cycle, and is a marker of cell proliferation, Ki67 levels could discriminate within ER positive tumors, those with poor recurrence-free and disease specific survival. This formed the basis for the St Gallen panel of experts recommendation to use the Ki67 to guide treatment decisions in early breast carcinoma, and to define luminal A versus luminal B immunohistochemical surrogate subtypes (Kos and Dabbs, 2016).

A predictive role of Ki67 has also been proposed; addition of chemotherapeutic agents in the adjuvant treatment of ER-positive, high Ki67-expressing tumors have shown benefit in terms of survival, although these data require further evaluation. Furthermore, Ki67 levels assessed as a pharmacodynamic intermediate end-point during neoadjuvant endocrine therapy have been used to discriminate the sub-optimally responding patients, who need to switch to neoadjuvant chemotherapy. Low levels of Ki67 at diagnosis indicate tumors unlikely to benefit from neoadjuvant endocrine treatment, and have been used as an exclusion criterion for such treatment (Kos and Dabbs, 2016).

Agboola et al. (2013) they reported no association between tumor phenotype and ki 67.

2.5.5 E-cadherin:

E-Cadherin is a single-span transmembrane glycoprotein that establishes homophilic interactions with adjacent E-cadherin molecules expressed by neighboring cells, thereby forming the core of the epithelial adherens junction. In its cytoplasmic domain, E-cadherin associates with a number of proteins, including three catenins α, β, and p120), which link E-cadherin to the actin cytoskeleton (Onder, *et al.*2008).

Loss or reduction of E-cadherin expression can be caused by somatic mutations, chromosomal deletions, proteolytic cleavage, and silencing of the *CDH1* promoter (Wildenberg, *et al.*2006). E-cadherin loss ostensibly promotes metastasis by enabling the first step of the metastatic cascade: the disaggregation of cancer cells from one another. However, it has been unclear whether E-cadherin loss also supports the successful completion of additional steps of the invasion-metastasis cascade (Perez and Fuchs ,2006).

Gamallo, C., *et al.* (1993), reported that the frequency of tumors with reduced E-cadherin expression was significantly higher ($P = 0.0233$) in histological grade 2 and 3 breast carcinomas than in grade 1 tumors. No correlation was observed with nuclear atypia, hormonal receptor levels (estrogen and progesterone), lymph node status, or tumor size.

Shiozaki, *et al.* (1991), have reported that nine of 20 cases (45%) displayed reduced E-cadherin expression in infiltrating ductal carcinomas.

Chapter three

3. Materials and Methods

3.1 Study design :

This is a descriptive hospital based retrospective study aimed to study the expression of ki67 and E.cadherin in breast cancer in Sudanese patients using immunohistochemical technique.

3.2 Study area:

This study was carried out in the department of histopathology, Radiation and isotopes center - Khartoum (RICK). The study conducted during period from February to October 2018. Patient identification data and other information were obtained from patient's file.

3.3 The sample:

Eighty cases with relevant histopathologic and clinical information sampled from February to October (2018) breast cancer results were studied. were selected and classified into two categories, 40(50%) triple negative and 40(50%) non triple negative breast cancer, all cases previously diagnosed as follow: 66 (82.5%) are invasive ductal carcinoma ,7 (8.8%) were invasive lobular carcinoma, 2 (2.2%) were invasive mammary carcinoma, 2 (2.5%) were metaplastic carcinoma, 2 (2.5%) were papillary carcinoma and1 (1.2%) was mucinous carcinoma.

3.4 Immunohistochemical staining:

The immunohistochemical procedure was done as follows: Following deparaffinization in xylene, TMA slides were rehydrated through a graded series of alcohol and were placed in distilled water. TMAs were steamed for antigen retrieval for E-cadherin and ki 67 using high PH (9) by water bath at 95C for 40 min. After washing with PBS for 3 min Endogenous peroxides activity were

blocked with 3% hydrogen peroxide and methanol for 10 min, and after washing with PBS for 3 min, then each TMA slide were treated separately with 100 µ L) of (mouse monoclonal antibody against E-cadherin, Dako), and 100 µ L) of mouse monoclonal antibody against Vimentin, Dako) 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 dextran labeled polymer (Dako). Finally, the sections washed in three changes of PBS, followed by adding 3, 3 diaminobenzidinetetrahydrochloride (14) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. After washing with distal water for 3 min Slides were counterstained with haematoxylin Mayer's) for one minute and washed in running tap water for several minutes 7-10 (bluing), then dehydrated, cleaned, and mounted in DBX.

3.5 Statistical analysis:

Data were analyze using version 11.5 SPSS computer program. Frequencies , means , and chi –square test values were calculated.

3.6 Ethical considerations:

Hospital administration agreement were taken ethically for medical records and patients data collection.

Chapter four

4. Results

A total of 80 cases had complete data concerning Ki67 and E.cadherin immunostaining. These cases are classified in to 40 (50%) were triple negative and 40(50%) were non triple negative as showed in table (4.1).

The age of study population rang between 20 and 80 with mean age 48 years most patients were more than 40 years representing 57 (71.2%) and the remaining 23 (28.8%) were less than 40 years as indicate in table (4.2).

The histological diagnosis of study population includes 66 (82.5%) were invasive ductal carcinoma , 7 (8.8%) were invasive lobular carcinoma, 2 (2.2%) were invasive mammary carcinoma, 2 (2.5%) were metaplastic carcinoma, 2 (2.5%) were papillary carcinoma and1 (1.2%) was mucinous carcinoma as showed in table (4.3).
The tumor grade of study sample revealed 4 (5.0%) were grade I , 30 (37.5%) were grade II , 47 (57.5%) were grade III as showed in table (4.4).
Table (4.5) showed ki67 index were more than 20 representing 48 (60%) and the remaining 32 (40%) were less/equal 20.
E.cadherin was positive in 68 (80%) of cases and negative in 12 (20%) of other as showed in table (4.6).
Triple negative cases showed ki67 index more than 20 in 29 (36.2%) of them and ki67 less/ equal 20 in 11 (13.8%) while non triple negative showed ki67 more than 20 in 19 (23.8%) of cases and the remaining 21(26.2%) were ki67 less/ equal 20 , these is association between ki67 index and tumor phenotype (P. value = 0.02) indicate in table (4.7).
Ki67 less / equal 20 was found in 2(2.5%) cases of grade I ,16(14.0%) cases of grade II , 14(17.5%) cases of grade III, while ki67 more than 20 revealed in 2(2.5%) cases

of grade I , 14(17.5%) cases of grade II , 32(40%) cases of grade III (P.value = 0.1) , no association between ki67 index and tumor grade as showed in table (4.8).

E.cadherin positive expression was found in 36 (45%) cases of triple negative breast cancer , 32(40%) cases of non triple negative breast cancer , while negative expression in 4(5%) cases of triple negative,8(10%) cases of non triple negative (P.value =0.2). No association between E.cadherin and tumor phenotype as showed in table (4.9).

E.cadherin positive expression was found in 3 (3.8%) cases of grade I , 23 (28.8%) cases of grade II , 42 (52.5%) cases of grade III , while E.cadherin negative expression was found in 1(1.2%) case of grade I , 4(5.0%) cases of grade II , 32(40%) cases of grade III (P.value = 0.1) , no association between E.cadherin and tumor grade as showed in table (4.10).

Table(4.1) : Distribution of tumor types among study population :

Tumor types	Frequency	Percent
triple negative	40	50.0%
non triple negative	40	50.0%
Total	80	100.0%

Table(4.2) : Distribution of age group among study population :

Age group	Frequency	Percent
less / equal 40	23	28.8%
more than 40	57	71.2%
Total	80	100.0%

Table(4.3) : Distribution of histopathological diagnosis among study population:

histopathological diagnosis	Frequency	Percent
invasive ductal carcinoma	66	82.5%
invasive mammary carcinoma	2	2.5%
mucinous carcinoma	1	1.2%
papillary carcinoma	2	2.5%
metaplastic carcinoma	2	2.5%
invasive lobular carcinoma	7	8.8%
Total	80	100.0%

Table(4.4) : Distribution of tumor grade among study population:

Tumor grade	Frequency	Percent
grade I	4	5.0%
grade II	30	37.5%
grade III	46	57.5%
Total	80	100.0%

Table(4.5) :frequency of ki67 index among study population:

ki67 index	Frequency	Percent
less / equal 20	32	40.0%
more than 20	48	60.0%
Total	80	100.0%

Table(4.6) :Frequency of E.cadherin expression among study population:

E.cadherin	Frequency	Percent
+ ve	68	85.0%
- ve	12	15.0%
Total	80	100.0%

Table (4.7) : Relation between ki67 index and breast cancer phenotypes :

Ki67 index	Tumor types (phenotype)		Total	P.value
	triple negative	non triple negative		
less / equal 20	11 13.8%	21 26.2%	32 40.0%	.02
more than 20	29 36.2%	19 23.8%	48 60.0%	
Total	40 50.0%	40 50.0%	80 100.0%	

Table(4.8) :Relation between ki67 index and tumor grade of breast cancer:

Ki67	Tumar grade			Total	P.value
	grade I	grade II	grade III		
less / equal 20	2 2.5%	16 20.0%	14 17.5%	32 40.0%	0.1
more than 20	2 2.5%	14 17.5%	32 40.0%	48 60.0%	
Total	4 5.0%	30 37.5%	46 57.5%	80 100.0 %	

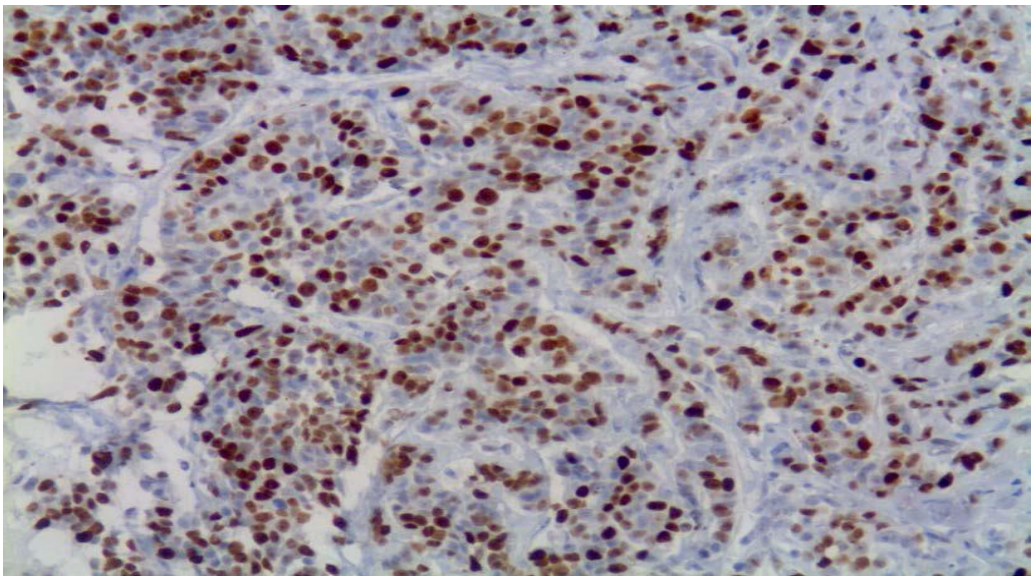
Table (4.9) : Relation between E.cadherin expression and breast cancer phenotypes :

E.cadhri-n	Tumor phenotype		Total	P.value
	triple negative	non triple negative		
+ ve	36 45.0%	32 40.0%	68 85.0%	0.2
- ve	4 5.0%	8 10.0%	12 15.0%	
Total	40 50.0%	40 50.0%	80 100.0%	

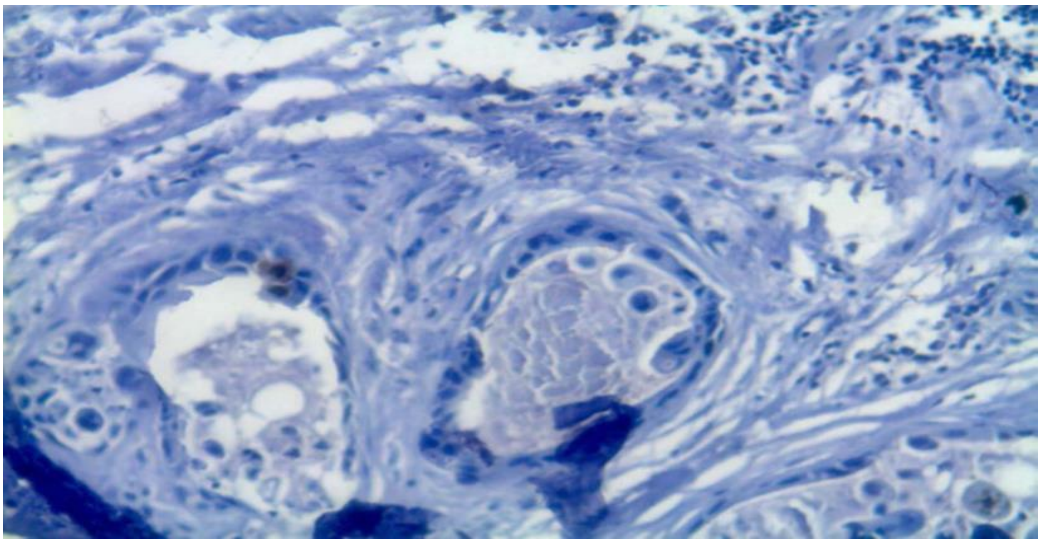
Table (4.10) : Relation between E.cadherin expression and tumor grade of breast cancer:

E.cad-herin	Tumar grade				P.value
	grade I	grade II	grade III	Total	
+ ve	3 3.8%	23 28.8%	42 52.5%	68 85.0%	0.1
- ve	1 1.2%	7 8.8%	4 5.0%	12 15.0%	
Total	4 5.0%	30 37.5%	46 57.5%	80 100.0%	

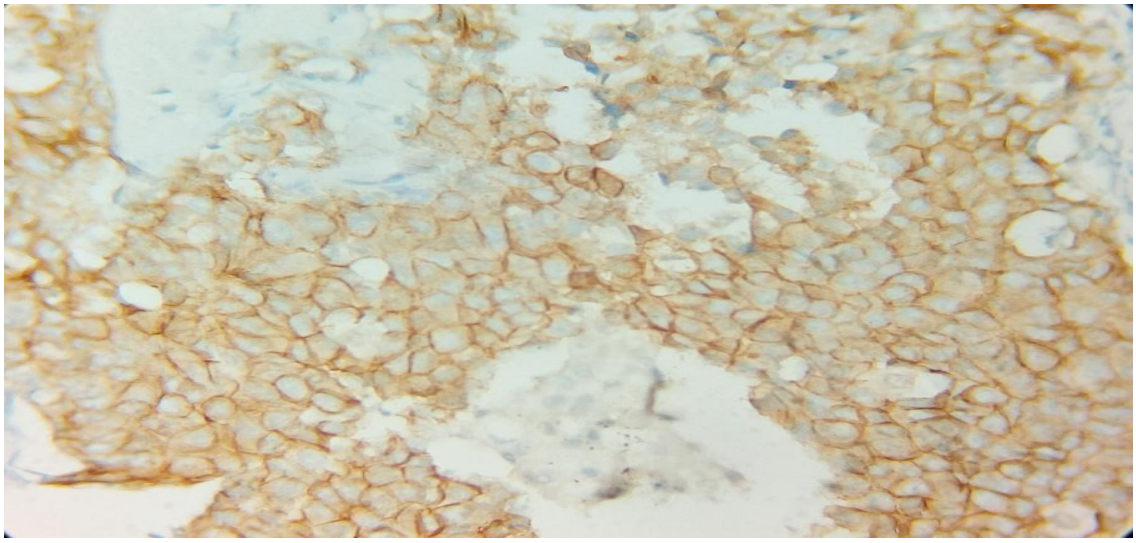
Microphotograph (4.1): Tumor shows strong expression of ki 67:



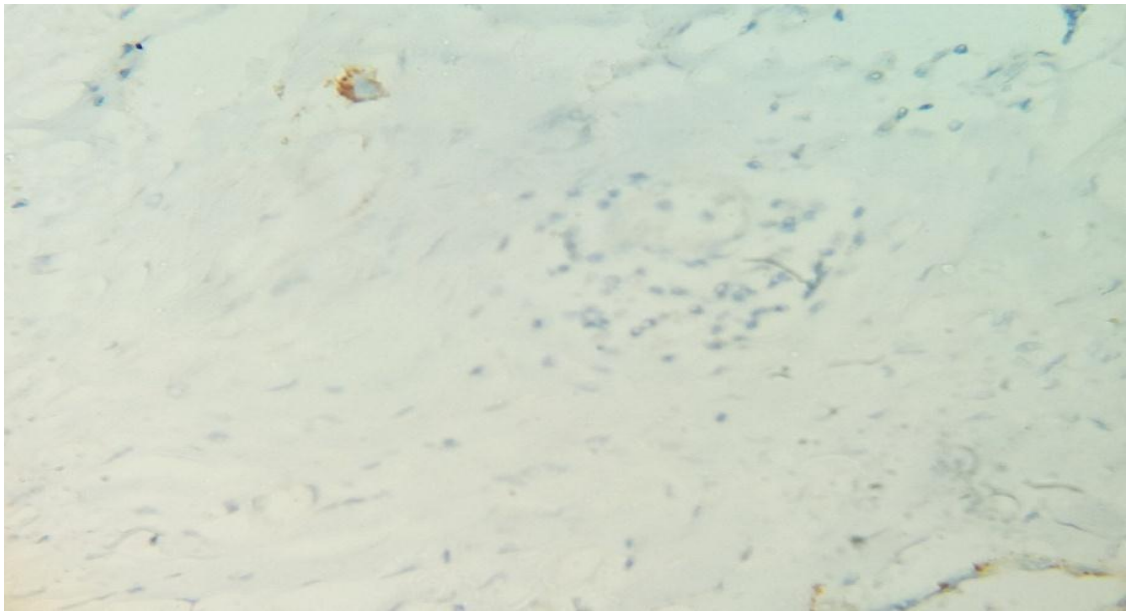
Microphotograph (4.2): Tumor shows low expression of ki 67:



Microphotograph (4.3): Tumor shows strong positive expression of E-cadherin:



Microphotograph (4.4): Tumor shows negative expression of E-cadherin:



Chapter Five

5. Discussion, Conclusion and Recommendation

5.1 Discussion:

The present study focused on detection of expression of Ki 67 and E-cadherin, and correlating their expression with various diagnostic parameters of breast cancer. It involved 80 cases with breast cancer stained by immunohistochemistry.

Regarding the age group of the study population, the study revealed that majority of patients was more than 40 years (80%), indicating that older women are more susceptible to breast cancer than younger women. This result agrees with Hemalatha et al.(2013), they reported that age is an important factor in occurrence of carcinoma, breast with carcinoma rarely occurring in young. Also compatible with Bakhet et al.*(*2016), they reported that risk of developing breast cancer increases with age.

Regarding the histopathological diagnosis of the study cases, the study revealed that the majority of diagnosed samples were invasive ductal carcinoma 40/56 (71.4%), and this finding agrees with Domagala *et al,(*1990), they reported that 214/262 (81.7%) cases of breast cancer is invasive ductal carcinoma, also agrees with Bakhet *et al.(*2016), they reported that most frequent type is invasive ductal carcinoma.

Regarding the histological grade of the study cases, the study revealed that, most frequent grade is grade III 46(80%), indicating that delay in diagnosis lead to delay in the treatment. This result is compatible with Bakhet *et al,(*2016), they reported that grade III were more frequent malignant tumor grade, and this associates with poor prognosis. But it's

not compatible with Hemalatha *et al.(*2013), they reported that (22/50) cases were of grade I.

The expression of Ki-67 is conventionally detected by Immunohistochemistry (IHC) in order to evaluate cell proliferation in tissue. The study investigated the immunohistochemical expression of Ki67 proteins in breast cancer. The study reveal that that 48 cases (60%) had high Ki67 protein (ki67 more than 20) expression this result is different to Guth et al. (2017) they reported that high Ki67 in 45% of cases and compatible with Agboola et al., (2013) they reported that high Ki67 in 82.6% of cases. This result showed significant relation between ki67 index and tumor phenotype (triple negative and non triple negative) of breast cancer (P.value= .02) this is agree with Elkablawy et al. (2016) they reported this observation and disagree with Agboola et al. (2013) they reported no association between tumor phenotype and ki 67. In this study there is no association of ki67 and tumor grade (P.value = 0.1) this is compatible with Inwald et al., (2013) thery reported no association between them and incompatible with Awadelkarfim et al. (2012) they reported grade 3 tumor, ki-67 is positive in 78% of cases.

The present study found that, the expression of E-cadherin was positive in 68 cases (85%), Negative expressed in 12 cases (15%) ,There is no significant correlation between the tumor type and grade of the study cases and E-cadherin expression (*p*-value = 0.1), this result may be due to the limited number and types of the study cases, suggesting that use of all types of breast cancer with large sample size may reveal the correlation. The result of current study disagrees with Qureshi et al. (2006), they

reported that E-cadherin expression correlates with histological type in breast carcinomas.

Conclusion and recommendations

5.2 Conclusion:

From this study we conclude that:

- Invasive ductal carcinoma is the commonest histologic subtype.
- There is significant relation between ki67 index and tumor phenotype (triple negative and non triple negative) and no significant between ki67 index and tumor grade of breast cancer.
- There is no significant association between E.cadherin and tumor phenotype and also tumor grade of breast cancer.

5.3 Recommendation:

On the base of this study , we recommend that :

Quantitative immunohistochemical methods should be done to determine

the degree of E.cadherin expression .

Quantitative immunohistochemical methods should be done to determine

the degree of ki 67 expression .

Application of markers in the diagnosis.

Future research should include larger samples of women to be able to confirm our

finding in large patient cohorts and include women from all regions to address different ethnic backgrounds.

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Appendices

Appendix 1:

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

- Disposable gloves
- Microtome knife
- Positively charged slides (Thermo)
- Cover glass
- Dry oven
- Water bath
- Embedding center
- Coplin jar
- Humidity chamber
- Ethanol (100%, 90%, 70%, 50%)
- Mayer's haematoxylin (haematoxylin , D.W, K or ammonium alum ,sodium iodated ,citric acid, chloral hydrate)
- Citrate buffer (pH 6.8)
- Primary antibody (Ki67 , E-cadherin)

- Secondary antibody (Dextran polymer conjugated secondary antibody – HRP)
- Tris EDTA buffer (pH 9)
- Phosphate buffer saline (pH 7.4)
- Peroxides blocker(3% hydrogen peroxide in methanol)
- DAB (3,3 diaminobenzidinetetrahydrochloride) substrate chromogen
- Bluing Reagent
- Xylene
- DPX

Appendix 2:

Name: XXXXX

Age: 45 years.

Sex: female.

Nationality: Sudanese

Block No: 590-2018

Specimen: left brest.

Clinical remark: known casebof lift side breast carcinoma

Diagnosis by Dr: Omayma Mohamed Osman as : invasive ductal carcinoma.

Micrposcopy :

- Estrogen receptors protein (ER): Negative
- Progesterone receptors protein (PR): Negative
- Her 2 neu: Negative
- E.cadherin: Positive
- Ki 67:40%

Regards,,,,,

Dr, Nazik Elmalaila O.S.Husain
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