Sudan University of Science and Technology

College of Graduate Studies

Immunohistochemical Detection of Laminin Protein in Sudanese Women with Breast Tumors

الكشف النسيجي الكيميائي المناعي عن بروتين اللامينين في أورام الثدي لدى النساء السودانيات

A Dissertation Submitted in Partial Fulfillment for the Requirement of Master Degree in Medical Laboratory Sciences (Histopathology and cytology)

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الآيـة

قال الله تعـــالى:

(تِلْكَ ٱلْجَنَّةُ ٱلَّتِى نُورِثُ مِنْ عِبَادِنَا مَن كَانَ تَقِيّاً۞
وَمَا نَتَنَزَّلُ إِلَّا بِأَمْرِ رَبِّكَ لَهُ مَابَيْنَ أَيْدِينَا وَمَا خَلْفَنَا
وَمَا بَيْنَ ذَلِكَ وَمَا كَانَ رَبُّكَ نَسِيّاً)

صدق الله العظيم

سـورة مـريـم (63-64)

Dedication

To the person who taught me a lot and made me proud of him in all times and places

My father soul

To the woman who taught me and helped me

My Mother

To those who gave me the support and encouragement

My brothers and sisters

To those who stood beside me to complete this research

My dear friends

To all my colleagues in Sudan university

I dedicate this simple effort with my love and best wishes

Acknowledgement

All praise &thanks to my lord Allah who blessed me with the courage for completion of this study

Then

Iwould like to thank my

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Dr: Abu Elgasim Abass AwadAlkareem

Who helped and supported me patiently to complete this work.

I would like to thank all people who helped me to perform this research.

Abstract

This is hospital based descriptive retrospective case study conducted in Omdurman Teaching Hospital in Khartoum state, during the period from December 2018 to March 2019. The study aimed to detect the expression of laminin in breast tumors using immunohistochemical method. Forty paraffin embedded blocks from patients samples previously diagnosed as breast tumors were collected. One section of 3 micron thickness was cut from each paraffin block by rotary microtome and stained by immunohistochemical method (avidin biotin technique) for detection of laminin. Data collected from patient's files and results were analyzed using SPSS computer program.

Samples include 20 (50%) samples were malignant tumors, including invasive ductal carcinoma 16 (40%) samples, lobular carcinoma 2(5%) samples, micro papillary carcinoma 1 (2.5%) sample and metaplastic squamous cell carcinoma 1(2.5%) sample. And 20 (50%) samples were benign tumors; all of them were fibroadenoma samples. Grade of malignant tumors were 1 (5%)sample was grade 1, 9 (45%) samples were grade 11, 6 (30%) samples were grade 111, 4 (20%) were not graded.

The patients age range between 18 to 90 years with mean age of 40 years.

Malignant breast tumors revealed strong positive expression of laminin in 7 (35%) samples, and weak positive in 13 (65%) samples, while all benign tumors showed strong positive expression of laminin with significant association (P=0.000).

This study concludes that breast tumor tissue express laminin with high expression in benign tumors.

الخلاصة

أجريت دراسة الحالة الوصفية الإسترجاعية في مستشفى أم درمان التعليمي في ولاية الخرطوم ، خلال الفترة من ديسمبر2018إلى مارس 2019، وقد هدفت الدراسة للكشف عن تعبير اللامنين في أورام الثدي باستخدام كيمياء الأنسجة المناعية .

جمعت أربعون قالب مطمور بشمع البارفين من عينات مرضى تم تشخيصهم مسبقا بأورام الثدي.قطع مقطع واحد من كل عينة بسمك 3 ميكرون بواسطة جهاز المشراح وتم صبغ العينات بواسطة كيمياء الانسجة المناعية باستخدام طريقة (افيدين بيوتين) للكشف عن اللامينين. جمعتالبيانات من ملفات المرضى وحللت النتائج باستخدام برنامج الحزمة الاحصائية للعلوم الاجتماعية SPSS.

شملت العينات 20 (50%) عينة لأورام خبيثة تشمل سرطان الاقنية الغازية 16 (40%) عينة، السرطان المفصص 2 (5%) عينة، السرطان الحليمي الصغير 1 (2.5%) عينة وسرطان الخلايا الحرشفية الحؤولة 1 (2.5%) عينة. وكانت 20 (50%) عينات الاورام الحميدة جميعها الاورام الغدية الليفية. كانت درجة الاورام الخبيثة كالآتي 1 (5%) عينة من الدرجة الاولى و 9(45%) عينات من الدرجة الثانية و 6 (30%) عينات من الدرجة الثالثة و 4 (20%) عينات لم تدرج.

تراوحت أعمار المرضى بين 18 إلى 90 سنة ومتوسط العمر 40 سنة.

اظهر التعبير الكيميائي في أورام الثدي الخبيثة في 7 (35%) عينة و تعبير إيجابي ضعيف في 13 (65%) عينة، بينما أظهرت جميع عينات الأورام الحميدة تعبيرإيجابي قوي للامينين، مع وجود علاقة احصائية (القيمة الإحصائية =0.000).

خلصت هذه الدراسة إلى أن ظهور تعبير اللامينين في أنسجة أورام الثدي بالتعبير العالي في الأورام الحميدة.

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Chapter One

Introduction

Chapter One

Introduction

1.1 Introduction:

Breast tumors usually start from the ductal hyper proliferation, and then develop into benign or even metastatic carcinomas after constantly stimulation by carcinogenic factors (Sun, *et al*. 2017).

Breast cancer is the leading cause of cancer death in women worldwide. It is the main cause of cancer-related death in women in developing countries and second leading cause in women in developed countries. (Seely and Alhassan,2018*).*

Breast cancer in Sudan is the most frequent type of cancer in adult females with median age (36-45 years) (Saeed, *et al*.2016).

Breast cancer risk factors common ones include early age of menarche, late age of menopause, short breastfeeding duration, late age of first full term pregnancy (Tan, *et al*. 2018).

Diagnosis of breast cancer requires the sampling of breast epithelial cells by fine needle aspiration, and\or core biopsy, ultrasonically, or magnetic resonance imaging (MRI) (Cheuk, *et al*.2017).

The main types of treatment for breast cancer are surgery, radiation therapy, chemotherapy, hormone therapy and targeted therapy (Nouno,2015).

Laminins are large extracellular glycoprotein's that are expressed by basal epithelium and are important components of basement membranes. Laminins are heterotrimers containing alpha, beta, and gamma chains forming a cross –shaped structure (Carpenter, *et al*.2016).

At least sixteen isoforms of Laminins have been described and named according to their specific trimeric combination of its chains (Pouliat,*et al*.2013).

Laminin is involved in breast cancer invasion and metastasis, were assessed in a study by Xia Qiu for expression by immunohistochemistry and reported that it was positive expressed in normal breast tissue and strongly positive expressed but lost its continuity in the breast cancer tissue (Qiu, *et al*.2014).

On other study by Aoj showed that 146 of 255 (57%) from cases were positive for laminin expression with majority from premenopausal women (p=0.005) (Aoj, *et al*.2016).

1.2 Objectives:

1.2.1 General objective:

To study Laminin expression in breast tumors among Sudanese women.

1.2.2 Specific objectives:

1- To detect the expression of laminin in breast tumors using immunohistochemical method.

2- To correlate the expression of laminin with histopathological diagnosis of breast tumors.

Chapter Two

Literature Review

Chapter Two

Literature Review

2.1 Scientific background:

Breast cancer is a major health problem and was among the top three most common malignancies globally in 2012 (Zahoor, *etal*.2017). Breast cancer is by far the most frequent cancer among women with an estimated 1.38 million new cases diagnosed every year (Wang, *et al*.2018).

2.2 Structure of the breast:

The breast is described as being composed of glandular and adipose tissue held together by a loose framework of fibers called Cooper's ligaments. Histological studies demonstrate that the lobes are composed of lobules, which consist of clusters of alveoli containing mammary secretory epithelial cells. The alveoli are connected to very small ducts that join to form larger ducts draining the lobules. These larger ducts finally merge to a unique duct for each lobe. Under the areola, this single duct is depicted as widening into a lactiferous sinus before narrowing at the base of the nipple and terminating at its orifice on the surface of the nipple. Adipose tissue of the breast is situated between lobes rather than within lobules (Matthes, *et al*.2016).

2.3 Disorders of the breast:

2.3.1 Benign breast tumors:

Benign breast diseases are defined as any non-malignant breast condition and encompass a wide range of clinical and pathologic disorders. It is one of the most common diseases in the females of any society. Up to 30% of women suffer from (BBD)in anytime of their life (Selvakumaran, *etal*.2017).

2.3.1.1 Inflammatory andrelated lesions of the breast tumor:

Inflammatory breast, sometimes referred to as red breast syndrome, is a classic but rare complaint in women consulting at gynecology emergency centers. It usually presents as a red and hot breast and is often associated with breast pain. It is usually classified into one ofthree main groups: malignant, infectious, or inflammatory without infection (or mastitis) (Dabi, *etal*.2017).

Mastitis refers to inflammation of the breast parenchyma, often presenting with pain, heat and redness that may be a debilitating illness with prolonged morbidity (Leong, *et al*.2017).

Granulomatous inflammatory changes in the breast can be related to specific infectious agents such as mycobacterium tuberculosis, non-infectious disease such as sarcoidoses, foreign material as silicon, paraffin or suture material or trauma. It is present in premenopausal age (Aslam, *et al*.2013).

Non-puerperal subareolar mastitis and breast abscess, also known as Zuska's disease is an uncommon and recurring disease. It frequently affects middle-age females in their 40s but may affect a wide range ofages and may even be observed in males (Leong, *etal*.2018).

Fat necrosis is a benign inflammatory condition of the breast characterised histologically by interstitial infiltration macrophages and plasma cells fat necrosis can produce a myriad of appearances on mammography and ultrasound, so biopsy is often required for definitive pathological diagnosis (Gilliever, *etal*.2018).

2.3.1.2Fibrocystic changes:

Fibrocystic breast change (FBC), also termed fibrocystic breast disease, and is the general, all-inclusive term, for a whole range of common and benign breast disorders. FBC is most common among premenopausal women aged 20–50 years old. Such changes comprise all types of benign

conditions such as cysts, papilloma, apocrine, metaplasia, epithelial hyperplasia, and adenosis (Chen, *etal*.2018).

2.3.1.3 Tumors of the breast:

Fibroadenomas are the most common breast masses in adolescents. Fibroadenomas generally present as 2 to 3 cm in size (Cerrato, *et al*. 2013). Lipomas of the breast are usually small but a giant lipoma of the breast is uncommon (Rajamohammed, 2017). Tubular adenomas, also known as pure adenomas, are rare epithelial tumors of the breast accounting for 0.13 - 1.7% of benign breast lesions (Salemis, *et al*.2011). Breast hamartoma is an uncommon breast tumor that accounts for approximately 4.8% of all benign breast masses (Sevim, *et al*.2014). Granular cell tumor (GCT) is a benign rare tumor that usually affects the head and neck and sometimes also appears in the female breast (Lara, *et al*.2017).

2.3.2 Symptoms of breast cancer:

Symptoms are classifying into three main categories: (a) breast lump, (b) non-lump breast symptoms (including breast pain, breast skin or shape abnormalities and nipple abnormalities), and (c) non-breast symptoms (including fatigue, breathlessness, axillary symptoms, neck lump, and back pain (Koo, *etal*.2017).

2.3.2.1 Types of breast cancer:

2.3.2.1.1 Non-invasive breast cancers:

Ductal Carcinoma in Situ (DCIS) it is a neoplastic proliferation of epithelial cells limited to the ducts or lobules, characterized by cellular and nuclear atypia and potential malignant capacity (Makki, 2015).

2.3.2.1.2 Invasive breast cancer:

Invasive ductal carcinoma (IDC) is the most common form of invasive breast cancer. Invasive ductal carcinomas are breast cancers having malignant ductal proliferation along with stromal invasion in the presence or absence of DCIS, apart from their relative proportion. The appearance of the invasive component should be determined from the subtypes of IDC rather than from the types of DCIS or its grade. IDC is classified into many histological subtypes according to awide range of criteria, including cell type (as in apocrine carcinoma), amount, type and location of secretion (as in mucinous carcinoma), architectural features (as in papillary, tubular, and micropapillary carcinoma),and immunohistochemical profile (as in neuroendocrine carcinoma) (Makki, 2015).

2.3.2.1.3Special forms of breast cancer:

Paget's disease of the breast is a rare histological breast cancer, representing 1–3% of female breast cancers(Dubar, *etal*2017). Metaplastic carcinoma (MBC) is a rare histological subtype of carcinoma breast. It is classified into low-grade adenosquamous carcinoma, fibromatosis-like metaplastic carcinoma, squamous cell carcinoma, spindle cell carcinoma (Ghosh,*etal*.2017).

2.3.2.1.4 Other types of breast cancer:

The rarer histological types of breast cancer–including mucinous, tubular, medullary, and papillary carcinomas and other epithelial tumors.Other rare typesmicropapillary carcinoma, adenoid cystic carcinoma, secretory carcinoma, apocrine carcinoma, solid neuroendocrine carcinoma, neuroendocrine small cell carcinoma, metaplastic carcinoma with squamous cell differentiation (Gudaviciene, *etal*.2015).

2.4 Epidemiology of breast cancer:

Breast cancer is the most common cancer in women, with an estimated 1.67 million new cases diagnosed in 2012 (25% of all cancers in females). It ranks fifth among the most common causes of cancer-related mortality worldwide, while it is the most frequent cause of cancer death in less developed countries (Zahoor, *et al*.2017). Breast cancer is the most common type of cancer among women in the US with the incidence rate of 12.5% (Ataollahi, *etal*.2015).

Over 1.5 million women (25% of all women with cancer) are diagnosed with breast cancer every year throughout the world. In America, it is estimated that 30% of all new cancer cases (252,710) among women are breast cancer in 2017 (Sun, *et al*.2017).

2.5 Risk factors of breast cancer:

2.5.1Age:

Aging is one of the most important risk factors of breast cancer, because the incidence of breast cancer is highly related to the increasing age (Sun, *et al*.2017).

2.5.2 Sex:

Most breast cancer occurs in women and the number of cases is 100 times higher in women than that in men (Sun, *et al*.2017).

2.5.3 Family history:

A woman's risk of breast cancer is increased if she has a family history of the disease, the highest risk is associated with increasing number of first degree relatives diagnosed with breast cancer at a young age (under age 50) Compared with women who had no affected relatives (Shah, *etal*.2014).

2.5.4 Alcohol consumption:

The risk may increase as a result of drinking even a small amount of alcohol as it affects estrogen metabolism in the liver (Kamińska, *etal*.2015).

2.5.5 Fat intake:

Modern western diet contains too much fat and excess intake of fat, especially the saturated fat, is associated with mortality (RR=1.3) and poor prognosis in breast cancer patients (Sun, *etal*.2017).

2.5.6 Obesity and lack of exercise:

Obesity, specifically in postmenopausal women, has also been shown to increase a woman's risk of breast cancerConsistent physical activity has been shown to reduce the risk of breast cancer (Shah, *et al*.2014).

2.5.7 Hormones:

The cycles of endogenous estrogen levels throughout a woman's lifetime have implications for the development of or the protection against breast cancer. High endogenous sex hormone levels increase the risk of breast cancer in both premenopausal and postmenopausal women (Shah, *etal*.2014).

2.5.8 Radiation:

Radiation exposure from various sources including medical treatment and nuclear explosion increases the risk of breast cancer (Shah, *etal*.2014).

2.5.9 Smoking:

Women exposed to cigarette smoke during childhood or married to a cigarette smoker are more prone to breast cancer. The onset of smoking in younger age increases the risk of cancer breast. Women who started smoking at 10-14 years were more proneto breast cancer (Attaollahi, *etal*.2015).

2.5.10 Medical conditions:

Proliferative lesions of benign character occurring in mammary glands they are benign; however, they can significantly increase the risk of occurrence of malignant lesions (Kamińska,*etal*.2015)

2.6 Diagnosis of breast cancer:

2.6.1 History:

The clinical history is directed at assessing cancer risk and establishing the presence or absence of symptoms indicative of breast disease. It should include age at menarche, menopausal status, previous pregnancies and use of oral contraceptives or post-menopausal hormone replacements (Shah, *etal*.2014)

2.6.2 Physical examination:

Physical examination should include a careful visual inspection with the patient sitting upright. Nipple changes, asymmetry and obvious masses should be noted. The skin must be inspected for changes such as; dimpling, erythema, peaud' orange (Shah, *etal*.2014).

2.6.3 Mammography:

Mammography is the current standard breast screening technique, but it is less effective for subjects under 40 years old and dense breasts, less sensitive to small tumors (less than 1 mm, about 100,000 cells), and does not provide any indication of eventual disease outcome (Wang, *etal*.2017).

2.6.4 Ultrasonography:

Ultrasound has been applied as an additional medical imaging tool for mammography Breast ultrasonography has been recommended as a supplement to mammography for subjectswith high breast cancer risk pregnant women and subjects who cannot to have mammography (Wang, *e*t *al*.2017).

2.6.5 Fine needle aspiration:

FNA is considered a diagnostic method for cystic lesions and lesions suspected of lymph node metastases. It can also be conducted for lesions located near the chest wall. Advantages of FNAB include: ready availability, simplicity of the technique, low cost and, most of all, low risk of complications.It requires no anesthesia, is minimally-invasive and relatively patient-friendly(Lukasiewicz, *etal*.2017).

2.6.6 Core needle biopsy:

Core-needle biopsy is an invasive procedure conducted under local anesthesia and with image guidance (US, MMG, MRI). The equipment needed includes a biopsy instrument and a needle with a large diameter (Lukasiewicz, *etal*.2017).

2.6.7 Magnetic resonance imaging (MRI):

MRI is a powerful imaging tool that produces high-resolution images without requiring the application of harmfulradiation(Nounou,*etal*.2015).

2.7 Treatment:

The main types of treatment for breast cancer are surgery, radiation therapy (RT), chemotherapy(CT), endocrine (hormone) therapy (ET), and targeted therapy(Nounou,2015).

2.7.1 Surgery:

Depending on the stage and type of the tumor, just lumpectomy may be all that is necessary, or removal of larger amounts of breast tissue may be necessary, surgical removal of the entire breast is called mastectomy (Kabel, *etal*.2015).

2.7.2 Hormone blocking therapy:

Hormone receptor-positive cancers are often treated with hormone-blocking therapy overseveral years (Kabel, *etal*.2015).

2.7.3 Chemotherapy:

Estrogen receptor is a major target for chemotherapy because more than 70% of breast cancers are ER-positive breast cancers(Sun, *etal*.2017).

2.7.4 Radiation therapy:

Radiation therapy is an adjuvant treatment for most women after lumpectomy or mastectomy. The purpose of radiation is to reduce the chance of recurrence. Radiation therapy involves using high-energy X-rays or gamma rays that target a tumor or tumor site(Kabel, *etal*.2015).

2.7.5 Monoclonal antibodies:

Monoclonal antibodies or other immunomodulators may be given in advanced stages with distant metastasis(Kabel, *etal*.2015).

2.8 Laminin:

Laminins (LMs) are abundant extracellular matrix (ECM) proteins present predominantly in basement membranes (BM). At least 16 isoforms have been described and named according to their specific trimeric combination of a, β and c chains using the new nomenclature (Pouliot, *etal*.2012).

Laminin is a heterotrimeric glycoprotein with diverse functions during embryonic development and in mature tissues. This extracellular matrix protein is involved in the mediation of attachment, migration and organization of cells into tissues during embryosis. It also has a role in cellular differentiation and cell survival, and in supporting the growth of embryonic stem cells. In mature on the cell surfaces. In addition to its physiological roles, laminin expression has been implicated in tumor tissue, laminin is a component of the epithelial and vascular basement membrane where it is involved in the maintenance of cell adhesion and cohesion. It is secreted by both epithelial and stromal cells and binds to integrin receptors progression. Cancer cells express laminin on their cell membranes during invasion to evade anoikis (apoptosis which is

triggered by cell detachment from the basement membrane). Indeed, laminin expression has been implicated in the hallmarks of carcinogenesis; including cell proliferation, invasion metastases and the epithelial-mesenchymal transition (Aoj, *etal*.2016).

Qiu, *etal*. (2014) reported that laminin is involved in breast cancer invasion and metastasis, and can use this to determine whether the integrity of a basement membrane for differential diagnosis of benign and malignant breast tumors

Kwon, *etal*. (2012) reported that positive expression of laminin 332 was identified in the tumor cells of 56 cases (70%) of the 80 TN cases; expression was identified in only 15.2% of the non-TN cases.

Pellegrini, *etal*. (1995) reported that of 887 cases of primary breast carcinoma tested, 244 (28%) were found to be positive in the cytoplasm, with a positivity ranging from 30% to 100% of tumor cells.

Chapter Three

Materials and Methods

Chapter Three

Materials and Methods

3.1 Materials:

Archived tissue blocks of breast tumors samples were used in this study.

3.2.1 Study design:

This is a hospital based descriptive retrospective case control study aimed to detect expression of laminin in breast tumor using

immunohistochemical method.

3.2.2 Study samples:

Forty tissue blocks were obtained from breast tumor samples. Twenty samples were previously diagnosed as malignant breast tumor and twenty samples were diagnosed as benign breast tumor.

3.2.3 Study area:

This study was held in Omdurman teaching hospital during the period from December 2018 to March 2019.

3.2.4 Immunohistochemical staining:

Section of 3 micron thickness was obtained from each formalin fixed paraffin embedded tissue using a rotary microtome. Then stained using monoclonal antibodies new indirect technique as follow: Sections were dewaxed and cleared in two changes of xylene for two minutes. Then hydrated through descending concentrations of ethanol (100% 90% 70% 50%) and water two minutes for each. Then Ag retrieval technique for thirty minutes at 95 Cº (Colin jar contain citrate buffer pH6.0), washed in phosphate buffer saline (pH 7.4) for five minutes, after

that treated with hydrogen peroxidase solution for ten minutes. Washed in phosphate buffer saline (pH7.4) for five minutes, treated with laminin primary antibody for twenty minutes, then rinsed in phosphate buffer saline then binding of antibody was detected by incubating for twenty minutes with biotin followed by fifteen minutes with strepto avidin (Thermo kit), the sections were washed in three changes of phosphate buffer saline, then treated with substrate and 3,3-diaminobenzidene tetra hydrochloride (DAB) chromogen for seven minutes, washed in phosphate buffer saline, counterstained in Mayer's haematoxylin for one minute, washed and blued in running tap water, then dehydrated through ascending concentration of ethanol (50% 70% 90% 100%), cleared in xylene and mounted in DPX mountant (Bancroft and Marilyn, 2008).

3.2.5 Result interpretation:

All quality control measures were adopted during sample staining and immunohistochemical results assessment. Positive and negative controls were used to confirm location of positivity of laminin expression that was confirmed by five cells per one filed.

3.2.6 Statistical analysis:

Data were analyzed using SPSS version 11.5 computer program, frequencies, means and Chi-square tests were calculated.

3.2.7 Ethical considerations:

Hospital administration agreements were taken ethically for archive samples and patients data collection.

Chapter Four

Results

Chapter Four

Results

4. Results:

A total of 40 samples collected from patients samples affected with breast tumors were investigated, 20(50%) of them were malignant tumors, including invasive ductal carcinoma 16(40%) samples, lobular carcinoma 2 (5%) samples, micro papillary carcinoma 1 (2.5%) sample, metaplastic squamous cell carcinoma 1 (2.5%) sample, the remaining were benign tumors samples all of them were fibroadenoma 20 (50%) samples, as indicated in table (4.1). The description of cancer grade revealed that 1 (5%) samples were grade 1, 9 (45%) samples were grade 11, 6 (30%) samples were grade 111, 4 (20%) samples were not graded, as indicated in table (4.2). The age of study population showed that 40 and less years were 20 (50%) patients and more than 40 years were 20 (50%) patients, as indicated in table (4.3). Malignant breast cancer revealed strong positive expression of laminin in 7(35%) samples and weak positive expression in 13 (65%) samples, while all benign tumors showed strong positive expression of laminin , this result showed significant association (P.value=0.000), as indicated in table (4.4).

Table (4.1): Histopathological diagnosis of the study samples:

Histopathology diagnosis		Frequency	Percent
Malignant	Invasive ductal carcinoma	16	40%
	Micro papillary carcinoma	1	2.5%
	Metaplastic squamous cell carcinoma	1	2.5%
	Lobular carcinoma	2	5%
Benign	Fibroadenoma	20	50%
Total		40	100%

Table (4.2): Distribution of cancer grade amongmalignant breast tumors:

Grade	Frequency	Percent
Grade 1	1	5%
Grade 2	9	45%
Grade 3	6	30%
Not graded	4	20%
Total	20	100%

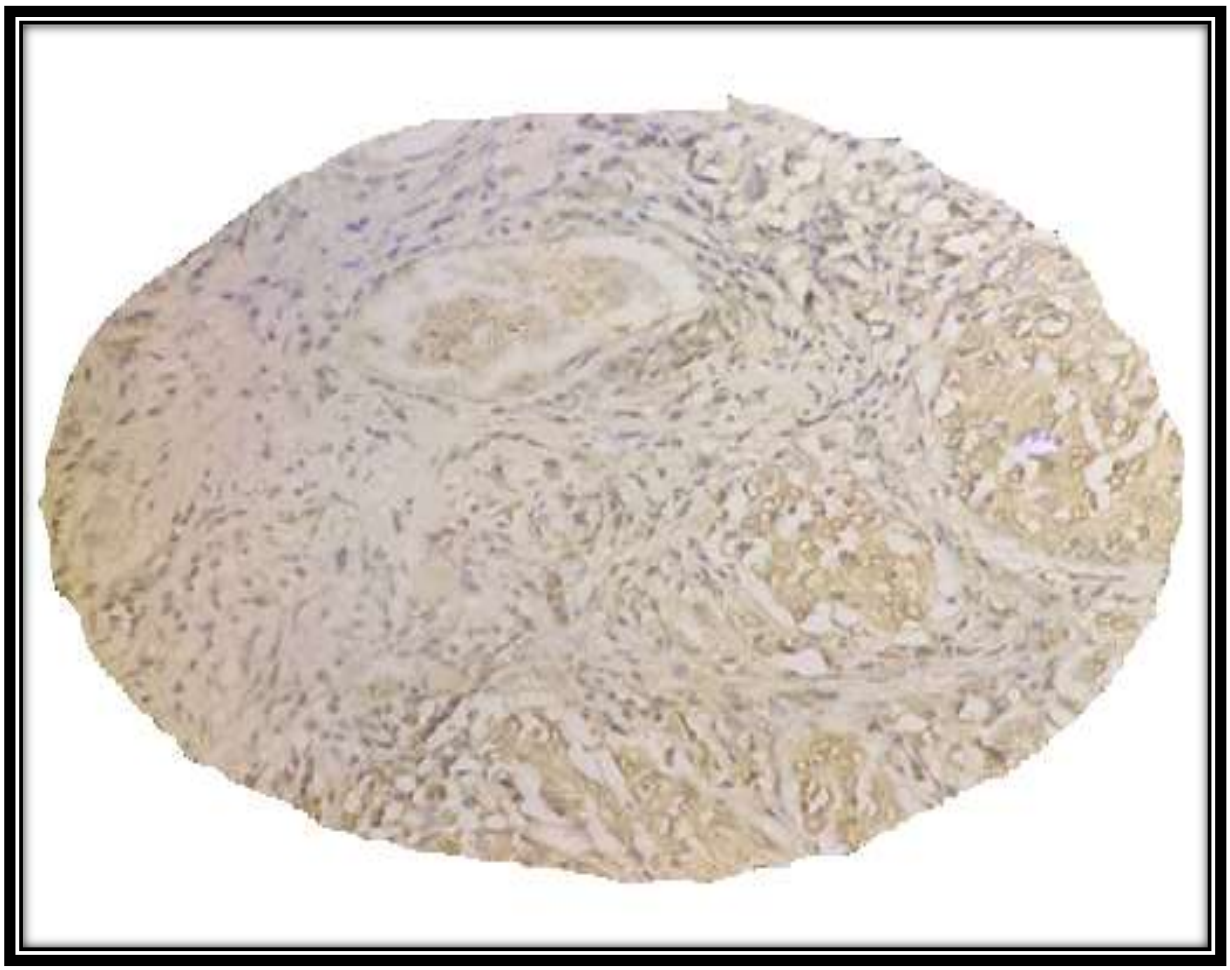
Table (4.3): Distribution of age group among studypopulation:

Age	Frequency	Percent
40 and less	20	50%
More than 40	20	50%
Total	40	100%

Table (4.4): Relation between laminin expression and histopathological diagnosis of breast tumors:

Histopathology diagnosis	Laminin expression		Total	P.value
	Strong positive N (%)	Weak positive N (%)		
Malignant	7 (35%)	13 (65%)	20 (50%)	0.000
Benign	20 (50%)	0 (0%)	20 (50%)	
Total	27 (67.5%)	13 (32.5%)	40 (100%)	

Graph (4.1): Invasive ductal carcinoma of breast grade 111 showing weak cytoplasmic expression of laminin (40 x).



Graph (4.2): Benign breast tumor showing strong cytoplasmic expression of laminin (40 x).

Chapter Five

Discussion ,

conclusion and recommendations

Chapter Five

Discussion

In this study forty samples from patients affected with breast tumor were investigated by immunohistochemical method for detection of laminin expression. The study revealed that the age of study population range from 18 to 90 years with mean age of 40 years. Most malignant type patients were more than 50 years; this is due to the exposure to carcinogens over a longer period oftime and the decreasing power of the immune system with age. This result was agreed with Sun, *et al*. (2017), who reported that the incidence ofbreast cancer is highly related to the increasing age. Also compatible with result observed by Shah, *et al*. (2014), who reported that the risk of developing breast cancer increases with age.

This result was disagree with Parsa, *et al*. (2016), who reported that breast cancer occurred before the age of 40 is clinically more aggressive and has a higher possibility of metastasis and lower survival of older patients.

Most type from malignant samples founded was invasive ductal carcinoma; this result agreed with Zangouri, *et al*. (2018), who reported that invasive ductal carcinoma was the most common subtype of breast carcinoma and responsible for significant breast cancer mortality.

Laminins are large extracellular glycoprotein's that are expressed by basal epithelium and areimportant components of basement membranes that enhances themigration and invasion of breast carcinoma cells. In this study strong expression of laminin is observed in malignant breast tumors 7/20, and all benign breast tumors showed strong expression also. This relation showed significant association (P.value=0.000), this finding is compatible with result observed by Aoj, *et al*. (2016), who reported that

laminin was expressed in 146 (57.3%) cases wereconsidered positive for laminin expression. Also compatible with result observed by Pellegrini, *etal*. (1995), who reported that of 887 cases of primary breast carcinoma tested, 244 (28%) were found to be positive in the cytoplasm, with a positivity ranging from 30% to 100% of tumor cells.

5.2 Conclusion: On the basis of this study we conclude:

Laminin is highly expressed in benign breast tumors.

5.3Recommendations:

Further studies should be done on the expression of laminin in breast tumors.

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Appendix

Appendix

MATERIALS AND INSTRUMENTS

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

Disposable gloves.

Rotary microtome.

Microtome knifes.

Coated slides.

Cover glasses.

Oven.

Water path.

Re embedding paraffin block.

Humidity chamber.

Ethanol (100%, 90%, 70%, 50%).

Xylene.

Mayer's haematoxylin (1 gm haematoxylin, 50 gm aluminum ammonium sulphate, 0.2 sodium iodate, 50 gm chloral hydrate, 1 gm citric acid and 1 liter distilled water).

Sodium citrate buffer :(10 Mm sodium citrate, 0.05% Tween20, PH 6.0 prepared as the following: Tri-sodium citrate (dehydrate) 2.94 g, 1 liter distilled water mix to dissolve and add 0.05 ml of Tween20 and mix).

Phosphate buffer (PH 7.4).

Hydrogen peroxidase blocking solution.

Primary antibody Laminin.

Secondary antibody.

DAB (3.3 diaminobenzidine).

DAB substrate buffer.