Sudan University of Science and Technology

College of Graduated Studies

Immunohistochemical Expression of Breast Cancer Antigene1 Mutant Gene among Sudanese Women

التعبير النسيجي الكيميائي المناعي لطفرة الجين المسبب لسرطان الثدي 1 لدى النساء السودانيات

A dissertation submitted in partial fulfillment of the requirement of sM.Sc degree in medical laboratory science (Histopathology and Cytology).

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الآيـة

قال تعالى :

(اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1)خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (2)اقْرَأْ وَرَبُّكَ الْأَكْرَمُ (3)الَّذِي عَلَّمَ بِالْقَلَمِ (4)عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (5))

صدق الله العظيم

سورة العلق الآيات من (1-5)

Dedication

I dedicate my dissertation work to

My mother and my family

My friends Sara Hassan and Wegdan Abed Alrheem

Acknowledgment

Firstly, I would like to thank Allah for giving me the strength and support to complete this work.

I would like to thank my supervisor Dr. Abu Elgasim Abass Awad Elkareem for his help and patient.

I would like to thank all the staff of histopathology and cytology department in college of medical laboratory in Sudan University of Science and Technology.

Finally, I thank my family for support and encouragement.

Abstracts

This is hospital-based case study conducted in Radiation and Isotopes Center (Khartoum) during the period from April 2018 to July 2019. The study aimed to detect BRCA1 mutant gene expression in breast cancer among Sudanese women.

Fifty-eight formalin fixed paraffin embedded blocks previously diagnosed as breast cancer were collected. One section of three microns thickness was cut from each block and stained by immunohistochemical method for detection of BRCA1 mutant gene. The data was collected from patient's files and the results obtained were analyzed by using SPSS computer program version 16.

The diagnosis of malignant tumors subtypes was 54 (93.1%) ductal carcinoma, 3 (5.2%) medullary carcinoma, and 1(1.7%) papillary carcinoma.

The grading of malignant tumors was 7 (12.1%) grade I, 12 (20.7%) grade II, 35 (60.3%) grade III, while 4 (6.9%) of samples were not graded.

Patients age ranged between 27 to 79 years with the mean age of 48 years, 42 (72.4%) of samples were over 40 years while the remaining 16 (27.6%) were less than 40 years.

The expression of BRCA1 mutant gene among the study samples was 5/58 (8.6%) show positive expression, while 53/58 (91.4%) of samples showed negative expression of BRCA1 mutant gene.

The relation between BRCA1 mutant gene expression and malignant tumors subtypes was 4 samples ductal carcinoma and 1 sample of

Medullarycarcinoma, showed positive expression of BRCA1 mutant gene, while negative expression of BRCA1 mutant gene inpapillary carcinoma, with insignificant correlation between BRCA1 mutant gene expression and breast cancer subtypes (P value 0.284).

The relation between BRCA1 mutant gene expression and grading of malignant tumors was 4 grad III and one sample of not graded tumors showed positive expression of BRCA1 mutant gene, while negative expression of BRCA1 mutant gene in grad I and grad II of the malignant tumors, with insignificant correlation between BRCA1 expression and grading of malignant tumors (P value 0.320).

The study concludes that BRCA1 mutant gene expression not correlated with the breast cancer subtypes and grades.

المستخلص

اجريت هذه الدراسة دراسة الحالة فى مركز العلاج بالأشعة والطب النووى)الخرطوم (فى الفتر من ابريل 2018الى يوليو 2019هدفت هذه الدراسة للكشف عن التعبير لطفرة الجين المسبب لسرطان الثدي 1في سرطان الثدي لدى النساء السودانيات .

جمع ثمانية وخمسون قالب شمعي مطمور فى شمع البرافين كانت مشخصة مسبقا كأورام خبيثة للثدي ,قطع من كل قالب شمعي شريحة بسمك ثلاثة مايكرون وصبغت العينات بإستخدام تقنية الكشف النسيجي الكيميائي المناعي ,المعلومات التي جمعت من ملفات المرضى والنتائج التي تم الحصول عليها حللت بإستخدام برنامج الحزمة الإحصائية للعلوم الإجتماعية الإصدار 16.

اشتملت انواع الأورام الخبيثة على (93.1%) 54عينة سرطان الأقنية (5.2%) 3 ,عينة سرطان الثدى النخاعي وعينة واحدة (1.7%)كانت سرطان الثدى الحليمي.

مراحل الأورام الخبيثة كانت (12.1%) 7عينة في المرحلة الأولي و (20.7%) 12عينة في المرحلة الثاتية و (60.3%) 35عينة في المرحلة الثالثة و (6.9%) 4عينات لم يتم تصنيفها لمراحل.

تراوحت أعمار المرضى بين 27الى 79عام مع متوسط عمر 48عاما (72.4%) 42 ,عينة كانت فوق 40عام والباقي (27.6%) 16عينة اقل من 40عاما .

التعبير لطفرة الجين المسبب لسرطان الثدي 1في العينات كانت (8.6%) 58/5 من العينات اظهرت تعبير ايجابي لطفرة الجين المسبب لسرطان الثدي 1بينما (91.4%) 58/53من العينات لم تظهراي تعبيرلطفرة الجين المسبب لسرطان الثدى .1

العلاقة بين التعبير لطفرة الجين المسبب لسرطان الثدي 1ونوع الورم الخبيث كانت كالأتي 4 عينات من سرطان الأقنيه وعينة واحدة من سرطان الثدى النخامي اظهرت تعبير ايجابي لطفرة الجين المسبب لسرطان الثدي ,1بينما لوحظ تعبيرسلبي لطفرة الجين المسبب لسرطان الثدى 1 في الورم الحليمي ,مع عدم وجود علاقه بين التعبير لطفرة الجين المسبب لسرطان الثدي 1 ونوع الورم الخبيث بالثدي)القيمة الإحتمالية تساوي (0.284

العلاقه بين التعبير لطفرة الجين المسبب لسرطان الثد ي1و مراحل الورم الخبيث كانت 4من عينات من المرحله الثالثه و عينة واحدة من العينات الغير مصنفة لمراحل اظهرت تعبير ايجابي

لطفرة الجين المسبب لسرطان الثدي 1 بينما لوحظ تعبير سلبي لطفرة الجين المسبب لسرطان الثدي1 في العينات بالمرحله الأولي والثانيه من الورم ,مع عدم وجود علاقه بين التعبيرلطفرة الجين المسبب لسرطان الثدي 1ومراحل الورم الخبيث بالثدي(القيمة الاحتمالية تساوي 0.320) قلصت هذه الدراسة الي عدم وجود علاقة بين التعبير عن طفرة الجين المسبب لسرطان الثدي 1 و بين انواع ومراحل الأورام السرطانية بالثدي .

List of abbreviations

BRCA1	Breast cancer antigen one
DCIS	Ductal carcinoma insitue
LCIS	Lobular carcinoma insitue
DAB	3,3 diaminobenzidene tetra hydrochloride
DPX	Distyrene plasticizer xylene
RICK	Radiation and isotope center Khartoum
SPSS	Statistical package for social science

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Introduction

Chapter one

Introduction

1.1 Introduction

Breast cancer is complex cellular tumor of the breast in which the cells are rapidly, constituently, and aggressively degenerated leads to increases of tumor size (Comanescu and Popescu, 2009).

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).

In Sudan breast cancer is the most common type of cancer with incidence rate 25.1 per 100,000 women (Saeed, *et al*. 2014).

There is many factors that increase the chance to develop breast cancer in women includes, age, benign disease of the breast, menstrual history, family history, pregnancy and germ line mutation of BRCA1 (Kumar, *et al*. 2018).

Common technique used for diagnosis of breast cancer is physical examination of breast; mammography, fine needle aspiration and paraffin-embedded block contain tissue obtained by surgical procedure from tumor site using histopathology and immunhistochemical stain to detect estrogen, progesterone receptors and HER2/ Neu (Kumar, *et al*. 2013) (Dabbagh, *et al*. 2015).

The treatment of breast cancer includes estrogen deprivation blockage (anti- estrogen), HER2 cytotoxic therapy (Herceptin), radiotherapy and chemotherapy (Kumar, *et al*.2018).

BRCA1 is encoded protein required for repair of certain kind of DNA damage and normally express in different cell and tissue, the germ-line mutation of BRCA1 has close association with breast cancer especially triple negative breast cancer (Kumar, *et al.*2018).

In study of BRCA1 mutant gene expression among Iraqian women with breast cancer the result was 39.02% of samples showed positive expression of BRCA1 mutant gene (Dabbagh, *et al.* 2015).

Another study to detect BRCA1 mutant gene among Indian women with breast cancer the result was 38% of samples showed positive expression of BRCA1 mutant gene (Sharma, *et al.* 2016).

1.2 Objectives:

1.2.1 General objective:

To study the expression of BRCA1mutant gene in breast cancer among Sudanese women.

1.2.2 Specific objectives:

1-To detect BRCA1 mutant gene expression in breast cancer tissue among Sudanese women using immunohistochemical method.

2-To correlate BRCA1 mutant gene expression with breast cancer subtypes and grades.

Chapter two

Literature review

Chapter two

Literature review

2.1 Historical background:

Breast cancer is common cause of death in women, the survival rate is depend on many factors include, stage of diagnosis, type of breast cancer, and treatment. The survival rate is poor in developing countries comparing to Weston world (Dabbagh, *et al*. 2015).

2.2 Structure of the breast:

The lobules are the functional unit of the breast supported by interlobular stromal cells. The breast contain inner luminal epithelial cells which secret milk during lactation and contain myoepithelial cells that support basement membrane of the breast, also contain acini and ducts that reach to the nipple to conduct milk during lactation (Kumar, *et al*. 2018)

2.3 Disorders of the breast:

2.3.1 Benign disorders of the breast:

2.3.1.1 Fibrocystic changes:

It causes by proliferation of connective tissue forming fibrosis and flow by epithelium proliferation (Vorheer, 1986).

Fibrocystic changes subdivided into:

2.3.1.1.1Non proliferative changes:

It is the most common type of benign disease of the breast, it is cystic formed by dilation and obstruct of collecting ducts formed by fibrosis over grow. It characterized by formation of many cysts with different size (Mohan, 2010).

2.3.1.1.2Prolifreative changes:

2.3.1.1.2.1 Epithelial hyperplasia:

It is epithelial hyperplasia of ducts and lobules of the breast, which lined by more than two layers of epithelial cells instead of two layers. The spectrum of epithelial hyperplasia ranging from mild to atypical hyperplasia (Kumar, *et al*. 2013).

2.3.1.2 Sclerosing adenosis:

It is benign proliferative breast disease associated with disorder of the acinar and present of myoepithelium and connective tissue in the terminal ductal lobules (Chen, *et al.* 2016).

2.3.1.3 Mastitis:

It is inflammatory process of the breast; it is common in lactating women. It usually cause by staphylococcus aurous bacteria (Kataria, *et al.* 2013).

2.3.1.4 Fibro adenoma:

It is the most common surgical treated breast disease, characterized by firm non-tender clearly demarcated mass about 2 to 3 cm in size. The tumor may enlarge slowly during menstrual cycle without pain or change in the skin or nipple (Cerrato and Labow, 2013).

2.3.1.5 Phyllodes tumor:

It is fibro epithelium lesion represents 0.3% to 0.5% of female breast tumors. It is giant type of fibro adenoma; usually occur in women between 45 up to 49 years (Mishra, *et al.* 2013).

2.3.1.6 Intraductal papilloma:

It is benign breast tumor comes from the epithelium of lactiferous ducts. It is common in women between 30 to 77 years. There are two type of intraductal papilloma, central and peripheral. The central intraductal papilloma is solitary tumor arise during premenopausal period.

The peripheral intraductal papilloma is appearing in younger women and rising within the terminal duct- lobular units (Tarallo, *et al.* 2012).

2.3.2 Malignant disorders of the breast:

2.3.2.1 Noninvasive tumors of the breast:

2.3.2.1.1 Ductal carcinoma insitue (DCIS):

It represents about 20% 0f all breast cancer, usually asymptomatic and the lesion may develop to invasive disease. Ductal carcinoma insitue have excellent prognosis and 15 years mortality rate (Votavec, *et al.* 2014).

2.3.2.1.2 Lobular carcinoma insitue (LCIS):

It represents 5.3% of insitue carcinoma. It has lower risk to develop invasive lobular carcinoma comparing to ductal carcinoma insitue. Mammographic picture of breast is very necessary to detect micro calcification (Cheng, *et al.* 2017).

2.3.2.2 Invasive tumors of the breast:

2.3.2.2.1 Invasive ductal carcinoma:

The term used to describe all carcinoma that not sub classified into specific group. Usually associated with ductal carcinoma insitue and rarely associated with lobular carcinoma insitue. Most of ductal carcinoma replaces normal breast fat with hard palpable mass, the invasion to lymph -vascular space may notice. Most of invasive ductalcarcinoma expresses ER, PR hormone receptor and one third express HER2/Neu (Kumar, *et al*. 2013).

2.3.2.2.2 Invasive lobular carcinoma:

It is common type of breast cancer represent up to 15% of breast cancer cases. The tumor have good prognosis and usually positive for hormone receptors, HER2/Neu and P53. This tumor is lake expression of E-cadherin which responsible of cell adhesion. Invasive lobular carcinoma has good respond to endocrine therapy (Reed, *et al.* 2015).

2.3.2.2.3 Papillary carcinoma:

It is papillary malignant tumor surrounded by myoepithelium cells; it is difficult to diagnosis and grading. Papillary carcinoma tumor is solid and produces mucins. It is usually seeing in post-menopausal women (Ingle, *et al.* 2016).

2.3.2.2.4 Inflammatory carcinoma:

It is rare and aggressive form of breast cancer. It characterized by edema and redness of the breast with or without palpable mass in the breast and present of lymphatic metastasis (Raghare, *et al.* 2016).

2.3.2.2.5 Adencystic carcinoma:

It is type of the breast cancer comprised about 0.1% to 1% of all breast cancer. It characterized by present of proliferative glands and stromal basement membrane, the lymphatic metastasis is uncommon. The tumor has good prognosis. It is common in women in fifth and sixth decade (Kocaay, *et al*. 2016).

2.3.2.2.6 Medullary carcinoma:

It is represent 5% of invasive breast cancer, characterized by lymphatic metastasis. This tumor usually not express ER, PR, or HER2/Neu, often referred to triple negative breast cancer, but 30% - 40% of cases express estrogen and progesterone receptors (Huober, *et al*. 2012).

2.3.2.2.7 Colloid (mucinous) carcinoma:

It is associated with ductal or lobular neoplasm. Pure mucinous breast cancer is rare, it characterized by present of extracellular mucins (Dumitru, *et al.* 2015).

2.3.2.2.8 Secretary juvenile carcinoma:

Malignant breast tumor occurs in children and infant in rare cases. It is asymptomatic mass in breast and uncommon behavior and appearance (Karl, *et al*. 1985).

2.3.2.2.9 Tubular carcinoma:

It is will differentiated invasive carcinoma surrounded with tubules and fibro hyaline stroma. Tubular carcinoma has good prognosis and five years survival rate (Min, *et al*. 2013).

2.3.2.2.10 Metaplastic carcinoma:

It represent 1% of all breast cancer, is malignant of two or more cellular type, and commonly the tumor is mixture of epithelium and mesenchymal tissue (Bostrom, *et al.* 2017).

2.3.2.3 Paget's disease:

Malignant disease of the nipple, it is rare and associated with insitue or invasive ductal carcinoma, the nipple is crusted, retracted and red in color with pain (Karaka, *et al.* 2011).

2.4 Epidemiology of breast cancer:

Breast cancer is the most common type of cancer in women and the commonest cause of death in developing countries. In India between 1982 and 2005 the breast cancer cases have more than doubled in the last 10 years (Sharma, *et al.* 2014).

In Sudan breast cancer is the commonest type of cancer with incidence rate 25.1 per 100,000 women (Saeed, *et al*. 2014).

In England and America approximately one in every ten women will develop breast cancer in their life time (Dabbagh, *et al*. 2015).

2.8 Role of BRCA1 in breast cancer:

BRCA1 is important factor in DNA repair system; hereditary breast cancer is associated with BRCA1 mutation account 5% of all breastcancer (Dabbangh, *et al*. 2015).

BRCA1 mutation is responsible for majority of hereditary breast cancer, women with BRCA1 mutation have higher risk to develop breast cancer comparing to women without BRCA1 mutation (Sharma, *et al*.(2014).

In study of BRCA1 mutant gene expression amonge Sudanese women with breast cancer the result was 18% of samples showed positive expression of BRCA1 mutant gene (Ahmed, *et al* . 2019).

In study of BRCA1 mutant gene expression among Tunisian women with hereditary breast cancer the result was 66% of samples showed positive expression of BRCA1 mutant gene (Troudi, *et al*. 2007).

Other study of BRCA1 mutant gene among Korean women with breast cancer the result was 36.5% of samples showed positive expression of BRCA1 mutation (Kim, *et al*. 2011).

Also another study of BRCA1 mutant gene among Indian women wih breast cancer the result was 35% of samples showed positive expression of BRCA1 mutant gene (Sharma, *et al*. 2014).

Chapter three

Materials and methods

Chapter three

Materials and methods

3.1Materials:

Archived tissue blocks obtained from breast samples previously diagnosed as breast cancer were selected for this study.

3.2 Method:

3.2.1 Study design:

This is hospital based case study of BRCA1 mutant gene in breast cancer among Sudanese women using immunohistochemical method.

3.2.2 Study samples:

Fifty-eight samples in paraffin blocks previously diagnosed as breast cancer selected for this study from (RICK) hospital. Patient identification data (age, grading of malignant tumor, and subtypes of breast cancer) are obtained from patients files.

3.2.3Study area and duration:

This study was performed in Radiation and Isotope Center (Khartoum) hospital in the period from April 2018 to July 2019.

3.2.4 Samples preparation:

Microarray technique was performed to obtain three blocks from 58 samples,one section of three micron were cut and mounted in positively charged slides, the section deparaffinied in xylene and rehydrated through series graded alcohol and water.

3.2.5 Immunohistochemical stain:

The section steamed for antigen retrieval in water bath.

Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 minutes, then the slides were incubated with primary antibodies (anti-BRCA1) for 20 minutes. After washing with PBS the sections were incubated for 10 minutes with secondary antibody. Then the sections were washed in PBS. The binding antibody was detected by adding 3.3 diaminobenzidine tetra hydrochloride (DAB) for 5 minutes as chromogen to produce the characteristic brown stain for the visualization. The slides counter stained with Mayer's haematoxylin for 1 minute then washed and rehydrated through graded series alcoho, cleared in xylene and mounted in DPX (Bancroft and Marilyn, 2008)

3.2.5 Quality control:

For each batch of stain positive and negative control slide were prepared. The positive control section is obtained from known positive sample and the negative control slide was obtained from the same tissue block, but was incubated with PBS instead of the primary antibody. Each slide was evaluated with investigator then the result confirmed by histopathologist.

3.2.6 Result interpretation:

BRCA1 mutant gene expression result classified as flows: negative staining showed no staining or staining in less than 10% of the lesion, positive staining showed staining in more than 10% of the lesion (Vaz, *etal*.2007)

3.2.7 Data analysis:

Data was analyzed by SPSS computer program (chi square test, frequency, mean, P value) were calculated.

3.2.8 Ethical consideration:

The study performed after approval to use tissue blocks and patients files from the (RICK) hospital.

Chapter four

Results

Chapter four

Results

4. Results:

Fifty-eight of formalin fixed tissue blocks previously diagnosed as breast cancer were selected and patient's information was obtained from hospital files.

The diagnosis of malignant tumors subtypes was 54 (93.1%) ductal carcinoma, 3 (5.2%) medullary carcinoma, and 1(1.7%) papillary carcinoma as indicated in table (4-1).

The grading of malignant tumors was 7 (12.1%) grade I, 12 (20.7%) grade II, 35 (60.3%) grade III, while 4 (6.9%) of samples were not graded as indicated in table (4-2).

Patients age ranged between 27 to 79 years with the mean age of 48 years, 42 (72.4%) of samples were over 40 years while the remaining 16 (27.6%) were less than 40 years as indicated in table (4-3).

The expression of BRCA1 mutant gene among the study samples was 5/58 (8.6%) show positive expression, while 53/58 (91.4%) of samples showed negative expression of BRCA1 mutant gene.

The relation between BRCA1 mutant gene expression and malignant tumors subtypes was 4 samples ductal carcinoma and1 sample of medullarycarcinoma, showed positive expression of BRCA1 mutant gene, while negative expression of BRCA1 mutant gene in papillary carcinoma, with insignificant correlation between BRCA1 mutant gene

expression and breast cancer subtypes (P value 0.284) as indicted in table (4-4).

The relation between BRCA1 mutant gene expression and grading of malignant tumors was 4 grad III and one sample of not graded tumors showed positive expression of BRCA1 mutant gene, while negative expression of BRCA1 mutant gene in grad I and grad II of the malignant tumors, with insignificant correlation between BRCA1 expression and grading of malignant tumors (P value 0.320) as indicated in table (4-5).

Table (4-1): Diagnosis of malignant tumor subtypes among the study samples:

Histopathological diagnosis	Frequency	Percentage
Ductal carcinoma	54	93.1%
Medullary carcinoma	3	5.2%
Papillary carcinoma	1	1.7%
Total	58	100%

Table (4-2): Grading of malignant tumors among the study samples:

Grading	Frequency	Percentage
Grade I	7	12.1%
Grade II	12	20.7%
Grade III	35	60.3%
Not graded	4	6.9%
Total	58	100%

Table (4-3) : Distribution of age group among the study population:

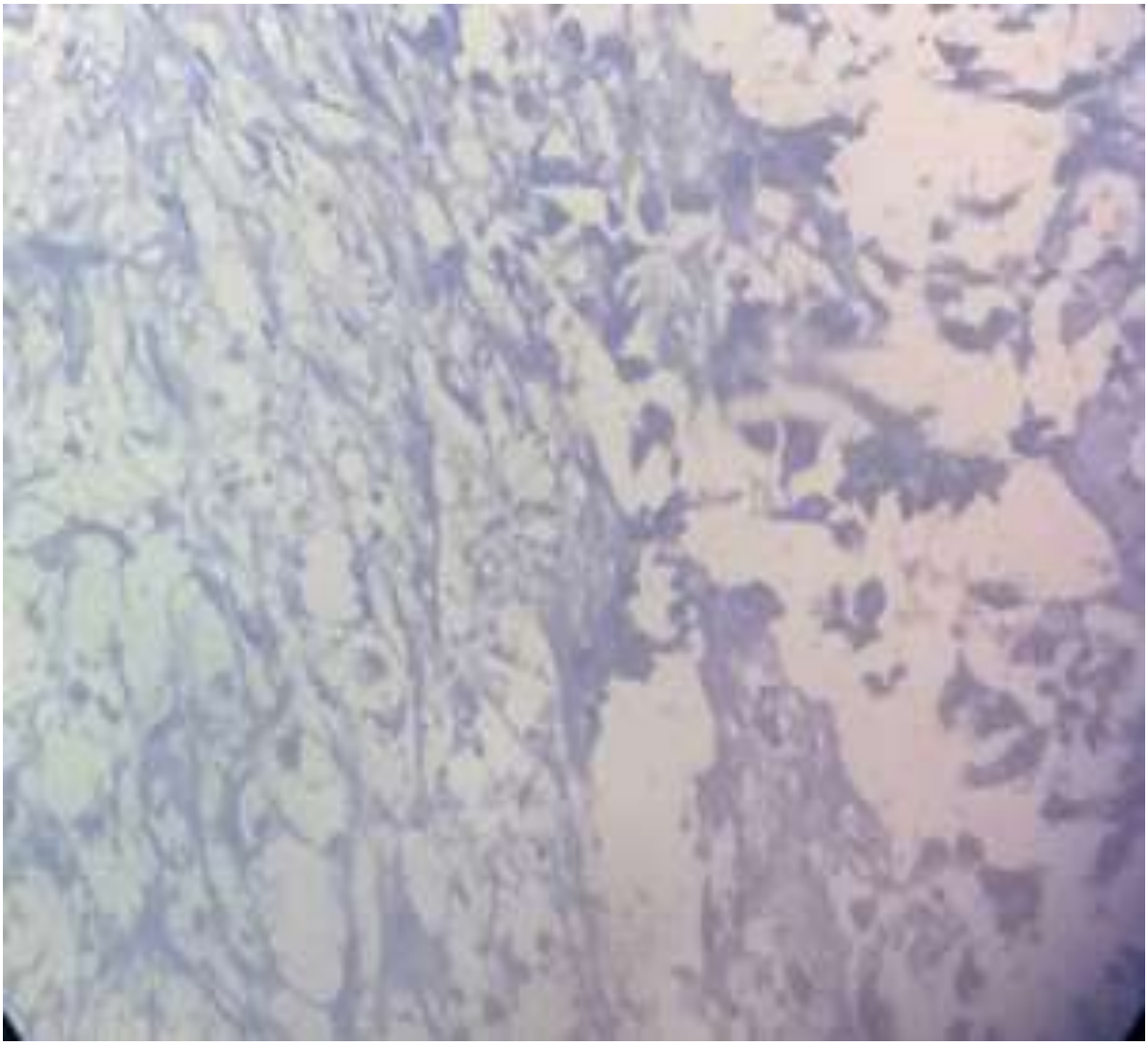
Age group	Frequency	Percentage
40 years and less	16	27.6%
More than 40 years	42	72.4%
total	58	100%

Table (4-4): Relation between BRCA1mutant gene expression and breast cancer subtypes among the study samples:

Breast cancer subtypes	Expression of BRCA1		P value
	Positive N (%)	Negative N (%)	
Ductal carcinoma	4 (6.9%)	50(86.2)	0.284
Medullary carcinoma	1 (1.7%)	2 (3.4%)	
Papillary carcinoma	0 (0%)	1 (1.7%)	
Total	5 (8.6%)	53 (91.4%)	

Table(4-5): Relation between BRCA1 mutant gene expreesion and grading of malignant tumors among the study sample:

Grading of malignant tumors	Expression of BRCA1		P value
	Positive N (%)	Negative N (%)	
Grad I	0 (0%)	7 (13%)	0.320
Grad II	0 (0%)	12 (22.2%)	
Grad III	(%7.4) 4	(%57.4) 31	
Total	4 (7.4%)	(%92.6) 50	



Figure(1): Ductal carcinoma of the breast show negative expression of BRCA1 mutant gene (by 40 X).

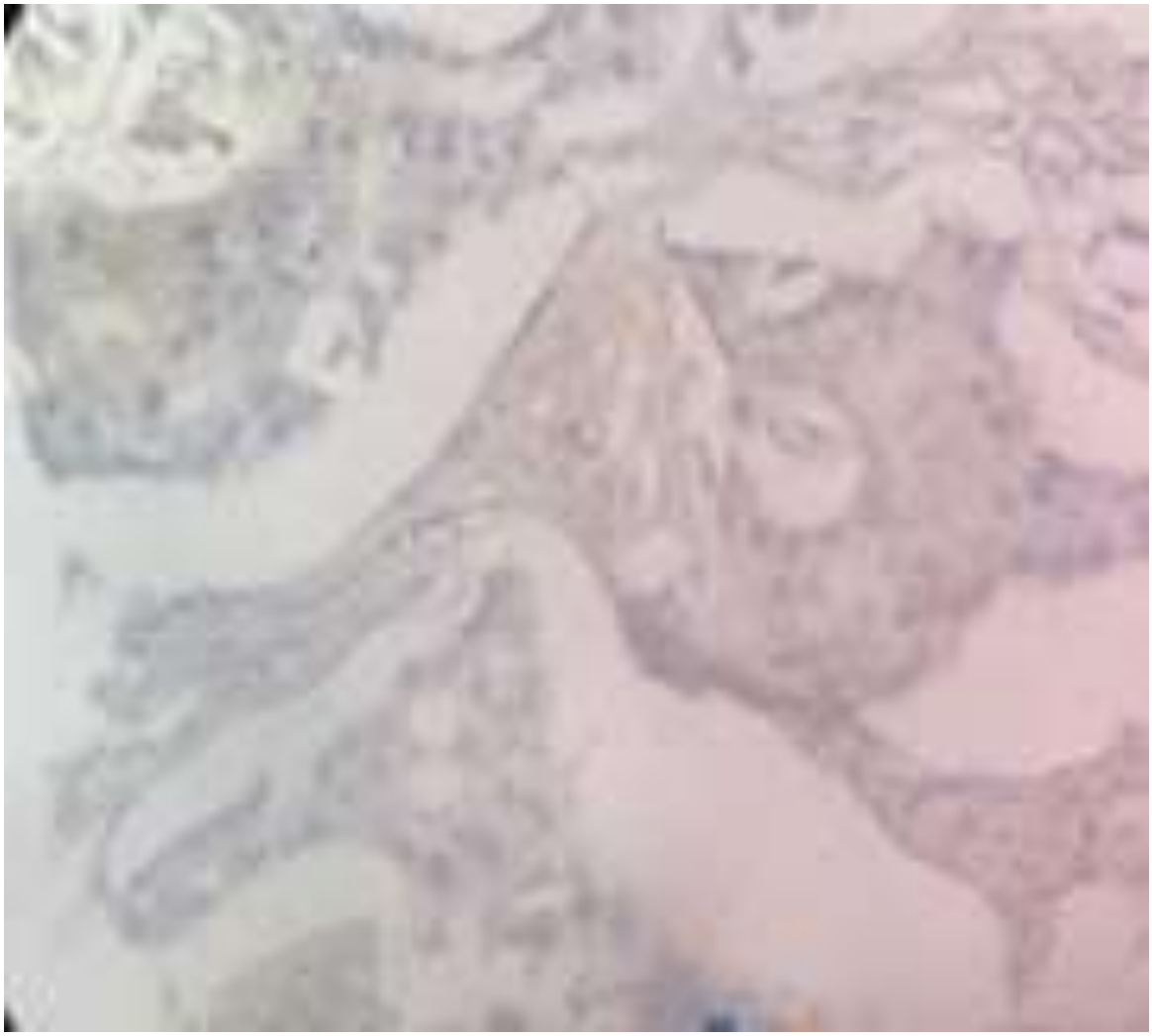


Figure (2): Ductal carcinoma of the breast show positive expression of BRCA1 mutant gene (by 40 X).

Chapter five

Discussion, Conclusion and Recommendation

Chapter five

Discussion, Conclusion and Recommendation

5-1Discussion:

This study involves fifty-eight samples of breast cancer for immunohistochemical staining of BRCA1 mutant gene expression. Regarding the age of the patients, the study revealed that most of patients were over 40 years, this result is compatible with Gabriel, *et al*. (2010) who reported that majority of women with breast cancer were diagnosed over 40 years. Also Kmietowicz, (2004) reported that women between 40 and 49 years have higher risk to develop breast cancer.

Regarding the correlation between expression of BRCA1 and breast cancer subtype, the study revealed that insignificant correlation between BRCA1 expression and breast cancer subtypes (P value 0.284), this result is incompatible with Kumar *et al* (2013) who reported that medullary carcinoma is commonly associated with BRCA1 mutation, also Mohan (2010) reported that BRCA1 mutant gene commonly seen in medullary carcinoma, this incompatibility may due to small sample size.

Regarding the correlation between expression of BRCA1 and grading of malignant tumor, the study revealed that no correlation between BRCA1 expression and grading of malignant tumor, this result is incompatible with Jouhadi *et al* (2016) who report that patient with BRCA1/2 mutation tend to develop early breast cancer with high grade tumors, this incompatibility may due to small sample size**.**

5.2 Conclusion:

On basis of this study, we conclude that:

- BRCA1 expression not associated with breast cancer subtypes and tumors grades.

5.3 Recommendation:

On basis of this study, we recommended:

Further studies should be done to detect BRCA1 mutant gene expression in breast cancer using molecular techniques with larger sample size.

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Appendix

Appendix

Instrument:

Puncher o.5 cm.

Microarray Mold.

Rotary microtome.

Microtome knife.

Water bath.

Positive charged slide.

Oven.

Daco pen.

Cover glass.

Coplin jars.

Staining rack.

Humidity chamber.

Gloves.

Material:

Xylene.

Ethyl alcohol (Absolut, 90%, 70%, 50%).

Distell water Peroxidase blocker.

Primary antibodies (BRCA1).

Secondary antibodies (biotenylated secondary antibodies).

3.3 di amino benzedrine tetra hydrochloridein substrate buffer.

DPX mounting media.

Phosphate (PH 7.4) component:

Solution A (0.2 M sodium di hydrogen orthophosphate, 3.02 g di sodium hydrogen orthophosphate, 100 ml DW)

Solution B (0.2 M sodium di hydrogen orthophosphate, 2.83g di sodium hydrogen orthophosphate, 100 ml DW.

(9.5 ml from solution A + 40.5 ml from solution B)

Citrate buffer (PH 6.8) component:

Solution A (0.2 ml sodium di hydrogen orthophosphate, 2.83g di sodium hydrogen orthophosphate, 100 ml DW).

Solution B (2.1 citric acid, 100 ml DW).

(72.2 ml from solution A + 22.8 ml from solution B).

Mayer‘s heamatoxylin component:

Heamatoxylin powder 1g

Potassium alum or ammonium alum	50g
Sodium iodide	0.2
Citric acid	1g
Chloral hydrate	50g
Distell water	1000 ml