Sudan University of Science and Technology

College of Graduate Studies

Immunohistochemical Detection of Gene Bcl2 in Breast Tumors

الكشف النسيجي الكيميائي المناعي عن الجين Bcl2 في اورام الثدي

A dissertation submitted in partial fulfillment for the requirement of

M.Sc Degree in

(Histopathology and cytology)

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الآيــــــــة

بسم اللهُ الرحمن الرحيم

قال تعالى:

لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا ۚ لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ ۗ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا ۚ رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إِصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا ۚ رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ ۖ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا ۚ أَنْتَ مَوْلَانَا فَانْصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ

صدق الله العظيم

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I

Dedication

To my father

To my mother

To my husband

To my brothers and sisters

To all my family

To all my teachers

To all my colleagues and friends

With love and respect.

Acknowledgement

ABSTRACT

This is analytical retrospective study conducted in Omdurman Teaching Hospital and Soba Teaching Hospital in Khartoum state, during the period from September 2015 to May 2016. The study aimed tostudythe role of Bcl2 expression in differentiation between malignant and benign breast tumors.

Forty paraffin embedded blocks previously diagnosed as breast tumors were collected. Samples include 30(75%) malignant tumors, including invasive ductal carcinoma 27(67.5%) samples, micro papillary carcinoma 1(2.5%) sample, metaplastic squamous cell carcinoma 1(2.5%) sample,low grade sarcoma 1 (2.5%) sample. And 10(25%) samples were benign tumors, includeingfibroadenoma 7(17.5%) samples, gynaecomastia 1(2.5%) sample, ductal ectasia 1(2.5%) sample, and granulomatous mastitis 1(2.5%) sample.

The patient's age ranged between 16 and 70 years with mean age of 43 years, most patients were less than 40 years representing 24(60%) and the remaining 16 (40%) patients were more than 40 years.

One section of 3µm thickness was cut from each paraffin block by rotary microtome and stained by immunohistochemical method (modified new indirect method) for detection of Bcl2. Data collected from patients files and results were analyzed using SPSS computer program frequencies mean and chi-square test values were calculated.

Immunohistochemical expression of Bcl2 was revealed positive result in 14/30 samples and negative result in 16/30 samples in malignant, while all benign tumors gave negative result for Bcl2, with significant statistical association between Bcl2 expression and histopathology diagnosis (P=0.007).

13 breast cancers Bcl2 positive samples, 3(11.1%) samples were grade 1, 3(11.1%) samples were grade11 and 7(25.9%) samples grade111, and negative in 10(37%)

samples. With statistical association between Bcl2 expression and grade of cancer (P=0.035).

This study concludes that there is association between Bcl2 expression and malignant tumors of breast. There is association between Bcl2 expression and the grade of cancer.

الخلاصة

اجريت هذة الدراسة الوصفية الاسترجاعية في مستشفي امدرمان التعليمي ومستشفي سوبا الجامعي في ولاية الخرطوم خلال الفتره من سبتمبر 2015 الي مايو 2016 .هدفت الدراسة للكشف عن بروتين Bcl2 في اورام الثدي باستخدام كيمياء الانسجة المناعية .

جمعت اربعين عينة مطمورة بشمع البارفين من عينات مرضي تم تشخيصهم مسبقا باورام الثدي.كانت كالاتي 30 (75%) عينه لاورام خبيثة ، تشمل سرطان الأقنية الغازية 27 (67.5 ٪) عينة ، السرطان الحليمي الصغير 1 (2.5 ٪) عينة ، استحالي سرطان الخلايا الحرشفية 1 (2.5 ٪) عينة ، وانخفاض ساركوما الصف 1 (2.5 ٪) عينة. و كانت 10 (25 ٪) عينات الأورام الحميدة ،تشمل ورم غدي ليفي 7 (17.5 ٪) عينة ، تثدي 1 (2.5 ٪) عينة، توسع وعائي الأقنية 1 (2.5 ٪) عينة ، و التهاب الثدي الحبيبي 1 (2.5 ٪) عينة .

تراوحت اعمار المرضي بين 16الي 70 سنة ومتوسط العمر 43 سنة,اغلب المرضي (24) كانت اعمارهم اقل من 40 سنه بنسبة 60% وبقية المرضي 16 (40%) كانت اعمارهم اكثر من 40 سنة.

قطع مقطع واحد من كل عينة بي سمك 3µm بواسطه جهاز المشراح الدوار.تم صبغ العينات بواسطة كيمياء الانسجة المناعية (باستخدام الطريقة المعدلة غير المباشره الجديدة) للكشف عن Bcl2 تم جمع البيانات من ملفات المرضي .تم استخدام الحزمة الاحصائية للعلوم الاجتماعية SPSS لتحليل البيانات بواسطة اختبارchi-square .

اظهرت الدراسة في الكشف عن التعبير المناعي للواسمة Bcl2 انها موجبه الظهور في14\30 وسالبة الظهور في 16\30 عينة من عينات الاورام الخبيثة .بينما كل عينات الاورام الحميدة اظهرت نتائج سالبة لي Bcl2 .مع وجود علاقة احصائية بين Bcl2 ونوع الورم (القيمة الاحتمالية = 0.007) .

من اصل 13 عينة ايجابية وجد ان3 عينات من الدرجة الاولي و3عينات من الدرجة الثانية و7 عينات من الدرجة الثالثة مع عدم وجود علاقة احصائية بين ظهور Bcl2 ونوع السرطان (القيمة الاحتمالية =0.035).

خلصت الدراسة الي ان هناك علاقة بين ظهور BCL2 والاورام الخبيثه في الثدي مع وجود علاقة مع نوع الورم ودرجة تمايز السرطان.

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CHAPTER ONE

INTRODUCTION

CHAPTER ONE
INTRODUCTION

1.1 introductions:

Breastcancer is caused by thedevelopment of malignantcells in thebreast.Themalignantcellsoriginate in thelining of themilkglands or ducts of thebreast(ductalepithelium).Cancercellsarecharacterized by uncontrolleddivisionleading to abnormalgrowthandtheability of thesecells to invadenormaltissuelocal or to spreadthroughoutthebody, in a processcalledmetastasis(Link and John, 2007).

Cancer is a leading cause of death worldwide and accounted for 7.6 million deaths (around 13% of all deaths) in 2008 (Hussain, *et al*. 2014). Breast cancer mortality is high in Sudan and most patients are detected at later stages of the disease due to the lack of awareness and absence of screening programs (Ahmed, *et al.* 2010). During 2009–2010, 6771 new cancer cases were registered(Intisar, *et al* .2014).

The risk of getting breast cancer increases with age*(Margolese, et al. 2000).*Male individuals have a much lower risk of developing breast cancer than females.In 5% of breast cancer cases there is a strong inherited familial risk*(Malone, et al .1998).* Smoking tobacco (Lyon, 2007). Gaining weight after menopause can increase a woman's risk (Nelson,2011). Persistently increased blood levels of estrogen are associated with an increased risk of breast cancer*(Ligibel,2011).*

Thediagnosis of breastcancer is accomplished by thebiopsy of anysuspiciouslump or mammographicabnormalitythathasbeenidentified (Link and John, 2007).Surgery, radiation, and chemotherapy all used in the treatment of breast cancer.(Kenneth and Miller, 2008).

Bcl-2 protein is a member of the Bcl2 family that regulates apoptosis. The Bcl2 gene encodes a MR 26000 protein that is mainly localized in the mitochondrial membrane and, to a lesser extent in the nuclear membrane and the endoplasmic

reticulum(McDonnell and Korsmeyer, 1991).Bcl2 gene has been identified as a cause of a number of cancers, including breast cancer. Bcl2 expression has been consistently associated with a better prognosis of breast cancer patients (Kumar, *et al*. 2000).

Bcl2 protein is frequently expressed in breast cancer, and its expression is associated with favorable clinicopathological features (Joensuu and Pylkkänen, 1994).

1.2 Objectives:

1.2.1 General objective:

To study the role of Bcl2 expression in differentiation between malignant and benign breast tumors.

1.2.2 Specific objective:

1. *To detect Bcl2 expression in breast tumors by immunohistochemical method.*

2. *To correlate the Bcl2 expression with histopathological diagnosis and cancer grade.*

CHAPTER TWO

LITREATURE REVIEW

CHAPTRE TWO
LITREATURE REVIEW

2.1 Scientific background:

Breast cancer is the commonest cause of cancer death in women worldwide. Rates vary about five-fold around the world, but they are increasing in regions that until had low rates of the disease (Timothy, 2001).

2.2 Structure of the breast:

Breasts are made up of fat and breast tissue, along with nerves, veins, arteries and connective tissue. Breast tissue is a complex network of lobules and ducts in a pattern that looks like bunches of grapes. These bunches are called lobes. Adult women have 15 to 20 lobes in each breast (Rosen, 2001).

Each lobe has 20 to 40 lobules, small milk ducts are attached to the lobules, and these ducts join together like branches of grape stems into increasingly larger ducts. There are about 10 duct systems in each breast, each with its own opening at the nipple (Osborne and Boolbol, 2014).

Muscle tissue in the nipples allows them to become erect in response to stimulation or breastfeeding. Muscle tissue around the lobules helps squeeze milk into the ducts. Glands on the areola release small amounts of fluid during breastfeeding to lubricate the nipple (Rosen, 2001).

2.3 Disorder of breast:

2.3.1 Benign breast tumors:

Benign breast diseases constitute a heterogeneous group of lesions including developmental abnormalities, inflammatory lesions, epithelial stromal proliferations, and neoplasm (Merih, *et al.* 2006).

2.3.1.1 Inflammatory and related lesionsof the breast tumor:

Mastitisa variety of inflammatory and reactive changes can be seen in the breast. While some of these changes are a result of infectious agents. Acute

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mastitisusually occurs during the first three months postpartum as a result of breast feeding (Foxman, *et al*. 2002).

Granulomatous mastitisgranulomatous reactions resulting from an infectious etiology, foreign material, or systemic autoimmune diseases*(Erhan, et al.* 2000). Foreign body reactions such as silicone and paraffin (van, *et al*. 1998).Recurring subareolar abscess(Zuska's disease) is a rare bacterial infection of the breast that is characterized by a triad of draining cutaneous fistula from the subareolar tissue (Passaro, *et al*. 1994).Mammary duct ectasia**,** also called periductal mastitis is a distinctive clinical entity that can mimic invasive carcinoma clinically (Furlong, *et al*. 1994)**.** Fat necrosis of the breast is a benign nonsuppurative inflammatory process of adipose tissue (*Rosai, 2004).*

2.3.1.2Fibrocystic changes:

Cysts are fluid filled, round or ovoid structures that are found in as many as one third of women between 35 and 50 years old(Donegan, 2002).Adenosisof the breast is a proliferative lesion that is characterized by an increased number or size of glandular components (Lee*, et al*.1996). Metaplasiaapocrine is characterized by the presence of columnar cells with abundant granular(Tavassoli, 1999). Epithelial hyperplasia (ductal or lobular type) is the most common form of proliferative breast disease. Ductal lesions are any increase in the cell number within the ductal space is regarded as epithelial hyperplasia(Tavassoli, 1999).Lobular lesions type epithelial proliferations, both atypical lobular hyperplasia and lobular carcinoma in situ (Page andSchuyler, 2003)**.**

2.3.1.3 Neoplasms:

Fibroadenoma is the most common lesion of the breast (El-Wakeel and Umpleby, 2003). Lipoma is a benign, usually solitary tumor composed of mature fat cells (Lanng, *et al*. 2004).   Adenoma is pure epithelial neoplasm of the breast (Silverberg and Masood, 1997). Hamartoma uncommon benign tumor like nodule,

of varying amounts of glandular, adipose, and fibrous tissue (Gatti, *et al*. 2005). Granular cell tumor is an uncommon, usually benign neoplasm that originates from Schwann cells of the peripheral nervous system (Montagnese, *et al*. 2004).

2.3.2   Breast cancer:

Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, or a red scaly patch of skin

(Saunders, et al .2009).

2.3.2.1 Types of breast cancer*:*

2.3.2.1.1 Non-invasive breast cancers

Ductal carcinoma in situ (DCIS)abnormal cells are contained in the milk ducts of the breast and have not spread to nearby breast tissue.

2.3.2.1.2 Invasive breast cancer

Invasive breast cancer has spread from the original site (either the milk ducts or the lobules) into the nearby breast tissue, and possibly to the lymph nodes and or other parts of the body. For this reason, invasive breast cancers have a poorer prognosis than DCIS, include Invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), tubular carcinoma, mucinous (colloid) carcinoma, carcinomas with medullary features, Invasive papillary carcinoma(Dillon, *et al*. 2014).

 2.3.2.1.3 Special forms of breast cancer

Inflammatory breast cancer (IBC) it is called inflammatory breast cancer because its main symptoms are swelling (inflammation) and redness of the breast. Paget disease of the breast (Paget disease of the nipple)is a rare cancer in the skin of the nipple or in the skin closely surrounding the nipple. Metaplastic breast cancer is rare, accounting for fewer than five percent of all invasive breast cancers

(Overmoyer and Pierce, 2014).

2.3.2.1.4 Other types of breast cancer

In rare cases, other types of cancer such as lymphomas (cancer of the lymph system) and sarcomas (cancer of the soft tissues) (Hansen, 2014).

2.4 Epidemiology of breast cancer:

Worldwide breast cancer is the most common invasive cancer in women. It affects about 12% of women worldwide (*McGuire, et al. 2015*). Incidence rates are generally highest in North American and Northern European countries, intermediate in Southern and Eastern European and South American countries and lowest in Asia and Africa (Kelsey and Horn. 1993). Almost 1.4 million women were diagnosed with breast cancer worldwide in 2008 and approximately 459,000 deaths were recorded. Incidence rates were much higher in more developed countries compared to less developed countries (71.7/100,000 and 29.3/100,000 respectively (Danny, *et al*. 2012). Study from Sudan NCR, 6771 incident cancer cases were recorded among Khartoum State residents during 2009–2010 periods (Intisar, *et al.* 2014).

2.5 Risk factors of breast cancer:

2.5.1 Age:

The risk of getting breast cancer increases with age. A woman is more than 100 times more likely to develop breast cancer in her 60s than in her 20s*(Margolese, et al.2000).*

2.5.2 Sex:

Male individuals have a much lower risk of developing breast cancer than females *(Margolese, et al. 2000).*

2.5.3 family history:

In 5% of breast cancer cases, there is a strong inherited familial risk BRCA1 and BRCA2 gene*(Malone, et al.1998).*

2.5.4 consumption:

There is sufficient scientific evidence to classify alcoholic beverages as a group 1 carcinogen that causes breast cancer in women*(Allen, et al. 2009).*

2.5.5 Fat intake:

Low fat diets may significantly decrease the risk of breast cancer as well as the recurrence of breast cancer *(Chlebowski, et al .2006).*

2.5.6 Obesity and lack of exercise:

Gaining weight after menopause can increase a woman's risk. Lack of exercise has been linked to breast cancer (Nelson, 2011).

2.5.7 Hormones:

Persistently increased blood levels of estrogen are associated with an increased risk of breast cancer. Increased blood levels of progesterone are associated with a decreased risk of breast cancer in premenopausal women *(Yager and Davidson, 2006).*

2.5.8 Radiation:

Women who have received high dose ionizing radiation to the chest have a relative risk of breast *cancer (Yager and Davidson, 2006).   .*

2.5.9 Smoking**:**

Tobacco appears to increase the risk of breast cancer, with the greater the amount smoked and the earlier in life that smoking began *(Johnson, et al. 2011).*

2.5.10 Medical conditions:

Breast changes like atypical ductal hyperplasia and lobular carcinoma in situ, found in benign breast conditions such as fibrocystic breast changes, are correlated with an increased breast cancer risk. Diabetes mellitus might also increase the risk of breast cancer*(Anothaisintawee, et al. 2013).*

2.6 Diagnosis of breast cancer:

2.6.1 History:

Include the characteristics of symptoms and their timing in relation to menstrual cycles (Gail, *et al*. 1989).

2.6.2 Physical examination:

Include inspection and palpation, inspection of the breasts can be done with the woman sitting with hands on her hips, palpated for evaluation of texture and detection of masses (Barton, *et al*. 1999).

2.6.3 Mammography:

It performed as an adjunct to the physical examination in evaluating breast lumps or as a screening tool. Mammography is not generally useful in women younger than 35 years who present with a lump (Hindle, *et al*. 1999).

2.6.4 Ultrasonography:

It is very useful for evaluating breast lumps and in further defining mammographic abnormalities. It is especially useful in women younger than 35 years (Sickles, *et al*. 1984).

2.6.5 Fine needle aspiration:

Itperformed to aspirate a palpable suspected cyst. A 22- or 24-gauge needle is inserted into a cyst that has been stabilized with the other hand. If non bloody fluid is obtained, it can be discarged, because no cancer was found in non bloody cyst fluid in a large series (Ciatto, *et al*. 1987).

2.6.6 Core needle biopsy:

A larger needle (14- to 18-gauge) is used for core needle biopsy. It is mostly used for evaluating non palpable breast masses (Evans, 1996).

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2.6.7 Triple diagnosis:

The combination of the physical examination, mammography, and fine-needle aspiration biopsy for diagnosing palpable lumps is referred to as triple diagnosis. There is excellent sensitivity (99%) and specificity (99%) (Layfield*, et al.* 1989).

2.7 Treatment:

Breast cancer is usually treated with surgery, which may be followed by chemotherapy or radiation therapy, or both *(Saini, et al.2011).*

2.7.1 Surgery:

It involves the physical removal of the tumor. Standard surgeries includemastectomy,quadrantectomy, lumpectomy*(Saini, et al.2011).*

2.7.2 Drugs:

It used after and in addition to surgery arecalled adjuvant therapy. Chemotherapy or other types of therapy prior to surgery are called neoadjuvant therapy aspirin may reduce mortality from breast cancer*(Holmes, et al .2010).*

2.7.3 Hormone blocking therapy:

Some breast cancers require estrogen to continue growing. They can be identified by the presence of estrogen receptors (ER+) and progesterone receptors (PR+) on their surface (sometimes referred to together as hormone receptors) (*Ting, et al. 2011).*

2.7.4 Chemotherapy:

It predominantly used for cases of breast cancer in stages 2–4, and is particularly beneficial in estrogen receptor-negative (ER-) disease (*Ting, et al. 2011).*

2.7.5 Monoclonal antibodies:

Trastuzumab, a monoclonal antibody to HER2 (a cell receptor that is especially active in some breast cancer cells), positive breast cancers to about 87% (overall survival 95%) *(Jahanzeb, 2008).*

2.7.6 Radiation:

Radiotherapy is given after surgery to the region of the tumor bed and regional lymph nodes, to destroy microscopic tumor cells that may have escaped surgery (*Massarut,et al.2006*).

2.8 Bcl-2:

Bcl-2 (B-cell lymphoma 2), encoded in humans by the Bcl2 gene, is the founding member of the Bcl-2 family of regulator proteins that regulate cell death (apoptosis), by either inducing (pro-apoptotic) or inhibiting (anti-apoptotic) apoptosis specifically considered an important anti-apoptotic protein and is thus classified as an oncogene *(Tsujimoto,et al. 1984).*

Apoptosis is a physiological process following which normal cells die after a given number of replications. Tumor cells tend to interfere with this mechanism by activating genes which inhibit apoptosis, one of the main genes limiting apoptosis is Bcl2. Bcl2 expression has been consistently associated with a better prognosis of breast cancer patients (Krajewski, *et al*. 1999, Kumar, *et al* .2000)**.**

Bcl-2 is frequently expressed in normal breast epithelial cells and breast cancer cells, and is known to be upregulated by estrogen (*Leek, et al. 1994).*

*Sabourin, et al. (1994)*reportBcl2 expression in breast cancer has been reported to positively correlate with differentiated markers or favorable prognostic factors.

Julia, *et al*. (1994) report the Bcl2 protein was found within the tumor epithelial cell cytoplasm of 32/46 breast cancer specimens, inter-patient staining was heterogeneous. Immunostaining for steroid hormone receptors was strongly associated with that for the Bcl2 protein, and it is thus possible that this protein.

Olopade, *et al*.(1997) report Bcl2 protein was over expressed in 18 of 42 (43%) invasive breast cancers when compared with adjacent normal breast epithelium. Over expression of Bcl2 protein in these tumors was associated with higher

tumorgrade and increased number of positive nodes. In contrast <u>Bcl2protein</u> was over expressed in 19 of 42 tumors (45%).

<u>Bhargava</u>, *etal*. (1994) report grade 1 and 2 tumors were almost three times as likely to be Bcl-2 positive (90%) as grade 3 tumors (33%) (P = 0.0057).

<u>Hellemans</u>, *et al.* (1995) reportthe results were correlated with clinicopathologica data and prognostic variables. 63 (25%) tumours were scored Bcl-2 negative and 188 (75%) tumours were Bcl-2 positive.

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials:

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

3.2 Methods:

3.2.1 Study design:

This is a hospital based descriptive retrospective case control study aimed to detect expression of Bcl2 tumor marker in breast tumor using immunohistochemical method.

3.2.2 Study samples:

Forty tissue blocks were obtained from breast tumor. Thirty samples were previously diagnosed as malignant breast tissue and ten samples were diagnosed as benign cell.

3.2.3 Study area:

This study held in Omdurman teaching hospital and Soba teaching hospital during the period from September 2015 to May 2016.

3.2.4 Immunohistochemicalstainig:

The section of 3µm thickness was obtained from formalin fixed paraffin embedded tissue using a rotary microtome. Then immune stained using monoclonal antibodies new indirect technique as follows:

Section 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 by water bath retrieval technique for thirty minutes at 95°c (colin jar contain citrate buffer pH 6.0). Then washed in phosphate buffer saline (pH 7.4) for five minutes.Then treated with hydrogen peroxidase solution for ten minutes.Then washed in phosphate buffer saline (pH 7.4) for five minutes. Then treated with anti Bcl2 (Bcl2 –alpha Ab-1)

primary antibody for thirty minutes. Then rinsed in phosphate buffer saline (pH 7.4).Then treated with secondary polymer conjugated antibody for thirty minutes.Then rinsed in phosphate buffer saline (pH 7.4).Then treated with diaminobenzidine (DAB) for five minutes. Then counter stained in Mayer's haematoxylin for one minutes.Then washed and blued in 0.05% ammoniated water for 16 second.

Then washed in tap water. Then dehydrated through ascending concentration of ethanol (50% 70% 90% 100%).Then cleared in xylene and mounted in DPX mountant .

3.2.5 Result interpretation:

Detection of more than 5 cells with brown cytoplasm per one field considered as positive result.

All quality control measures were adopted positive and negative control slides were used during immunohistochemical staining.

3.2.6 Data analysis:

Data analysis was done using SPSS 11.5 computer program frequencies mean and chi-square test values were calculated.

3.2.7 Ethical consideration:

Sample collected after taking ethical acceptance from hospital administration.

CHAPTER FOUR

RESULTS

CHAPTER FOUR
RESULTS

A total of 40 samples collected fromo patients with breast tumors were investigated, 30(75%) of them were malignant tumors, including invasive ductal carcinoma 27(67.5%) samples, micro papillary carcinoma 1(2.5%) sample, metaplastic squamous cell carcinoma 1(2.5%) sample, low grade sarcoma 1(2.5%) sample. And the remaining benign tumors 10(25%) samples, include fibroadenoma 7(17.5%) samples, gynaecomastia 1(2.5%) sample, ductal ectasia 1(2.5%) sample, granulomatous mastitis 1(2.5%) sample. As indicated in table (4.1). The sex of study population revealed that 2(5%) samples were males and 38(95%) samples were females, as indicated in table (4.2). The description of cancer grade revealed that 13(43.3) samples were grade 1, 5(16.7%) samples were grade 11, 9(30%) samples were grade 111, 3(10%) samples were not graded, as indicated in table (4.3). The age study population showed that 40 and less years were 24(60%) patient and more than 40 years were 16(40%) patients, as indicated table (4.4). Malignant breast cancer revealed positive expression of Bcl2 in 14(35%) samples and negative expression in 16(40%) samples, while all benign tumor showed negative expression of Bcl2, this result showed significant association (P.value=0.007), as indicated in table (4.5) The comparison between Bcl2 expression and the grade of tumor showed that Bcl2 expression was positive in 3(11.1%) samples were grade1, 3(11.1%) samples were grade 11, 7(25.9%) samples were grade 111. And negative in 10(37%) samples were grade 1, 2(7.4%) samples were grade 11, 2(7.4%) samples were grade 111. With insignificant association (P.value=0.035), as indicated in table (4.6).

Table (4.1): Histopathology diagnosis among the study population

Histopathology diagnosis		Frequency	Percent
Malignant	Invasive ductal carcinoma	27	67.5%
	Micro papillary carcinoma	1	2.5%
	Met plastic squamous cell carcinoma	1	2.5%
	Low grade sarcoma	1	2.5%
Benign	Fibroadenoma	7	17.5%
	Gynaecomastia	1	2.5%
	Ductal ectasia	1	2.5%
	Granulomatus mastitis	1	`2.5%
Total		40	100%

Table (4.2): The distribution of sex among study population

Sex	Frequency	Percent
Female	38	95%
Male	2	5%
Total	40	100%

Table (4.3): Distribution of cancer grade among malignant breast tumors

Grade	Frequency	Percent
Grade 1	13	43.3%
Grade 2	5	16.7%
Grade 3	9	30%
Not graded	3	10%
Total	30	75%

Table (4.4): Distribution of age among study population

Age	Frequency	Percent
40 and less	24	60%
More than 40	16	40%
Total	40	100%

Table (4.5): Relation between BCL2 expression and histopathology diagnosis

Histopathology diagnosis	Bcl2 expression		Total	P.value
	Positive n (%)	Negative n (%)		
Malignant	14 (35%)	16 (40%)	30 (75%)	0.007
Benign	0 (0%)	10 (25%)	10 (25%)	
Total	14 (35%)	26 (65%)	40 (100%)	

Table (4.6): Relation between BCL2 expression and the grade of breast cancer

Cancer grade	Bcl2 expression		Total	P.value
	Positive n (%)	Negative n (%)		
Grade 1	3 (11.1%)	10 (37%)	13 (48.1%)	
Grade 2	3 (11.1%)	2 (7.4%)	5 (18.5%)	0.035
Grade 3	7 (25.9%)	2 (7.4%)	9 (33.3%)	
Total	13 (48.1%)	14 (51.9%)	27 (100%)	
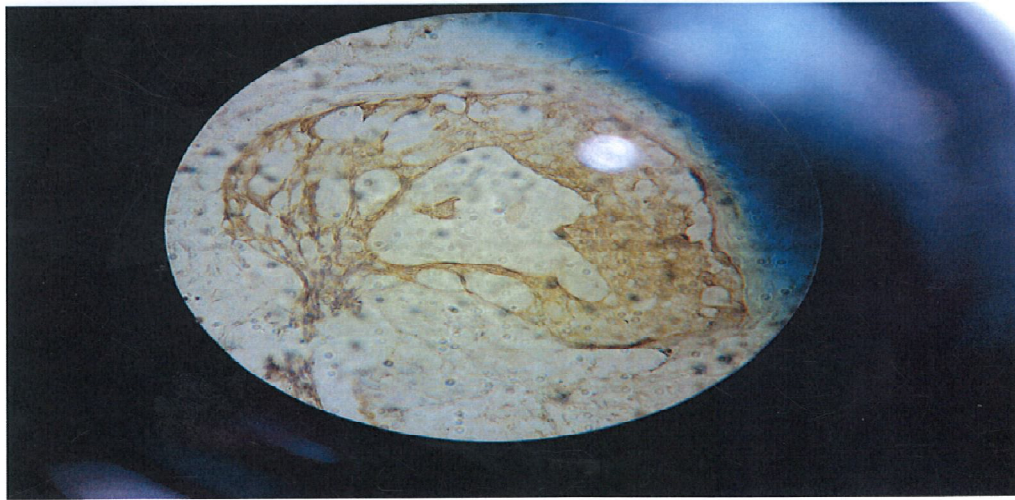
Graph (4.1): Invasive ductal carcinoma of breast grade II showing cytoplasmic expression of Bcl2 (40 x).
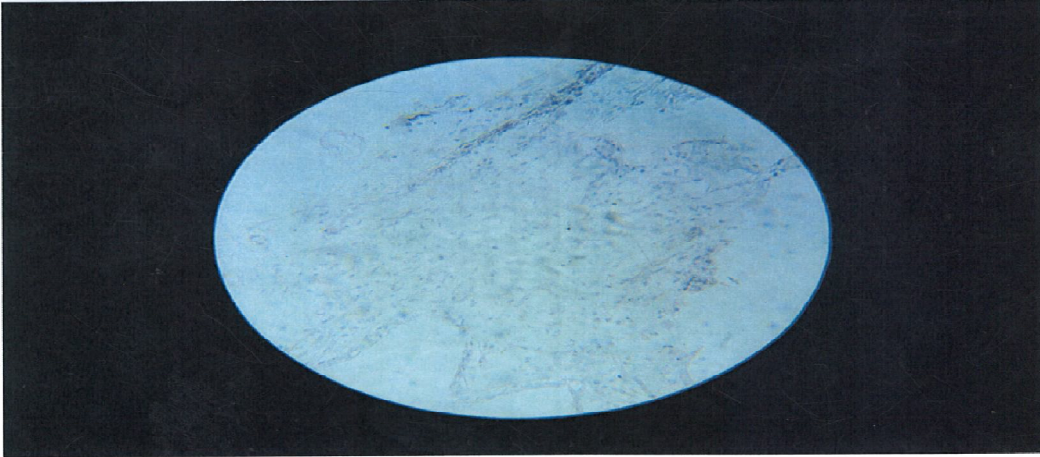
Graph (4.2): Bilateral breast mammoplastiy fibrocystic change showing
cytoplasmic negative expression of Bcl2 (40 x).

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

In this study forty samples from patients affected with breast tumor were investigated by immunohistochemical method for detection of Bcl2 expression . The study revealed that the age of the study population range from 16 to 70 years with mean age of 43 years. Most patients were less than 40 years, this is probably due to production of estrogen hormone in this age. This result was agree with Carey, *et al.* (2009), who reported that the young women generally face more aggressive cancers and lower survival rates. This result wasdisagree withHowlader, *et al.* (2015), who reported that the risk of getting breast cancer increases with age. The study revealed that the majority of the study population sex, this result explain males wear less expected than females with breast cancer, this probably due to reproductive risk factors, this result was agree with Anderson, *et al.* (2004), who reported that the gender specific incidence trends differed, most likely reflective of female related changes in surveillance and or reproductive risk factors. Also compatible with result observed byBagley*, et al.* (1987), who reported that male breast cancer is an uncommon disease. Because this disease is rare, compared with female breast cancers.

Bcl2 is a mitochondrial protein associated with anti apoptotic function over expression of Bcl2 is found to be in a variety tumor due to degradation of Bcl2. In this study over expression of Bcl2 is observed in malignant breast tumors 14/30, while benign breast tumor showed no expression of Bcl2. This relation showed significant association (P.value =0.007), this finding is compatible with result observed by Samantha, *et al.* (2011), who reported that Bcl2 expression is associated with malignant condition. Also compatible with result observed by Leek, *et al* (1994), who reported that Bcl2 expression was then compared with the

established indicators of prognosis and biological behavior in malignant breast disease.

The study revealed that the Bcl2 expression and grade of cancer showed increase over expression in high grade tumors, this relation showed significant association (P.value=0.035) indicating that rising of cancer grade is affected by the Bcl2 over expression this finding is compatible with result observed by Lipponen, *et al.* (1995), who reported that the intensity of Bcl2 expression was inversely related to tumors grade (*P*<0·0001). Also compatible with result observed by Olopade, *et al.* (1997), who reported that these findings suggest that expression of Bcl2 <u>protein</u> is increased in a significant fraction of <u>invasive breast cancers</u>. This result was disagree with Hellemans, *et al.*(1995), who reported that no relationship could be observed between Bcl-2 status and tumor grade .

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion:

On the basis of this study we conclude that:

There is association between Bcl2 expression and malignant tumors of breast and there is association between Bcl2 expression and grade of breast cancer.

6.2 Recommendation:

On the basis of this study we recommend that:

Bcl2 could be used as marker for differentiation between malignant and benign breast tumors.

Molecular detection of tumor markers should be done.

Other tumor markers should be applied for breast tumors.

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MATERIALS AND INSTRUMENTS

Materials and instruments used for processing and staining s 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 sulfate,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 , 1Liter distilled water mix to dissolve and add 0.05 ml of Tween 20 and mix).

Phosphate buffer (PH 7.4).

Hydrogen peroxidase blocking solution.

Primary antibody Bcl2.

Secondary antibody.

DAB (3.3 diaminobenzidine).

DAB substrate buffer.