1. Introduction

1.1. Introduction:

Breast cancer is the most frequently diagnosed cancer in women, with an estimated 1.38 million new cases per year. Fifty thousand cases in women and 400 in men are recorded each year in the UK alone. There are 458,000 deaths per year from breast cancer worldwide making it the most common cause of female cancer death in both the developed and developing world (Ferlay,*et al.*, 2010).

In Sudan, breast cancer accounts for around a third of all cancers.In a descriptive study done in Sudan in 2010, the prevalence of advanced, stage III or worse metastatic disease was higher in women living in rural areas than it was in women living in urban areas (Abuidris, *et al*., 2013).Among breast cancer patients who visited Khartoum Teaching Hospital during a 5-year period from 1994 to 1999, invasive ductal carcinoma was the most common type (71.5%) and most patients (17.2%) had an advanced stage III and IV disease. Furthermore, among breast cancer patients attending National Cancer Institute**,** University of Gezira(NCI-UG) from 1999 to 2006.Invasive ductal carcinoma was the most common type (82%). And about 74% of those patients were less than 50 years old and presented with stage III and higher tumors expressing no estrogen or progesterone receptors that had already metastasized (Elamin,*et al*., 2015)

There are a number of factors that have been shown to increase a woman's risk of developing breastcancer**,** age is the best-documented risk factors for breast cancer, lifestyle and environmental factors, alcohol consumption , body mass index , hormone replacement therapy and prior history of neoplastic disease or hyperplasia in the breast (Singletary., 2003).

Mammographic screening for breast cancer is currently the best available approach for early detection in the general population.However, additional approachesare needed. (Paweletz,*et al.*, 2001).

The most commonly utilized treatment for breast cancer is surgical resection with adjuvant chemotherapy, hormone therapy or radiation. Radiation and chemotherapy are effective in killing or limiting the growth of actively dividing cancer cells through various mechanisms including the production of oxygen free radicals, DNA damage, and subsequent apoptosis (Davis,*et al*.,2014).

E-cadherin is a member of a family of transmembraneglycoproteinsresponsible for the calcium dependent cell-celladhesion mechanism and has been demonstrated to beinvolved in organogenesis and morphogenesis.Previous studies have shown reduced expression of Ecadherinin approximately 50% of mammary carcinomas inassociation with high histological grade and nodal metastasesHowever,not all studies confirmed these findings (Acs,*et al*., 2001).

1.2. Objectives:

1.2.1. Generalobjective:

-To study the expression of E-cadherin in breast tumors.

1.2.2. Specificobjective:

-To detect the expressionof E-cadherin in breast tumors by using immunohistochemistry.

2. Literature Review

2.1. Anatomy of the breast:

The breasts of an adult woman are milk-producing, tear-shaped glands. They are supported by and attached to the front of the chest wall on either side of the breast bone or sternum by ligaments. They rest on the major chest muscle (Martini,*et al.*, 2008).

The breast has no muscle tissue. Alayer of fat surrounds the glands and extends throughout the breast. The breast is responsive to a complex interplay of hormones that cause the tissue to develop, enlarge and produce milk. The three major hormones affecting the breast are estrogen, progesterone and prolactin, which cause glandular tissue in the breast and uterus to change during the menstrual cycle (Martini,*et al.*, 2008).

The breast is composed of 15-20 lobes that radiate from nipple. Each lobe is surrounded by fat and fibrous connective tissue and divided into many lobules. The lobule is the basic structure unit of the breast and is lined by epithelial cells. Each lobule subdivided into 10 to 100 alveoli, the milk producing unit of the breast. Milk flows from the alveoli of the lobules into the ducts. The ducts gradually coalesce into 10 to 15 major ducts, each lobe containing one major duct terminating in the nipple (Ramsay,*et al.*,2005).Breast also contain blood and lympatic vessels, most lymphatic vessels within the breast lead to axillary lymph node, some also connect to supra-or infraclavicular nodes, and internal mammary nodes. Beneath the tissue of the breast lie the muscles of the chest wall and between the two is the fascia. Two layers of suspensory ligaments link the breast to the fascia, providing support. As these ligaments stretch with age or weight gain, the breast loses some of it is firmness (Harris.,2004).

2.2. Pathology of the breast:

2.2.1. Benign changes:

Benign breast changes refer to heterogeneousgroup of lesions which can be divided into major types: inflammatory(including infection, traumatic) and benign epithelial lesions (also known as fibrocystic changes). Both hormones and genetics are believed to play causal roles in benign breast changes. Some lesions are palpable while other are detectable only with breast imaging or biopsy. Many appear similar to breast cancer, yet the vast majority of lumps, inflammation, nipple discharge and other breast changes are neither cancerous nor rare. In fact, benign changes of the breast are very common. The breast contain a number of cell and tissue types that make up the ducts, lobules and nipple, which support lactation. Thebreast also contains fat cells, blood vessels, connective tissue and lymph node that support breast structure and function. Inflammation of the breast tissue can affect any of these tissue types, and lead to swollen and painful. Breast inflammation may be a symptom of a serious underlying disease, which requires medical attention (Arriagade, *etal.*, 2005). Lactation mastitis is a form of breast inflammation characterized by infection and swelling of breast, usually occurring as a result of bacteria (mostlystaphylococcusaurous) entering the nursing mother breast through cracks in nipple (Schoenfeld and McKay.,2010).

The exact cause of fibrocystic changes is not completely understood but is known to be associated with hormone activity. These types of changes may vary during the menstrual cycle and usually subside with menopause; symptoms of fibrocystic include lumps, lumpiness, areas of thickening and tenderness. Fibrocystic changes are often grouped into three descriptive subtypes (based upon the appearance of tissue under microscope), non proliferative changes, atypia and proliferative changes with atypia. Each of these subtype confers differing relative risks for developinginto subsequent breast cancer (Santen and mansel., 2005).

2.2.2. Breast cancer:

Many breast cancer arise from a sequence that begin with an increase in number of breast cells to the emergence of atypical breast cells followed by carcinoma in situ and finally invasive cancer, not all breast cancer necessarily follow this progressive pattern; however, the speed of progression for those that do is highly variable, it also appears that cancer may never progress beyond in situ disease (Sariego., 2010).

2.2.2.1. Non invasive breast cancer:

2.2.2.1.1. Ductal carcinoma in situ (DCIS):

Ductal carcinoma in situ is the most common type of non-invasive breast cancer, accounting for about 15% of all new breast cancer cases in the U.S. so ductal carcinoma in situ refer to an uncontrolled growth of cells that are confined to the breast duct. As such, some experts believe DCIS to be a precancerous condition. Other, however; classify any cellular changes beyond a typical hyperplasia as cancer. Frequently a single lesion, DCIS is classified into several histological subtype associated with varying prognostic implication. Invasive cancer usually occurs within the same breast, but women with DCIS are also at higher risk of developing cancer in the opposite breast. Very few cases of DCIS are as palpable mass; most are diagnosed by mammography, usually as clustered micro calcification. DCIS may also present as pathological nipple discharge with or without mass, the frequency of diagnosis of DCIS has greatly increase with greater use of mammography, with early detection and treatment, the five-year survival rate for DCIS is nearly 100%, providing that the cancer has not spread past the milk ducts (Yen, *et al*.,2005)

2.2.2.1.2.Lobullar carcinoma in situ (LCIS):

Lobullar carcinoma in situ is characterized by abnormal changes in cells that line the milk-producing lobules of breast. LCIS is much less common accounting for only about 4,200 cases annually in the united state and carries slightly less risk of invasive cancer than DCIS. Also called lobular intraepithelial neoplasia, LCIS is actually considered by most medical experts to be neither cancer nor premalignant

lesion, but rather a marker that identifies women at increase of invasive cancer. Risk remain elevated beyond two decades and most subsequent breast cancers are ductal rather than lobular. LCIS typically includes multiple lesions and is frequently bilateral. It is usually discovered as an incidental finding from breast biopsy; there are rarely clinical or mammographic signs (Ryerson, *et al.*, 2009).

2.2.2.2. Invasive breast cancer:

2.2.2.2.1. Invasive ductal carcinoma (IDC):

The most common type of breast cancer, about 80% of invasive breast cancer is classified as invasive ductal carcinoma. Also called infiltrating ductal carcinoma, cancer cells have penetrated the ductal wall and invaded surrounding breast tissue. The cells may metastasize to other part of body through the bloodstream or lymphatic system. IDC may present as a hard and firm palpable mass or as mammographic abnormality. Tumor can cause skin and nipple reaction. IDC is most commonly encountered in pure form, although a substantial minority of IDC cases exhibits a combination of histological types. As with all invasive breast cancer, it is important to detect and treat invasive ductal carcinoma early, before it has had an opportunity to metastasize (Varga and mallon.,2009).

2.2.2.2.2. Invasive lobular carcinoma (ILC):

Begins in the milk-producing lobules where it extends into the adipose tissue of the breast. It is relatively uncommon, comprising about 10% of invasive breast cancers. As with IDC, ILC may breast as palpable mass, however, it tends to be less well-defined. Often, the only clinical evidence is that of a vague area of thickening. ILC can be more difficultto detect by mammogram. Compared with IDC, patients with infiltrating lobular carcinoma are more often prone to bilateral disease (Varga and mallon.,2009).

2.2.2.2.3. Tubular carcinoma:

Tubular carcinoma is a highly differentiated invasive carcinoma whose cells are regular and arranged in well-defined tubules. Before widespread use of mammography, tubular carcinomas were most often detected as palpable lesion. Now, most cases preasent as non palpable mammographic abnormalities, usually amass lesion, and only occasionally associated with micro calcification. Pure tubular carcinoma has limited metastatic potential and better than average prognosis (Varga and mallon.,2009).

2.2.2.2.4. Medullary carcinoma:

Medullary carcinoma is a relatively uncommon type of invasive carcinoma, accounting for less than 5% to 7% of all invasive breast cancers. Lesion has well-defined boundaries and can be quite large and soft on palpation. Histologically, the tumor is characterized by larger than average cancer cells, and with immune system cells present on the edges of the tumor. The prognosis for this type of breast is relatively favorable (Varga and mallon.,2009).

2.2.2.2.5. Mucinous carcinoma:

Mucinous carcinoma also called colloid carcinoma is an invasive form of breast cancer characterized by large amounts of extracellular mucin production. Less than 5% of invasive breast cancers show amucinous component. Usually occurring in postmenopausal women, tumors may or may not be palpable. Mammographically, pure mucinous carcinomas may mimic benign lesions with well-circumscribed and microlobulated margins. Like medullary carcinoma, mucinous carcinoma is associated with a relatively favorable prognosis (Varga and mallon., 2009).

2.2.2.2.6. Metaplastic carcinoma:

Metaplastic carcinoma is uncommon, representing less than 5% of all breast cancers. Lesion contain several different types of cells that are not typically seen in other form of breast cancer. Clinical presentation is frequently a single palpable lesion often associated with rapid growth. Mammographically, most metaplastic carcinomas are fairly circumscribed, noncalcified lesion which, in some cases,

appear benign. Prognostic implications of this type of breast cancer are variable (Varga and mallon., 2009).

2.2.2.2.7. Invasive papillary carcinoma:

Invasive papillary carcinoma is very rare, comprising less than 1% to 2% of an invasive breast cancer. Found predominantly in postmenopausal women, it is characterized by nodular densities that that may be multiple and are frequently lobulated, limited data suggests relatively favorable prognosis. Invasive micro papillary carcinoma is distinct but poorly recognized variant of breast cancer, usually presenting as afirm, immobile mass. Findings on mammography are of a speculated, irregular or round, high density mass with or without associated microcalification. Pure micropapillary carcinoma is uncommon, with an incidence of less than 3% limited research suggests that this type of cancer may be associated with a relatively poor prognosis (Edwin, *et al*., 1975).

2.2.2.3. Other type of breast cancer:

2.2.2.3.1. Inflammatory breast cancer:

Inflammatory breast cancer is a form of locally advanced breast cancer associated with a rapid onset of clinical features including breast inflammation, warmth, thickening or dimpling, and palpable ridge at the margin of induration. Often mistaken as an infection, symptoms result from the blocking of lymphatic vessels near the surface of the skin by cancer cells. Inflammatory breast cancer is relatively rare, representing about 1% to 5% of all breast cancer in U.S., and has a less favorablethan average prognosis (Kollmorgen, *et al*., 1998).

2.2.2.3.2. Paget's disease of nipple:

Paget's disease of nipple isbegins in the milk ducts as either an in situ or invasive cancer, prognosis is excellent when associated with carcinoma in situ. Early stage symptoms include erythema and milk scaling of nipple skin. Symptoms of more advanced disease may include nipple tingling, itching, increase sensitivity, burning, pain or oozing. Diagnosed by biopsy, Paget's disease of the nipple must be differentiated from eczema, contact dermatitis, basal cell carcinoma and a number of

other conditions. Paget's disease of nipple account for approximately 1% of all breast cancer (Kollmorgen, *et al*., 1998).

2.2.2.3.3. Phylloides tumors:

Phylloides tumorsalso spelled pyllodes can be benign, borderline or malignant. Malignant tumors are very rare. Phylloidestumors are biphasic and composed of benign epithelial elements and cellular connective tissue stroma. The stroma dictates whether the tumor will be benign, borderline or malignant. They can grow to a relatively large size within a few months, although rapid growth does not necessarily indicate malignancy. The gross appearance of most phylloides tumors, particularly those that are benign, is not distinctly different from fibroadenoma. Phylloides tumors are often painless (Kollmorgen, *et al*., 1998).

Some breast cancers are advanced or metastatic at the time of diagnosis. Other recurs or metastasizes months or years after treatment for primary tumor is completed. Approximately 20-30% of patients diagnosed with early breast cancer will eventually develop metastatic disease. The most site of metastasis are the bone, lung, liver and brain. Breast cancer that metastasize to other organ of the body are still breast cancers and treated as such, although the treatment will vary depending on site of the metastasis (Kollmorgen, *et al*., 1998).

2.3.Epidemiology of breast cancer:

Breast cancer is the most frequently diagnosed cancer in women, with an estimated 1.38 million new cases per year. Fifty thousand cases in women and 400 in men are recorded each year in the UK alone. There are 458,000 deaths per year from breast cancer worldwide making it the most common cause of female cancer death in both the developed and developing world (Ferlay,*et al.*, 2010).

Breast cancer is a leading cause of death among women in west africa with an approximately 30000 new cases in 2008 and more than 16000 deaths. The incidence appears to be significantly lower in eastern africa with approximately 18000 new cases and a corresponding 10000 deaths during the same year. In western europe,

the incidence is five times higher than that in west efrica. Furthermore, approximately 40000 deaths from breast cancer were recorded in 2008. The incidence is similar in central and eastern europe with approximately 115000 new cases and more than 47000 deaths in 2008. The incidence has also been shown to be significantly higher among women of european origin in the united states of america. Fejerman and colleagues reported that Greater European ancestry is associated with increased risk of breast cancer. They recorded a statistical significance when women with 51% to 75% and 76% to 100% european ancestry were compared with women with 0% to 25% european ancestry (Abdulrahman and Rahman., 2012)

In Sudan, breast cancer accounts for around a third of all cancers.In a descriptive study done in Sudan in 2010, the prevalence of advanced, stage III or worse metastatic disease was higher in women living in rural areas than it was in women living in urban areas (Abuidris, et al., 2013).Among breast cancer patients who visited khartoum teaching hospital during a 5-year period from 1994 to 1999, invasive ductal carcinoma was the most common type (71.5%) and most patients (17.2%) had an advanced stage III and IV disease. Furthermore, among breast cancer patients attending National Cancer Institute**,** University of Gezira(NCI-UG) from 1999 to 2006. Invasive ductal carcinoma was the most common type (82%). And about 74% of those patients were less than 50 years old and presented with stage III and higher tumors expressing no estrogen or progesterone receptors that had already metastasized (Elamin, *et al*., 2015)

This indicates that women in Sudan are inflicted with breast cancer at young age. However, the mean age of breast cancer in Sudan and the entire Africa is younger compared to other developed countries. The majority of the tumors were invasive ductal carcinoma that lacked hormonal receptors expressions, and present with advanced disease (Ahmed,*et al*., 2010).

The frequency of advanced breast cancer among women presenting with palpable lesions were very high in Sudan. Women patients who referred to a cytodiagnosis center in Khartoum (n = 200) with palpable breast lumps during a period of a year, 68 (34%) were diagnosed with malignant disease while 56 cases (28%) were fibroadenoma, 23 cases (11.5%) were fibrocystic change, 22 cases (11%) were inflammatory lesions (including mastitis and abscess formation), 12 cases (6%) were benign cysts, and the remaining 19 cases (9.5%) were with lactation changes, lipoma, gynecomastia, and phyllodes tumor(Ahmed,*et al.*, 2010).

2.4. Risk factor of the breast cancer:

Every woman is at risk for developing breast cancer 100 times more than man, because the breast tissue cells of women are more exposed to the growth promoting effect of female hormones estrogen and progesterone. Several risk factors have been known to contribute in breast cancer. However, the vast majority of breast cancer cases occur in women who have no identifiable risk factors other than their gender (Kriebs, *et al*., 2005).

2.4.1. Age:

Age is one of the best-documented risk factors for breast cancer and for many other cancers. The incidence of breast cancer is extremely low before age 30 (incidence <25 cases per 100,000), after which it increases linearly until the age of 80, reaching a plateau of slightly less than 500 cases per 100,000. If all women less than 65 years of age are compared with women aged 65 or older, the relative risk of breast cancer associated with increased age is 5.8(Singletary., 2003).

2.4.2. Lifestyle and environmental factors:

There is a keen interest on the part of the general public in risk factors that may lie under the direct control of the individual, as is the case with cigarette smoking and lung cancer. Researchers are investigating the exciting potential for preventing cancer through behavioral modifications or by the avoidance of carcinogenic agents. Unlike the case of cigarette smoking and lung cancer, however, no factors of this

kind have yet been identified that have a major effect on the risk of breast cancer. There are, however, several factors that have a more limited effect (Singletary.,2003).

2.4.3. Family history and genetic mutation:

About 15% of all breast cancer patients have appositive family history for this disease (Bock, *et al*., 2004). A family history of breast cancer can be divided into three categories. Weak family history occurs when two or less second degree have breast cancer. Strong family occurs when any first degree relatives have breast cancer and very strong family history which occur when two or more first degree relatives have breast or ovarian cancer (Carolin and Pass., 2000). Deleterious gremlin mutation in highly penetrate genes such as BRCA1 and BRCA2 are strong predictors of breast cancer development. These genes are found in 5-10% of women with breast cancer and in only 1% of general population. Nearly 80% of hereditary breast cancers are caused by mutations in one or two of these genes (Oldenburg, et al., 2007). Carrier's mutation in BRCA1 and/or BRCA2 has been associated with the life time risk of breast cancer of 45% to 87% (Kotssopoulos, et al., 2005). Inherited mutations in two other genes, tumor suppressor gene (TP53) and phosphatase and tensin homolog (PTEN) are associated with familial syndromes (Li-Fraumen and cowdens syndromes respectively) that include a high risk of breast cancer but both are rare (Mcpherson., 2000).

2.4.4. Radiation Exposure:

Although much of our early knowledge about the carcinogenic effects of radiation exposure in a human population was derived from studies of atomic bomb survivors, therapeutic radiation exposure to monitor or treat disease is now the most significant cause of radiation-induced carcinogenesis. Studies by Boice et al. involving tuberculosis patients have documented that multiple fluoroscopies are a significant risk factor for breast cancer. In a 1991 update, breast cancer incidence was tabulated for 2,573 women who were examined by x-ray fluoroscopy an average of 88 times during therapy for tuberculosis and who were followed for an average of 30 years.

Extrapolating from the data collected in this population, the relative risk for 1 Gy of radiation exposure at a latency period of 10 years was estimated to be 1.61. They found that younger women were at higher risk than older women, and that the increased risk for breast cancer, beginning 10 to 15 years after the initial exposure, remained high for the duration of the woman's lifetime.The risk of breast cancer is also increased in women receiving radiation therapy to the chest area for the treatment of Hodgkin's lymphoma, especially in women treated from the time of puberty to the age of 30. As with fluoroscopic exposure, there is again a long latency period (approximately 15 years), but the resulting cancers still often occur in women who have not yet begun regular mammographic screening. Clemons et al. reviewed 17 published studies examining the incidence of breast cancer in Hodgkin's disease survivors. They found a median relative risk of 5.2 (range 1.4–33, plus an outlier value of 75.3), at an average latency period of 14 years (range 5–15.1)(Singletary.,2003).

2.4.5. Body mass index (BMI) and Obesity:

Epidemiology studies indicated that overweight or obesity, usually reflected by BMI is a risk factor for development of postmenopausal breast cancer (Cleary and Crossman., 2009). The association can largely be explained by the increase in circulating levels of estrogens and decrease levels of serum hormone binding globulin (Endogenous Hormones Breast Cancer Collaborative Group., 2003). On the other hand, overweight and obesity or high BMI, for premenopausal women, may act as a protective factor, but this is not in women with family history of breast cancer. Therefore, the weight gain should be avoided in women with BRCA1mutatin carries (Kotsopoulos, *et al*., 2005).

2.4.6. Breast feeding:

It is not easy to assess the effect of breast feeding, but it is known that the breast feeding is correlated to parity and other reproductive related factors of breast cancer risk. A study by collaborative group in 2002 indicated that each year of breast feeding reduces the risk of breast cancer by 4.3% and the longer women breast feed

the more they are protected against breast cancer. The lack or short lifetime duration of breast feeding typical of women in developed countries makes a major contribution to the high incidence of breast cancer in these countries (Collaborative Group on Hormonal Factors in Breast cancer., 2002)(Gadducci, *et al*., 2005).

2.4.7. Prior history of neoplastic disease or hyperplasia in the breast:

Individuals who have a prior history of invasive carcinoma, carcinoma in situ, or atypical hyperplasia in the breast can have a significantly increased risk for the future development of invasive breast carcinoma. Most physicians prefer to manage such women conservatively with close surveillance, although a few women at very high risk may opt for prophylactic mastectomy (Singletary.,2003).

2.4.8. Other risk factors:

Many other risk factors may be associated with increase risk factors like induced abortion (Ozmen, et al., 2009), decreased vitamin A intake (Holmes and Walter.,2004) and longer period of night work (Schernhammer, *et al*., 2006).

2.5. Diagnosis of breast cancer:

2.5.1. Diagnostic mammogram:

This similar to a screening mammogram but includes additional views focused on the area of concern. In addition to the standard craniocaudal and mediolateral oblique views, diagnostic mammogram may include lateromedial, mediolateral, and exaggerated craniocaudal views, and other special views, such as spot compression and magnification. Mammography is the imaging method of choice for investigating microcalcifications (Berg, *et al.*, 2011).

The additional views of a diagnostic mammogram can be a source of concern for women who are not properly informed about the procedure. The primary care provider can help alleviate potential anxiety by explaining that the need for additional images does not imply that a malignancy has been found on screening mammography. In fact, a majority of cases where extra views are obtained do not result in recommendation for biopsy (Berg, *et al.*, 2011).

2.5.2. Breast ultrasound:

It is an imaging test that sends high-frequency sound waves through your breast and converts them into images on viewing screen showing dark irregular mass. The ultrasound technician places a sound-emitting probe on the breast to conduct the test. There is no radiation involved. Ultrasound is not used on its own as screening test for breast cancer. Rather, it is used to complement other screening tests, it cannot determine whether a solid lump is cancerous, nor can it detect calcifications (Berg, *et al*., 2011).

2.5.3 Magnetic resonance imaging (MRI):

Magnetic resonance imaging is a technology that uses magnets and radio waves to produce detailed cross-section images of the inside of the body. MRI does not use x-ray, so it does not involve any radiation exposure. Breast MRI has a number of different uses for breast cancer, including screening high-risk women and gathering more information about are of suspicion found on mammogram or ultrasound monitoring for recurrence after treatment. MRI is especially helpful for discriminating between cancer and scar tissue. Like ultrasound, it is also useful in evaluating dense breast tissue, may also be used to help define the size and extent of cancer within breast tissue and help spot multifocal disease (OConnor, *et al.*,2009).

2.5.4. Biopsy:

There are different types of biopsy used to take cells or tissue samples from a suspicious lump so they can be sent to a laboratory for analysis under a microscope. Fine needle aspiration is usually the first type of biopsy used. It is performed using a local anesthetic and involves inserting a fine needle into the lump and removing a small sample of cells and/or fluid. At the laboratory the sample is spread onto a glass slide and analyzed. The insertion of the needle may be guided by ultrasound. Core biopsy uses a large needle to remove a sample of tissue from the lump. A local anesthetic is used and a very small incision (1-2mm) is made in the skin over the lump. The needle is usually guided into the lump by ultrasound. At the laboratory the tissue sample is sliced very finely and placed on a glass slide for analysis.

Stereotactic core biopsy is a core biopsy performed on a special x-ray table allowing three dimensional computerized images of the lump to be taken and used to guide the biopsy needle into the lump. This is useful for testing lumps seen on a mammogram that cannot be felt or visualized using an ultrasound scanner. Excisional biopsy is aminor surgical procedure where part or all of the abnormal area is removed. It can performed using a local or general anesthetic. If the lump is unable to be precisely located using mammogram or ultrasound scanning, it may need to marked by thin wire called a hook wire. This is inserted under x-ray guidance using a local anesthetic just prior to surgery. If a diagnosis of breast cancer is made, blood test, x-ray and scans of the bones and liver may be performed to assess whether the cancer has spread to other organs (Kosters and Gotzsche., 2003).

2.5.5.Immunohistochemistry (IHC):

This is a special technique performed on fresh or frozen breast cancer tissue removed during biopsy. (Ramos-Vara., 2005).

2.5.6. Fluorescence In situ hybridization(FISH):

FISH is a test that maps the genetic material in a person's cells. This test can be used to visualize specific genes or proteins of genes (Sarrate, *et al*., 2010).

2.6. Treatment of breast cancer:

Method of treatment for breast cancer are local or systemic. Local treatments are used to remove, destroy, or control the cancer cells in specific area. Surgery and radiation therapy are local treatments. Systemic treatments are used to destroy or control cancer cells throughout the body. Chemotherapy and hormone therapy are systemic treatments. A patient may have just one form of treatment or combination. Different forms of treatment may given at the same time or one after another (Polgar and Major., 2010).

2.6.1. Surgery:

Most patients with breast cancer have surgery to remove the cancer from the breast. Some of the lymph nodes under the arm are usually taken out and looked at under a microscope to see if they contain cancer cells (Bear, *et al*., 2012).

Chemotherapy may be given before surgery to remove tumor. When given before surgery, chemotherapy will shrink the tumor and reduce the amount of tissue that needs to be removed during surgery. Treatment given called neoadjuvantherapy (Devita., 2008).

2.6.2. Radiation therapy:

Radiation therapy is a cancer treatment that uses high-energy x-ray or other types of radiation to kill cancer cells or keep them from growing.The way the radiation therapy is given depends on the type and stage of the cancer being treated (Robinson, *et al*., 2013).

2.6.3. Chemotherapy:

Chemotherapy is a cancer treatment that uses drug to stop the growth of cancer cells, either by killing the cells or by stopping them from dividing. The way the chemotherapy is given depends on the type and stage of the cancer being treated (Bear, *et al.*, 2012)

2.6.4. Hormone therapy:

This is for cases where the breast cancer hormonereceptor positive, hormone therapy may be prescribed to help prevent recurrence of the breast cancer. These medication work by blocking the hormone receptors on the breast cancer cells, preventing hormones binding to them and stimulating growth (Slamon, *et al*., 2010).

2.7. E cadherin:

E cadherin it is calcium dependent cell adhesion molecules and one of the most important molecules in cell-cell adhesion in epithelial tissues. It is localized on the surfaces of epithelial cells in regions of cell-cell contact known as adherent's junctions. The cadherin glycoproteins are expressed by a variety of tissues, mediating adhesion through homotypic binding such as E, N, K, P, R, OB. Classical cadherins, E and N cadherins being the best characterized play important roles in the formation of tissues during gastrulation, neurulation and organogenesis, can play a major role in malignant cell transformation, and especially in tumor development and proliferation. The suppression of E cadherin expression is regarded as one of the main molecular events responsible for dysfunction in cell-cell adhesion. Most tumors have abnormal cellular architecture, and loss of tissue integrity can lead to local invasion. Thus, loss of function of E cadherin tumor suppressor protein lead to increased invasiveness and metastasis of tumors, resulting in it being referred to as the suppressor of invasion gene. The human epithelial (E) cadherin gene CDH1 it is located in chromosome 16q22.1 (Shimazui *et al.,* 2000).

2.7.1 Role of E cadherin in normal cells:

E cadherin expression is very early in epithelial tissue at the two cell stage epithelial differentiation and polarization occur early in ontogeny in the morula stage, when the embryo compacts and each cell polarizes along it's apicobasal axis to generate an epithelial-like phenotype. E cadherin plays an important role in the adhesion junction of epithelial cell, and early embryo's ability to compact. E cadherin is expressed in the membrane even before compaction of the morula occurs, is distributed in a non-polar manner. The mechanism that renders E cadherin functional is unknown, but it does include phosphorylation of the protein (Katagiri*et al.,* 1995).

Epithelial tissue conversion is the most important exhibit of E-cadherin's function in development. Loss of epithelial adhesion and polarity causing mesenchymal cell

morphology occurs during mesoderm formation. Heterozygous mutant animals were normal and fertile but abnormal in human (Jeanes *et al.*, 2008).

2.7.2 Role of E cadherin in malignant cells:

Somatic mutations is very important to cause cancer in a number of different genes characterizes the process of tumorigenesis. Many genes involved in the process of tumorigenesis are components of one of a great many signal transduction pathways such as E cadherin and beta catenin. It is now apparent that epithelial malignancy can in certain aspects be explained by alterations in the adhesive properties of neoplastic cells, epithelial mesenchymal conversion is also observed in malignant tumors of epithelial origin. This process is similar to developmental events but with the important difference that it is uncontrolled. Malignant carcinoma cells are characterized in general by poor intercellular adhesion, loss of the differentiated epithelial morphology and increased cellular motility. Down regulation or a complete shutdown of E cadherin expression, mutation of the E cadherin gene, or other mechanisms that interfere with the integrity of the adherens junctions, are observed in carcinoma cells. In human tumors, the loss of E cadherin mediated cell adhesion correlates with the loss of the epithelial morphology and with the acquisition of metastatic potential by the carcinoma cells. Thus, a tumor invasion suppressor role has been assigned to E cadherin (Slaus., 2003).

3. Materials and methods

3.1. Study design:

This is analytical retrospective hospital based case control study, aimed to study the expression of E cadherin in breast tumors using immunohistichemistry.

3.2. Study area:

The study was conducted at the Radiation and Isotopes center –Khartoum and Sudan University of Science and Technology- College of Medical Laboratory Science during the period fromMarchtoAugust 2016.

3.3. Study population:

39 Patients ofSudanese women previously diagnosed as breast tumors of whom 13 werediagnosed as benign tumors, 13were diagnosed as ductal carcinoma and 13 were diagnosed as lobular carcinoma.

3.4. Sample collection:

Blocks were collected from hospital archived block,from each block onesection was cut (3µ) by using rotary microtome.

3.5. Sample processing:

One section (3µm) from formalin-fixed paraffin-embedded tumor was cut and mounted onto coated slides (Dako). Following deparaffinization in xylene, slides was rehydrated through a graded series of alcohol and placed in distilled water. Samples were steamed for antigen retrieval for E cadherin using PT link, slides were placed in the PT tank containing enough tris buffer (PH9.0) to cover the sections, then the machine was turn on and programmed as follow, 20 minutes to start heating from 65ºC till it reach 95ºc and then boiled at high temp (95ºC) for 20 minutes then allow sections to cool to 65ºC. Endogenous peroxidase activity was blocked with peroxidase blocking reagent (3% hydrogen peroxide and methanol) for 10 minutes.The sections were incubated with 100µl of primary antibody (E cadherin)(Dako) for 20 minutes at room temperature in moisture chamber, and then were rinsed in phosphate buffer saline, after washed with PBS for 3minutes, binding of antibodies were detected by incubating for 20 minutes with dextran labeled

polymer (Thermo –ultra vision) . Finally, the sections were washed in three changes of PBS, followed by adding 3,3diaminobenzidine tetra hydrochloride (DAB) as chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for 5 minutes. Slides were counter stained with haematoxylin (Mayer's) for one minute, then dehydrated, cleared and mounted in DPX.

3.6. Result interpretation:

Immunoreaction was assessed as positive(strong) ornegative(weak or absent), and the internal positivecontrol was represented by the neighboring normalglandular structures. Negative expression was statedwhen the immunohistochemical reaction was negative or less than 70% of the tumor cells show positivereaction with membrane pattern.Positive expression was stated when ≥70% of thetumor cells showed positive reaction with membranepattern (Suciu, *et al*., 2008).

3.7. Statisticalanalysis:

Data was analyzed using SPSS version 20 computer programs; mean, frequency and Chi square testwas calculated.

3.8. Ethical consideration:

The study was performed after approval to use tissue blocks from theRadiation and Isotopes –Khartoum center.

Results

The study involved 39 samples, previously diagnosed as breast tumors.Table (1) showed frequency of histopathological diagnosis, 26 (66.6%) malignant breast tumors distributed as follow 13 (33.3%) ductal carcinoma, 13 (33.3%) lobular carcinoma, and the remaining 13 (33.3%) benign breast tumors.

Figure (1) explains frequency of immunohistopathological result of benign and malignant tumors. In benign tumor two (15.4%) cases were negative and 11(84.6%) cases were positive, which in malignant tumors 10 (38.5%) cases were negative and 16(61.5%) case were positive.

Table (2) showed the compression of E cadherin expression in malignant and benign breast tumors, there was insignificant differences between E cadherin expression and breast tumors ($P = 0.141$).

Table 4.1: Frequency of histopathological diagnosis

Histopathological diagnosis		Frequency	Percentage
Benign		13	33.3
Malignant	Ductal carcinoma	13	33.3
	Lobular carcinoma	13	33.3
Total		39	100%

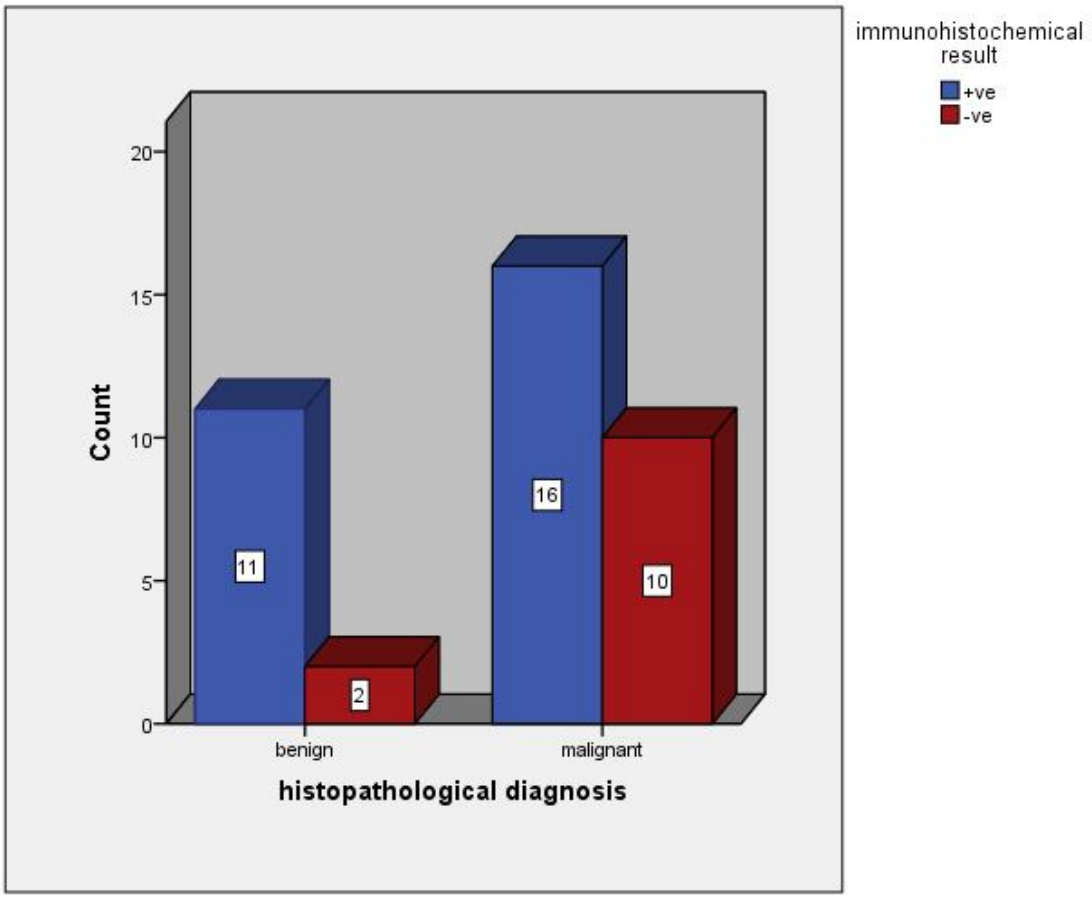
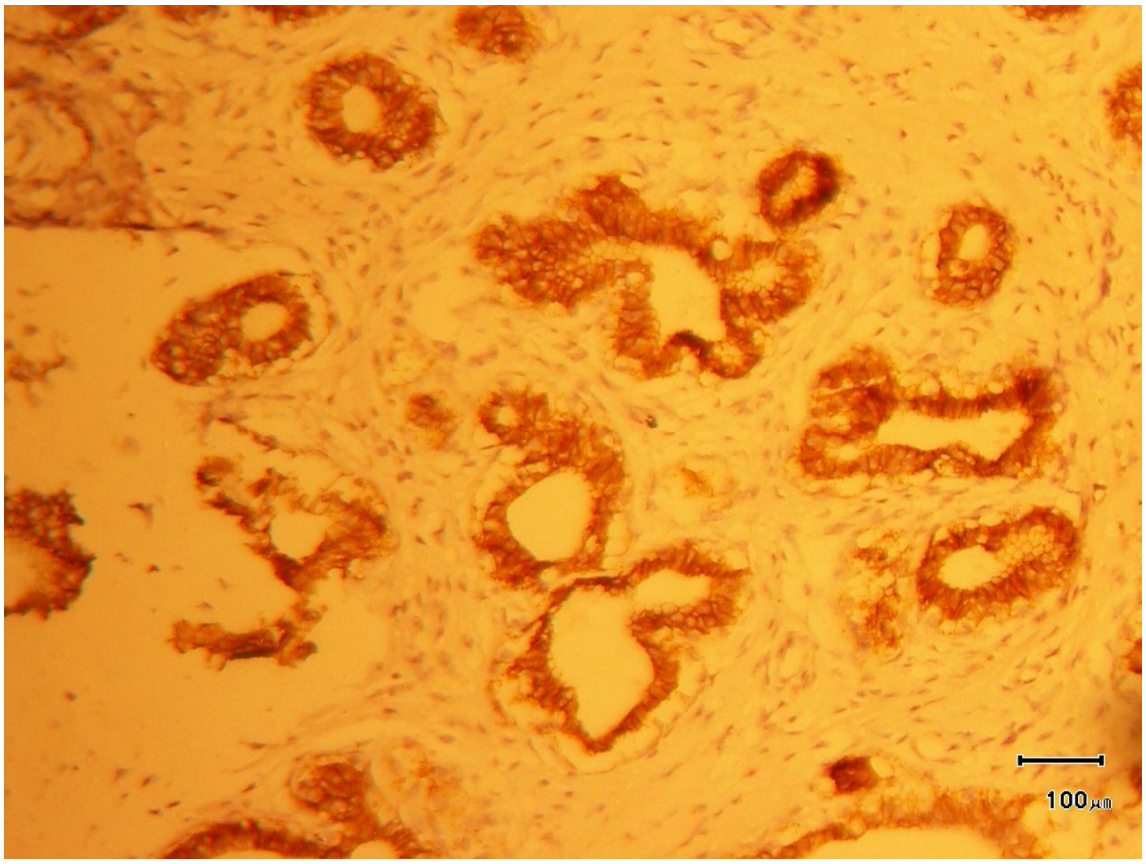


Figure 4.1: Frequency of immunohistopathological result of E cadherin in breast tumors

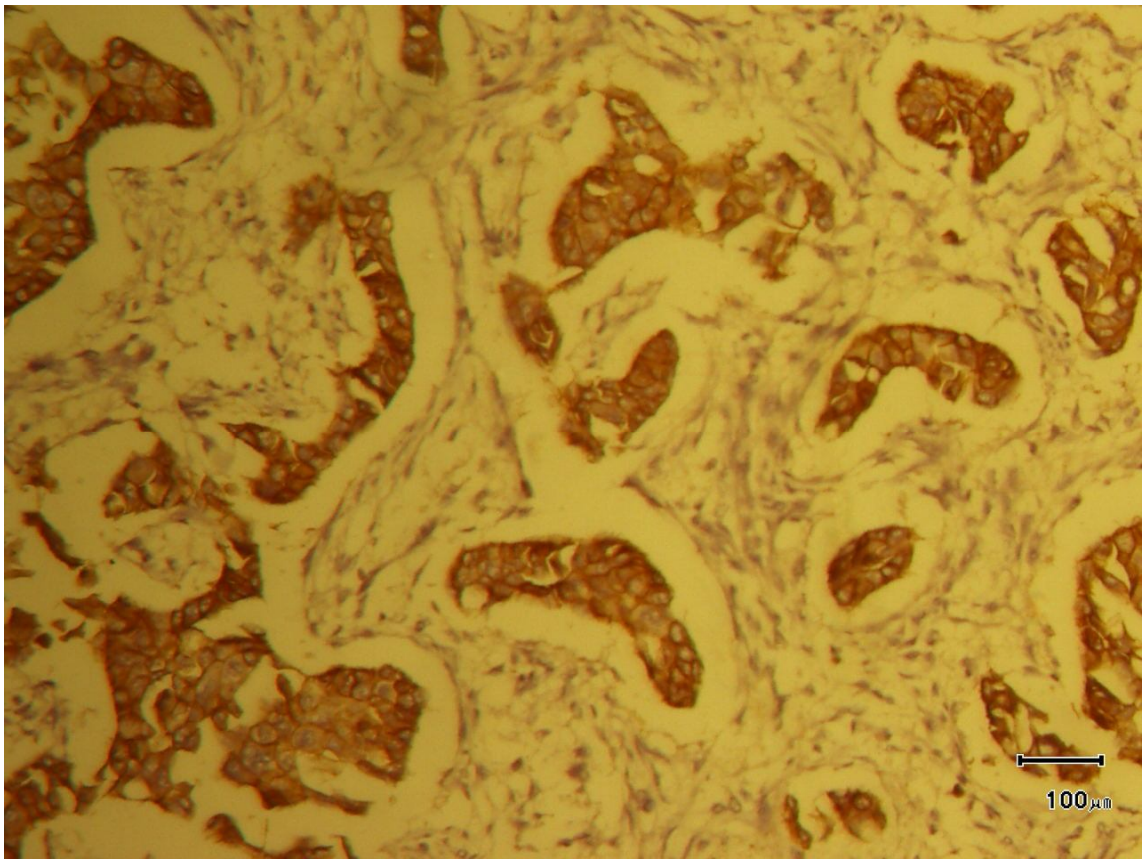
Table 4.2: Relation between E-cadherin expression and histopathologicaldiagnosis

histopathological diagnosis	immunohistochemical result		Total
	+ve	-ve	
Benign	11	2	13
Malignant	16	10	26
Total	27	12	39

P= 0.141



Photograph (4.1):Benign breast tumor show positive expression of E cadherin (20X)



Photograph (4.2):invasive ductal carcinoma show positive expression of E cadherin (20X)

5. Discussion

The present study found that When we compared the expression of E cadherin in malignant and benign breast tumors individuals, in benign tumors two (15.4%) cases were negative and 11(84.6%) cases were positive, which in malignant tumors 10 (38.5%) cases were negative and 16(61.5%) case were positive ,(*P*=0.141)this result explainthere was no significant difference between E cadherin expression in malignant and benign breast tumors.To our knowledge, there are no studies that agree with us, and this may be due to the size of our sample which is very small. Our study disagree with study of Ascand his colleagues they found a highly significant correlation of E-cadherin membrane expression with the histologic phenotype of the tumors (ASc, *et al*., 2001), and also disagree with study of Suciuand his colleagues they found thatInvasive breast carcinomas are characterized by the decrease of E cadherin expression, being found inalmost a half of the cases of invasive carcinomas (Suciu, *et al*., 2008).

6. Conclusion and Recommendations

6.1 Conclusion:

On the basis of these results the study concludes that:

- There is noassociation between E cadherin expression andbreast tumors.

6.2 Recommendations:

On the basis of this study we recommended:

- Farther studies should be done of E cadherin with another adhesion molecule such as beta catenin with large sample size.

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APPENDICES (1)

Instrument and materials:

1-Instrument:

-Rotary microtome

-Oven

-Coplinjare

-Staining racks

-Stainless microtome blade

-coated slides

-PT link

-Cover glass

-Water bath

-Moisture chamber

-Work station

-Pipettors

2. Materials:

-Xylene

-Ethyle alcohol

-Mayer’s haematoxylene

-Distilled water

-Citrate buffer

-Peroxidase blocker

-Anti E cadherin antibodies (primary antibody)

-Dextran polymer conjugated secondary antibodies and HRP

-3,3diaminobenzidinetetrahydrochloride in substrate buffer

-DPX mounting media

APPENDICES (2)

Kit leaflet

P02080 001

Dako

CE

FLEX
Monoclonal Mouse
Anti-Human
E-Cadherin
Clone NCH-38
Ready-to-Use
(Dako Omnis)

English
Code GA059

Intended use

For in vitro diagnostic use.

FLEX Monoclonal Mouse Anti-Human E-Cadherin, Clone NCH-38, Ready-to-Use (Dako Omnis), is intended for use in immunohistochemistry together with the Dako Omnis instrument. This antibody is useful for the identification of E-cadherin positive cells in normal and neoplastic tissues and for the differentiated diagnosis between ductal carcinoma and lobular carcinoma of the breast (1). The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Synonyms for antigen

E-CD, uvomorulin, L-CAM, Arc-1, or cell-CAM 120/180 (2-4)

Summary and explanation

E-cadherin is a 120 kDa transmembrane cell adhesion molecule. The gene has been localized on chromosome 16q22.1. In its extracellular domain, E-cadherin is involved in cell-cell adhesion through calcium-regulated homophilic interactions, whereas in its intracellular domain, E-cadherin connects to the actin cytoskeleton via catenins. E-cadherin has a significant function in intercellular adhesion of epithelial cells, the establishment of epithelial polarization, glandular differentiation, and stratification. It is localized mainly in the adherens junctions and concentrates the urokinase plasminogen activator and the epidermal growth factor receptor to cell contact sites (5, 6). Down-regulation of E-cadherin expression has been observed in a number of carcinomas and is usually associated with advanced stage and progression (5-8).

Refer to Dako's *General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures.

Reagent provided

Ready-to-use monoclonal mouse antibody provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L sodium azide.

Clone: NCH-38. Isotype: IgG1, kappa.

Immunogen

E-cadherin (uvomorulin) (9).

Specificity

Anti-E-cadherin, NCH-38 recognizes the 120 kDa mature form and 82 kDa fragment of E-cadherin in Western blots of A431 cells lysates (9).

Precautions

1. For professional users.
2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
3. As with any product derived from biological sources, proper handling procedures should be used.
4. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
5. Unused solution should be disposed of according to local, State and Federal regulations.

Storage

Store at 2-8 °C. During storage the cap should be closed. Do not use after expiration date stamped on vial. Onboard stability is 80 hours. Remaining on-board stability is tracked by the Dako Omnis software. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

Quick guide

Step		Comments
Fixation/embedding	Formalin-fixed, paraffin-embedded	Onboard deparaffinization
Pre-treatment	EnVision™ FLEX, High pH (Code GV804)	30 min HIER
Antibody	Ready-to-use	25 min incubation
Negative Control	FLEX Negative Control, Mouse (Code GA750)	25 min incubation
Visualization	EnVision™ FLEX (Code GV800) + EnVision™ FLEX+ Mouse LINKER (Code GV821)	Block: 3 min; Link: 10 min; Polymer: 20 min; Chromogen: 5 min
Counterstain	Hematoxylin (Code GC808)	3 min incubation
Control Tissue	Colon and liver	Cytoplasmic/membranous staining
Slides	FLEX IHC Microscope Slides (Code K8020)	Recommended for greater adherence of tissue sections to glass slides
Mounting	Non-aqueous, permanent mounting required	After staining, the sections must be dehydrated, cleared and mounted using permanent mounting medium
Instrumentation	Dako Omnis	Reagents are provided in instrument-specific vials

**The user must always read the package insert for detailed instructions of the staining procedure and handling of the product.*

Specimen preparation

Paraffin sections: The antibody can be used for labeling formalin-fixed, paraffin-embedded tissue sections. Tissue specimens should be cut into sections of 4 µm.

Pre-treatment: Pre-treatment of formalin-fixed, paraffin-embedded tissue sections with heat-induced epitope retrieval (HIER) is required. Pretreating tissues with HIER using diluted EnVision™ FLEX Target Retrieval Solution, High pH (50x) (Dako Omnis), Code GV804 is recommended. Deparaffinization, rehydration and target retrieval are performed onboard Dako Omnis. Please refer to Dako Omnis Basic User Guide.

The tissue sections should not dry out during the pre-treatment process or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of FLEX IHC Microscope Slides, Code K8020 is recommended.

Staining procedure

Program: The staining steps and incubation times are pre-programmed into the Dako Omnis software. Please refer to the Dako Omnis Basic User Guide for detailed instructions on loading slides and reagents. If the protocols are not available in the Dako Omnis system, please contact Dako Technical Services. All incubation steps are performed at 32 °C onboard Dako Omnis.

Visualization: The recommended visualization system is EnVision™ FLEX, High pH (Dako Omnis), Code GV800 in combination with EnVision™ FLEX+ Mouse LINKER (Dako Omnis), Code GV821. The visualization is performed onboard Dako Omnis.

Counterstaining: The recommended counterstain is Hematoxylin (Dako Omnis), Code GC808. The counterstaining is performed onboard Dako Omnis.

Mounting: After staining onboard Dako Omnis the sections must be dehydrated, cleared and mounted using permanent mounting medium.

Controls: Positive and negative controls should be run simultaneously using the same protocol as the patient specimens. The positive control tissue should include colon and liver the cells/structures should display reaction patterns as described for this tissue in the "Performance characteristics" section. The recommended negative control reagent is FLEX Universal Negative Control, Mouse (Dako Omnis), Code GA750.

Staining interpretation

The cellular staining pattern is cytoplasmic and membranous.

Performance characteristics

Normal tissues: In colon, the epithelial cells show a strong staining reaction. In liver, the hepatocytes and ductal cells show a moderate to strong reaction.

Summary of Normal Tissue Reactivity

Tissue Type (# tested)	Positive Tissue Elements	Tissue Type (# tested)	Positive Tissue Elements
Adrenal (3)	3/3 epithelium (50-80%), membrane and cytoplasm	Pancreas (3)	3/3 epithelial cells (100%), membrane and cytoplasm
Bone marrow (1)	0/1	Parathyroid (3)	3/3 epithelium (100%), membrane and cytoplasm
Breast (3)	3/3 epithelial cells (100%), membrane and cytoplasm	Pituitary (3)	0/3
Cerebellum (3)	0/3	Prostate (3)	3/3 epithelium (100%), membrane and cytoplasm
Cerebrum (3)	0/3	Salivary gland (3)	3/3 epithelium (100%), membrane and cytoplasm
Cervix (1)	1/1 epithelium (100%), membrane and cytoplasm	Skin (3)	3/3 epithelium (100%), membrane and cytoplasm
Colon (3)	3/3 epithelial cells (100%), membrane and cytoplasm	Small intestine (3)	3/3 epithelium (100%), membrane and cytoplasm
Esophagus (3)	3/3 epithelium (100%), membrane and cytoplasm	Spleen (3)	3/3 small vessel endothelium (50%), membrane and cytoplasm
Kidney (3)	3/3 tubular epithelium (80%), membrane and cytoplasm	Stomach (3)	3/3 epithelium (100%), membrane and cytoplasm

Liver (3)	3/3 hepatocytes, membrane and cytoplasm 3/3 bile ducts, membrane and cytoplasm	Testis (3)	0/0
Lung (3)	3/3 alveolar bronchial *epithelium cells (100%)*, membrane and cytoplasm	Thyroid (3)	3/3 epithelium (100%), membrane and cytoplasm
Muscle, cardiac (3)	0/3	Thymus (3)	Hassall's corpuscles - cortico reticular (100%), membrane and cytoplasm
Muscle, skeletal (3)	0/3	Tonsil (3)	3/3 epithelium (100%), membrane and cytoplasm
Nerve, peripheral (2)	2/2 nerve (60%), cytoplasm	Uterus (3)	3/3 epithelium (100%) membrane and cytoplasm
Ovary (3)	2/3 epithelium in primary follicles (<1-100%), membrane and cytoplasm		

Abnormal tissues: The antibody labeled 203/204 invasive ductal breast carcinoma, 5/49 invasive lobular breast carcinoma, 3/10 pleomorphic lobular carcinoma; 4/4 cases of tubulolobular breast carcinomas; and 5/ 9 invasive breast carcinomas, with uncertain classification between lobular and ductal type (1).