Sudan University of Science and
Technology
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Immunohistochemical Detection of EBV, BRCA1 and Ki67 in Triple Negative Breast Cancer

الكشف المناعى النسيجى الكيميائى عن فيروس الابشتاين ومستضد سرطان الثدى **1** وكي اى **67** فى
سرطان الثدى السالب الثلاثي

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الآيـــــة

بِسْمِ اللّهِ الرّحْمَنِ الرّحِيمِ

قال تعالي :

وَإِذْ أَخَذْنَا مِنَ النّبِيّينَ مِيثَاقَهُمْ وَمِنكَ وَمِن نُّوحٍ وَإِبْرَاهِيمَ
وَمُوسَىٰ وَعِيسَى ابْنِ مَرْيَمَ ۖ وَأَخَذْنَا مِنْهُم مِّيثَاقًا غَلِيظًا

صدق الله العظيم

سورة الأحزاب الآية 7

DeDicaTion

To my mother for herlife long love and encouragement.

To my husband, daughter, sisters and brother, whose love, support and people make every thingworth while.

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ABSTRACT

The aim of this study was to detect EBV, BRCA1 and ki67 in breast cancer using immunohistochemistry. One hundred formalin fixed paraffin embedded tissue blocks were collected fromfemale patients previously diagnosed with breast cancer, seventy samples were triple negative (case) and thirtysamples were non triple negative (control).Three section from each sample were taken and immunohistochemistery tech were held on and primary antibody applied for EBV,BRCA1,and Ki67 flowed by secondary antibody(supper sensitive one steps from thermo scientific reagent) The age of patients were ranged between 20 to 80 year with mean age48.6 years, age group were divided into two categories ≥40 of whom 30 were triple negative and 4 were non triple negative <40 of whom 39 were triple negative and 26 were non triple negative with siginficant relation to cancer status. Histopathological diagnosis reveals that invasive ductal carcinoma was the most common amongcases representing 88/100 (88%) and most of them were grade III 60%, 20% were grad II and 20% were grad I

Ki67 was positive in all samples 100% of cases while in controls were 16% (5/30) with relation to cancer status. EBV was identified in 24.3% (17/70) of cases and 13.3 (4/30) of controls with no relation to cancer status. BRCA1 gene was expressed in 22.9% (16/70) in case group, while no expression among control group (0/30) with relation to cancer status. The study concludedthat triple negative had high proliferation index, and small ages, there is association between BRCA1 gene expression and breast cancer status but less association with EBV infection.

المستخلص

هدفت هذه الدراسة للكشف عن فيروس الابستاين ومستضد سرطان الثدي و واسمة كاي67 في سرطان الثدي الثلاثي السالب باستخدام طريقة مناعة كيمياء الانسجة. تم جمع مائة قالب شمع برافين من نساء مريضات مشخصات مسبقا بسرطان الثدى . سبعون عينة كانت سالبة ثلاثية (ER,PR and Her2) كعينات حالة وثلاثون عينة كانت غير سالبة الثلاثية كعينات ضابطة وتم عمل ثلاث شرائح من كل عينة ليفيروس الابستاين ومستضد سرطان الثدي و واسمة كاي67 . تراوحت اعمار المرضى بين عشرون الى ثمانون سنة بمتوسط عمر48,6 سنة تم تقسيم اعمار المرضى الى فئتين اصغر من 40 ومثلت عينات حالة الدراسة 30 مريضا بينما 4 فقط من العينات الضابطة واكبر من 40وكانو39 مريضا من حالة الدراسة بينما 26 من العينات الضابطة مع وجود علاقة احصائية بسرطان الثدي الثلاثي السالب اظهرت نتيجة تشخيص الانسجة ان غالبية العينات كانت من نوع الغدي الغازي وكانت تمثل 88%ومعظمها كان في الدرجة الثالثة 60%من الدر جة اثانية 20% ومن الدرجة اولى 20% من حيث التصنيفات نتيجة كي أي 67 كانت موجبة من كل عينات حالة الدراسة 100% بينما في العينات الضابطة كانت موجبة فى 16%من الحالات مع وجود علاقة احصائية بسرطان الثدي الثلاثي السالب تم التعرف على فيروس الابستاين من 24,3% من الحالات بينما كان بنسبة 13,3%في العينات الضابطة مع عدم وجود علاقة احصائية بسرطان الثدي الثلاثي السالب جين مستضد سرطان الثدي اظهر تعبيرا موجبا من 22,9%من الحالات ولم يعطى نتيجة ايجابية في العينات الضابطة مع وجود علاقه احصائية بسرطان الثدي الثلاثي السالب خلصت الدراسة الى ان سرطان الثدي السالب الثلاثية لدية معامل انقسام عالى وتوجد علاقة بينه والاعمار الصغيرة و توجد علاقة كذلك بين افراز مستضد سرطان وحالة سرطان الثدى السالب الثلاثي وبدرجة اقل مع فيروس عدوى الابشتاين

List of Contents

List of tabLes

List of micro PhotograPhs

Chapter One
Introduction

Chapter One

1. Introduction:

Breast cancer is most frequently diagnosed cancer inwomen and one of the leading causes of cancer deathfor women. Worldwide, over 1.3 million cases ofinvasive breast cancer are diagnosed, and more than450,000 women die from breast cancer annually (Gonzale,*et al* .2010).Breast cancer is the second most common cancer inthe world and, by far, the most frequent cancer amongwomen with an estimated 1.67 million new cancercases diagnosed in 2012 (25% of all cancers). It is themost common cancer in women both in more and lessdeveloped regions.Incidence rates vary nearly fourfoldacross the world regions, with rates ranging from27 per 100,000 in Middle Africa and Eastern Asia to96 in Western Europe(Globocan,2012).

Breast cancer ranks as the fifth cause of death fromcancer overall (522,000 deaths) and while it is themost frequent cause of cancer death in women in lessdeveloped regions (324,000 deaths, 14.3% of total), itis the second cause of cancer death in moredeveloped regions (198,000 deaths, 15.4%) after lungcancer (Globocan,2012). Breast cancer in Sudan according to RICKrecord during the period from 2010 to 2012 is 16.7%,17.2% and 25% respectively. Several environmentalrisk factor that may contribute to or hastendevelopment of breast cancer include life style, age, race and family history (DattaK and BiswasJ.2009). Triple negative breast-cancer is defined as invasivecarcinoma of breast that lack staining for estrogenreceptor, progesterone receptor and her-2 neu.Approximately 15%-20% of breast cancer illustratedthis phenotype, the triple negative breast cancerincreased in black woman regardless age or bodymass (Carey,*et al* . 2006). These negative results mean that the growthof the cancer is not supported by the hormonesestrogen and progesterone, or by the presence of toomany HER2 receptors. Therefore, triple-negativebreast cancer

does not respond to hormonal therapy (such as tamoxifen or aromatase inhibitors) ortherapies that target HER2 receptors, such asHerceptin (chemical name: trastuzumab) (Xu,*et al* .2013)However, other medicines can be used to treat triplenegativebreast cancer. About 10-20% of breastcancers are found to be triple-negative. Triplenegative breast cancer can be more aggressive anddifficult to treat. Also, the cancer is more likely tospread and recur (Lara, *et al* .2011) .Triplenegative breast cancer is more likely to affectyounger people, African Americans, Hispanics, and/orthose with a BRCA1 gene mutation (Stead, *et al* .2009). Triple negative breast cancer is associated with high proliferative rate, earlyrecurrence and poor survival rate .This aggressivedisease is insensitive to widely used target therapiessuch as trastuzumab and tamoxifine aromataseinhibitor which have been effective to reduce breastcancer mortality (Carey,*et al* . 2006) .EBV is a lymph tropic herpes virus etiologicallyassociated with a number of human malignancies ofboth epithelial and lymphoid origin. A number ofstudies have reported a positive correlation betweenEBV and breast cancer, with up to 50% of casesgiving a positive signal (Murray, *et al* .2003) .KI-67 is a nuclear protein necessary for cellularproliferation. Furthermore it is associated withribosomal RNA transcription (Bullwinkel, *et al* .2006) .Ki-67 is an excellent marker to determine the growthfraction of a given cell population. The fraction of Ki-67-positive tumor cells (the Ki-67 labeling index) isoften correlated with the clinical course of cancer.The best-studied examples in this context arecarcinomas of the prostate, brain and breast (Kametaka, *et al* . 2002). Breast cancer gene 1(BRAC1) is located on long armsof chromosome 17 and it encodes a protein of 1863amino acids, the protein physically associated with p53 and involve homologous recombination anddouble –strand break repair in response to DNAdamage (Greenberg. 2008).

2. Rational:

Breast cancer is one of the most cancers found in females and it is leading cause of death among cancers disease. In Sudan breast cancer account 25% of all cancer and 34% of female cancer (Rick record, 2010).

In this study we intended to detect EBV in triple negative breast cancer association of EBV as marker of biological aggressiveness also inherited mutation in BRCA-1gene woman who carry these gene have low risk of breast cancer. 30% of breast cancer diagnosed triple negative in Africa and 16% in American it is likely genetic and epidemiological factor may be responsible of high incidence of triple negative in Africa. As there were no studies available in or region touch the relation between triple negative breast cancer and EBV Lmp as epidemiological factor and inherited BRAC-1 gene mutation .In this studies we try to explore these relationship.

3. Objectives

3.1. General objective:

To detect EBV, BRCA-1 gene and ki 67in triple negative breast cancer using imunnohistochemistry.

3.2. Specific objectives:

To correlate triple negative breast cancer with age group

To correlateEBV infection, BRACA-1 gene and ki 67 expression with grade of breast cancer.

Chapter Two

Literature Review

Chapter Two

2. Literature Review

2.1. Anatomy of 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, the pectoralis major (Martini,*etal.*2009).

The breast has no muscle tissue. A layer 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 the uterus to change during the menstrual cycle(Martini,*etal.*2009).

Each breast contains 15 to 20 lobes arranged in a circular fashion. The fat (subcutaneous adipose tissue) that covers the lobes gives the breast its size and shape. Each lobe is comprised of many lobules, at the end of which are tiny bulb like glands, or sacs, where milk is produced in response to hormonal signals(Marieb, *et al.*2006).

Ducts connect the lobes, lobules, and glands in nursing mothers. These ducts deliver milk to openings in the nipple. The areola is the darker-pigmented area around the nipple (Marieb, *et al.*2006).

The breast is made up of both fatty tissue and glandular milk-producing tissues. The ratio of fatty versus glandular tissue varies among individuals. In addition, with the onset of menopause (decrease in estrogen levels), the relative amount of fatty tissue increases as the glandular tissue diminishes (Colditz,*et al.*2005).

The soft tissues of the breast are supported by the suspensory ligaments of Cooper. These ligaments run throughout the breast tissue parenchyma from the deep fascia beneath the breast and attach to the dermis of the skin. Since they are not taut, they allow for the natural motion of the breast. Eventually, this results in breast ptosis since these ligaments relax with age and time (Connor,*et al* . 2002).

2.2 Etiology of breast cancer:

Risk factors for the development of invasive breast cancer include female gender, age, family history, age at the time of first parturition, personal history of previous breast cancer, estrogen exposure, history of atypical hyperplasia or noninvasive lesions, and genetics (Broet,*et al*. 2000).

2.3 Pathphysiology of breast tumors:

The World Health Organization classification of breast tumors organizes both benign and malignant lesions by histological pattern. Epithelial tumors comprise the largest group, including intraductal papilloma, adenomas, intraductal and lobular carcinoma in situ, invasive (ductal and lobular) carcinoma, and Paget disease of the nipple. Invasive ductal carcinoma is by far the most common type (Burstein,*et al*.2009) .

Phylloides tumor, benign and malignant, and carcinosarcoma are rare lesions grouped as mixed connective tissue and epithelial tumors. Other common nonadenocarcinoma lesions of the breast include angiosarcoma and primary lymphoma(Burstein,*et al*.2009).

2.4 Clinical presentation:

Physical examination include inspection of the patient in the upright as well as supine positions. With the patient upright, assessment for symmetry and changes in the nipple and skin may be performed. Obvious size discrepancy, nipple inversion, skin dimpling, scaling, and edema are suggestive findings. Supraclavicular, infraclavicular, and axillary lymph adenopathy also can be best detected in this position (Silverstein *et al*, 2000).

When the patient is in the supine position with the bilateral arm extended over the head, the breast parenchyma can be compressed against the chest wall and each quadrant assessed for masses. Benign lesions are more frequently smaller, rubbery, well-circumscribed, and mobile. Characteristics suggestive of malignancy include skin involvement, fixation to the chest wall, irregular border, firmness, and enlargement (Silverstein,*et al.* 2001).

Assessment for nipple discharge also should be a routine part of the examination. Concerning characteristics include unilateral discharge, non milky fluid, and origin from a single duct. Intraductal papilloma, a benign finding, is the most common cause of unilateral bloody nipple discharge. Other benign pathology associated with nipple discharge includes sub areolar duct ectasia and fibrocystic changes (Wingo,*et al.* 2003). Usually, malignant pathology presenting with nipple discharge also is associated with a palpable mass and/or suggestive mammographic findings (Slamon,*et al.* 2001).

Currently, breast self-examination, performed monthly, is recommended for women beginning at 18 years-40 years approximately every 3 years. Annual evaluation is advised for those older than 40 years, particularly if risk factors are present (Veronesi, *et al*. 1999).

2.5 Triple-Negative Breast Cancer

Triple negative' is a term used to describe a type of breast cancer that means the pathology report that the breast cancer cells tested negative for estrogen receptors (ER-), progesterone receptors (PR-), and (HER2-). Testing negative for all three means the cancer is triple-negative(Bosch,*et al.*2010).

These negative results mean that the growth of the cancer is not supported by the hormones estrogen and progesterone, nor by the presence of too many HER2 receptors. Therefore, triple-negative breast cancer does not respond to hormonal therapy (such as tamoxifen or aromatase inhibitors) or therapies that target HER2 receptors, such as Herceptin (chemical name: trastuzumab)(Jiao*et al.*2014)

Triple negative breast cancer can be more aggressive and difficult to treat. Also, the cancer is more likely to spread and recur. The stageof breast cancer and the grade of the tumor will influence the prognosis (Lara,*et al.*2011)

Triple negative breast cancer is more likely to affect younger people, African Americans, Hispanics, and/or those with a BRCA1 gene mutation.(Stead,*et al.* 2009).

TNBC is less likely to be found on a mammogram than some other types of breast cancer.It can also be aggressive. Compared to other types, it tends to grow faster. It can be treated, but it may recur (come back) early and spread to other parts of the body. Part of the reason is due to the lack of targeted treatments (Russo,*et al.*2013).

2.6 Ki 67:

Proliferation is a key feature of the progression of tumors and is widely estimated by the immunohistochemical assessment of the nuclear antigen Ki-67. The expression of Ki-67 correlates with other measurements of proliferation, including S-phase and bromodeoxyuridine uptake. High Ki-67 is a sign of poor prognosis associated with a good chance of clinical response to chemotherapy, but its independent significance is modest and does not merit measurements in most routine clinical scenarios. However, its application as a pharmacodynamic intermediate marker of the effectiveness of medical therapy holds great promise for rapid evaluation of new drugs(Rahmanzadeh, *et al.* 2007)..

Antigen KI-67 is a nuclear protein that is associated with and may be necessary for cellular proliferation. Furthermore it is associated with ribosomal RNAtranscription(Bullwink,.*etal,*2006). Inactivation of antigen KI-67 leads to inhibition of ribosomal RNA synthesis(Rahmanzadeh, *et al.* 2007).

The Ki-67 protein (also known as MKI67) is a cellular marker for proliferation(Scholze .*et.al,*2001). It is strictly associated with cell proliferation. During interphase, the Ki-67 antigen can be exclusively detected within the cell nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. Ki-67 protein is present during all active phases of the cell cycle (G_1, S, G_2, and mitosis), but is absent from resting cells (G_0)(Scholzen,*et al.*2000).

Ki-67 is an excellent marker to determine the growth fraction of a given cell population. The fraction of Ki-67-positive tumor cells (the Ki-67 labeling index) is often correlated with the clinical course of cancer. The best-studied examples in this context are carcinomas of the prostate, brain and the breast and nephroblastoma . For these types of tumors, the prognostic value for survival

and tumor recurrence have repeatedly been proven in uni- and multivariate analysis (Kametaka, *et al*. 2002).

2.7 EBV Biology:

EBV is a γ herpesvirus; its 184-kb DNA genome of which encodes approximately 100 genes .The virus is transmitted through saliva , and primary infection is thought to occur in the oral mucosa . typically early in life as a subclinical illness. When infection is delayed until later childhood or adolescence, it manifests in 20–75% of people as infectious mononucleosis By adulthood, more than 90% of the population has been infected Primary EBV infection has a replicative (lytic) component marked by production of new virions(Sally. et.al . 2004).

2.7.1 EBV and Cancer:

As the balance of EBV persistence, virion production, and immune control is well evolved, the vast majority of the world's population tolerates lifelong EBV infection with no adverse health consequences. However, EBV has been linked to the etiology of several cancers, including African Burkitt lymphoma, in which it was initially described (Epstein.*et al,*1964); Hodgkin, AIDS, and nasal NK/T-cell lymphomas; post-transplant lymphoproliferative disorder; nasopharyngeal carcinoma (NPC); lymphoepithelioma-like squamous cell malignancies; gastric adenocarcinoma; and leiomyosarcoma (Niedobitek, *et al.* 2001).

2.7.2 EBV and Breast Cancer:

Indirect support for an association of EBV with breast cancer comes from observations that: EBV is present in breast tissue, where it is detected in breast milk in some women (Junker,.*et al*.1991); transfection of EBV DNA stimulates growth of human breast milk cells (Xue.*et al,*2003); some EBV-associated lymphomas occur in the breast (Abhyankar,*et al*.1998); breast cancer has

epidemiological similarities to young-adult Hodgkin lymphoma(Yasui,et. al.2001), although evidence for breast cancer implicates timing of primary EBV infection rather than viral oncogenesis (Yasui.,*et al*.2001); EBV has been identified in benign breast tumors in immunosuppressed women (Kleer.*et al*,2002); and *in vitro*, breast epithelial cells can be infected by direct contact with EBV-bearing lymphoblastoid cell lines (Speck, *and Longneker*.2000).

2.8 BARCA1:

The BRCA1 gene belongs to a class of genes known as tumor suppressor genes. Like many other tumor suppressors, the protein produced from the BRCA1 gene helps prevent cells from growing and dividing too rapidly or in an uncontrolled way(Duncan,et.al.1998).

The BRCA1 gene provides instructions for making a protein that is directly involved in repairing damaged DNA. In the nucleus of many types of normal cells, the BRCA1 protein interacts with several other proteins, including the proteins produced from the genes, to mend breaks in DNA. These breaks can be caused by natural and medical radiation or other environmental exposures, and also occur when chromosomes exchange genetic material in preparation for cell division. By helping repair DNA, BRCA1 plays a role in maintaining the stability of a cell's genetic information(Casilli, et.al 2002).

BRCA1 is a human tumor suppressor gene(Yoshida,and Mik*i*. 2004) (to be specific, a caretaker gene), found in all humans; its protein, also called by the synonym breast cancer type 1 susceptibility protein, is responsible for repairing DNA(Check,. 2010).

BRCA1 are normally expressed in the cells of breast and other tissue, where they help repair damaged DNA or destroy cells if DNA cannot be repaired. They are involved in the repair of chromosomal damage with an important role

in the error-free repair of DNA double-strand breaks (Friedenson,.2007). If BRCA1 or BRCA2 itself is damaged by a BRCA mutation, damaged DNA is not repaired properly, and this increases the risk for breast cancer.(Friedenson..2007) Thus, although the terms "breast cancer susceptibility gene" and "breast cancer susceptibility protein" (used frequently both in and outside the medical literature) sound as if they describe an oncogene, BRCA1 and BRCA2 are normal; it is their mutation that is abnormal(Miki et.al. 1994).

The human BRCA1 gene is located on the long (q) arm of chromosome 17 at region 2 band 1, from base pair 41,196,312 to base pair 41,277,500 (Build GRCh37/hg19) (map) BRCA1 orthologs (Mazoyer. 2005).

The BRCA1 protein contains the differentdomains(Paterson,1998) Zinc finger, C3HC4 type (RING finger)

2.8.1BRCA1 C Terminus (BRCT) domain:

This protein also contains nuclear localization signal and nuclear export signalmotifs (Henderson,.2005)

The human BRCA1 protein consists of four major protein domains; the Znf C3HC4- RING domain, the BRCA1 serine domain and two BRCT domains. These domains encode approximately 27% of BRCA1 protein. There are six known isoforms of P38398 BRCA1, with isoforms 1 and 2 comprising 1863 amino acids each((Paterson,1998).

In the nucleus of many types of normal cells, the BRCA1 protein interacts with RAD51 during repair of DNA double-strand breaks.(Boulton,.2006) These breaks can be caused by natural radiation or other exposures, but also occur when chromosomes exchange genetic material (homologous recombination, e.g., "crossing over" during meiosis). The BRCA2 protein, which has a function similar to that of BRCA1, also interacts with the

RAD51 protein. By influencing DNA damage repair, these three proteins play a role in maintaining the stability of the human genome (Ding, et.al.2004)

BRCA1 is also involved in another type of DNA repair, termed mis-match repair. BRCA1 interacts with the DNA mismatch repair protein MSH2.(Narod, *et al*.2004) MSH2, MSH6, PARP and some other proteins involved in single-strand repair are reported to be elevated in BRCA1-deficient mammary tumors.(Warmoes,*et al*.2012)

2.8.2Mutations and cancer risk

Certain variations of the BRCA1 gene lead to an increased risk for breast cancer as part of a hereditary breast-ovarian cancer syndrome. Researchers have identified hundreds of mutations in the BRCA1 gene, many of which associated with an increased risk of cancer. Women with an abnormal BRCA1 or BRCA2 gene have up to an 80% risk of developing breast cancer by age 90; increased risk of developing ovarian cancer is about 55% for women with BRCA1 mutations and about 25% for women with BRCA2 mutations (Mazoyer.2005)These mutations can be changes in one or a small number of DNA base pairs (the building-blocks of DNA). Those mutations can be identified with PCR and DNA sequencing(Warmoes,*et al*.2012).

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A mutated BRCA1 gene usually makes a protein that does not function properly. Researchers believe that the defective BRCA1 protein is unable to help fix DNA damages leading to mutations in other genes. These mutations can accumulate and may allow cells to grow and divide uncontrollably to form a tumor. Thus, BRCA1 inactivating mutations lead to a predisposition for cancer.
BRCA1 mRNA 3' UTR can be bound by amiRNA, Mir-17 microRNA. It has been suggested that variations in this miRNA along with Mir-30 microRNA could confer susceptibility to breast cancer(Shen,*et al*.2009)

In addition to breast cancer, mutations in the BRCA1 gene also increase the risk of ovarian, fallopian tube, and prostate cancers. Moreover, precancerous lesions (dysplasia) within the Fallopian tube have been linked to BRCA1 gene mutations. Pathogenic mutations anywhere in a model pathway containing BRCA1 and BRCA2 greatly increase risks for a subset of leukemias and lymphomas (Friedens on,.2007)

Women having inherited a defective BRCA1 or BRCA2 gene have risks for breast and ovarian cancer that are so high and seem so selective that many mutation carriers choose to have prophylactic surgery. There has been much conjecture to explain such apparently striking tissue specificity(Levy, et.al. 2007). Major determinants of where BRCA1/2 hereditary cancers occur are related to tissue specificity of the cancer pathogen, the agent that causes chronic inflammation or the carcinogen(Scully,et.al ,2002). The target tissue may have receptors for the pathogen, become selectively exposed to an inflammatory process or to a carcinogen. An innate genomic deficit in a tumor suppressor gene impairs normal responses and exacerbates the susceptibility to disease in organ targets. This theory also fits data for several tumor suppressors beyond BRCA1 or BRCA2. A major advantage of this model is that it suggests there may be some options in addition to prophylactic surgery (Levin,*etal.*2012).

2.9 Diagnosis of breast cancer:

The introduction of mammographic screening has led to an increased detection of ductal carcinomas in situ (DCIS), which now constitute approximately 10–20% of all detected breast cancers .The single most common mammographic finding is the presence of micro calcification .The second most common mammographic finding is the presence of an irregular or ill-defined mass. The other mammographic categories identified included architectural disturbance and asymmetric density (Foxcroft *.et al.* 2004). Mammography has been considered relatively unsatisfactory in young women, and this apparent

insensitivity has been postulated to be a result of more rapidly growing and aggressive tumors in younger women (Gill,*et al.* 2004).

Fine-needle aspiration (FNA) is one of the first-line diagnostic procedures in the evaluation of a palpable breast mass. It is particularly useful in evaluation of cystic lesions. Aspirate from benign cystic lesions typically appears green tinged or serous and should result in collapse of the cavity. Persistence of a palpable mass and recurrence following a repeat aspiration are general indications for open biopsy. Bloody cyst fluid should be examined by pathology and warrants an open biopsy. Clearly malignant and suggestive lesions warrant an open biopsy. However, performing a biopsy of benign or indeterminate lesions depends on assessment of individual patient risk and correlation with physical examination findings (Kuske,*et al*. 2001).

Fine needle aspiration cytology (FNAC) is part of the triple assessment for the diagnosis of breast lesions. It is an established, highly accurate method for diagnosing breast cancer and has given rise to a reduction in the number of excision biopsies for benign breast disease. The FNAC report is scored into five categories: C5 malignant, C4 suspicious of malignancy, C3 atypical probably benign, C2 benign and C1 inadequate material (Mottahedeh*et al*. 2003).

Tru-cut (core) needle biopsy is an alternative that can provide more tissue to derive architectural information and determine the invasiveness of a lesion. This is a distinct advantage over FNA, particularly in patients with large palpable masses suggestive of cancer. In this instance, definitive diagnosis of malignancy by true-cut biopsy may eliminate the need for an open biopsy prior to definitive surgical treatment (eg, modified radical mastectomy, conservation) (Rosen,*et al*. 2005).

2.10. Breast cancer treatment:

There are different types of treatment for patients with breast cancer.

Six types of standard treatment are used:

2.10.1 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 icrmoscope to see if they contain cancer cells(Bear et al. 2012).

Chemotherapy may be given before surgery to remove the 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 is called neoadjuvanttherapy(Devita,*et al*. 2008).

2.10.2 Radiation therapy:

Radiation therapy is a cancer treatment that uses high-energy x-rays 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).

2.10.3 Chemotherapy:

Chemotherapy is a cancer treatment that uses drugs 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).

2.10.4 Hormone therapy:

Hormone therapy is a cancer treatment that removes hormones or blocks their action and stops cancer cells from growing. Hormones are substances made by glands in the body and circulated in the bloodstream. Some hormones can cause certain cancers to grow.Hormone therapy with tamoxifen is often given to

patients with early stages of breast cancer and those with metastatic breast cancer (cancer that has spread to other parts of the body).

For the treatment of early stage breast cancer, certain aromatase inhibitors may be used as adjuvant therapy instead of tamoxifen or after 2 or more years of tamoxifen. For the treatment of metastatic breast cancer, aromatase inhibitors are being tested in clinical trials to compare them to hormone therapy with tamoxifen(Birrell,et al . 1995).

Chapter Three

Materialsand Methods

Chapter Three

3.Materialsand Methods:

3.1 Study design:

This is a descriptive retrospectivehospital based study aimed to detect EBV in triple breast cancer and BRAC-1 mutation and ki67inTNBC using Immuohistochemistry.

3.2 Study area:

This study were conducted at Radiation and Isotope Center –Khartoum (RICK) during the period 2013-2015.

3.3 Study population:

Hundred paraffin wax tissue blocks were taken from RadiationandIsotope Center, Khartoum (RICK) archive that includes malignant breast cancer biopsies.

3.4 Selection criteria:

Paraffin blocks that were previously diagnosed as triple negatives breast cancer were selected for thise study.

3.5 sample processing:

From each paraffin block three sections were cut into 3µm thickness, section were floated into preheated floating water bath at 40˚C , three slides were coated with adhesive salinized glass slide for Immunohistochemistry, stained by EBV LMP,BRAC-1and Ki67 .

3.6 Immunohistochemical staining:

Sections were taken to distilled water. Antigen retrieval achieved by heat retrieval using water path briefly, slides were placed in Coplin jars containing enough 0.01 M sodium citrate solution (pH 9.9) to cover the sections, then were preheated at 95°C (high temperature) for 30 min. (Bancroft, 2002). Slides were incubated with peroxidase blocking reagent for 10min then slides were incubated in primary antibody for 20 minutes at room temperature in a humid chamber, and then were rinsed in Phosphate buffer saline. The primary antibody is ready to use (Thermo scientific USA). After washing, binding of antibodies were detected by incubation for 20 minutes with super sensitive secondary system (one step) the slides were then rinsed with three changes of PBS. Finally, followed by adding 3, 3 diaminobenzidine tetra hydrochloride -DAB (Thermo scientific) as a chromogen to produce the characteristicbrowencoulor then counter stain with mayerhamatoxline and clear and mount.positive control slides were prepared from breast known to be positive for the antigen under study.

The negative control slides were prepared from the same tissue block, but incubated with PBS instead of the primary antibody. Each slide was evaluated with investigator then the results were confirmed by supervisor.

3.7 Ethical consideration:

All samples were taken after take permission from hospital administration. Andethical clearance from Khartoum ministeryofhealth.

3.8Assessment of results:

immunohistochemistery result were diagnosed according to the expression of immune marker as cytoplasm , cell membrane and nuclear stain we accept the

positive reaction more than 5% of the tumor and also exclude any back ground stain or non- specific reactivity

3.9 Statistical analysis:

Data was analyzed using SPSS computer program;frequencies mean and chi^2test were calculated.

Chapter Four

Results

Chapter Four

4.1 The Result

In this studies100 women were intended with different age in table1 include pre and post menopause female divided into two group according to early one set of breast cancer suspected 40 years as in table1≥ (40) 35% and ≤ (40) 65%.

Histopathological diagnosis showed different type of breast cancer include 7 group the high frequency is invasive ductale carcinoma 88% in situ carcenoma3%,invasive lobular carcinoma 3%, mucinouse carcinoma 2%,squamouse cell carcinoma2%, tubular carcinoma and phylloid tumor 1%for each typeasintable2.

The frequency of caner grading in the sample collected were according to (Bloom Richardson Grade). Grade I 20%,gradeII 20% and gradIII 60% as in table3.

The result of immunohistochemistery for Ki67in the sample collectedshowed 75%show positive result and 25%is negative result as intable4.

The result of immunohistochemistery result of EBV in breast cancershowed 21sample is positive from 100 (21%) and 79 is negative (79%) as intable5.

The result of immunohistochemistery result ofBRCA-1in breast cancershowed 16sample is positive from 100(16%)and 84is negative(84%) as intable 6.

The relationbetweenage group andcancer status≥ (40) were30 from case study and for from control cases≤ (40) were39 from cases study and 26 from control with P.0.008 as in table7

:The relation between KI67 result and cancer statusshowed 70 sample of TNBC is positive out of 70 (100%) in spit of 5sample positive from 30 triple positives positive and 25 negative (5%) positive and(25%) negative with p.value: 0.000 which is significant for the result as intable8.

The relation between EBV result and cancer statusshowed 70 sample of TNBC 17(24%) is give positive reaction and 53 is negative and 4 sample show positive result from 30 triple positive and 26 is negative with p.value: 0.218as in table9

The relation between BRCA1 result and cancer statusshowed 70 sample of TNBC16 is positive for BRCA1 (22,9%)and 30 sample triple positive gives negative result from 30 (100%)with p.value: 0.004as in table 10

The relation betweenKI67 cancer &grading grade 1 showed just 2% positive and 18% is negative grade 11 showed 13% positive and 7% is negative grade 111 showed 60% positive with p.value : 0.000 as intabel11

The relation between BRCA1 result and grading cancer grade 1 showed no positive result and grade 11 showednopsitive result except grade 111 showed 16% positive and 84% negative result with significant p.value: 0.002as intable 12.

Table 4.1: Distribution of age group among study population

Age group	Frequency	Percentage
≥40	35	35%
<40	65	65%
Total	100	100%

Table4.2: Frequency of histopathological diagnosis among study of population

Diagnosis	Frequency	Percentage
In situ carcinoma	3	3%
Invasive ductal carcinoma	88	88%
Invasive lobular carcinoma	3	3%
Mucinous carcinoma	2	2%
Squamous cell carcinoma	2	2%
Tubular carcinoma	1	1%
Phylloid tumor	1	1%
Total	100	100%

Table4.3: Frequency of cancer Gradingamong study of population:

Grade	Frequency	Percentage
Grade I	20	20%
Grade II	20	20%
Grade III	60	60%
Total	100	100%

Table4.4: Immunohistochemical result of Ki 6among breast cancer patient:

Result of Ki67	Frequency	Percentage
Positive	75	75%
Negative	25	25%
Total	100	100%

Table4.5: Immunohistochemical result of EBVamong breast cancer patient:

Result of EBV	Frequency	Percentage
Positive	21	21%
Negative	79	79%
Total	100	100%

Table4.6: Immunohistochemical result of BRCA-1mutation:

Result of BRCA-1	Frequency	Percentage
Positive	16	16%
Negative	84	84%
Total	100	100%

Table4.7: Relation between Age group and cancer statusamong study of population:

Status	Age group		Total
	≥ (40)	≤ (40)	
Triple negative	31	39	70
Triple positive	4	26	30
Total	35	65	100

P.value: 0.008

Table4.8: Relation between KI67 result and cancer statusamong study of population:

Status	KI67		Total
	Positive	Negative	
triple negative	70	0	70
triple positive	5	25	30
Total	75	25	100

P.value: 0.000

Table4.9: Relation between EBVresult and cancer statusamong study of population:

Status	EBV		Total
	Positive	Negative	
Triple negative	17	53	70
Triple positive	4	26	30
Total	21	79	100

P.value: 0.218

Table4.10: Relation between BRCA-1 result and cancer statusamong study of population:

Status	BRCA-1mutation		Total
	Positive	Negative	
Triple negative	16	54	70
Triple positive	0	30	30
Total	16	84	100

P.value: 0.004

Table4.11: Relation between KI67 cancer and grading of tumor:

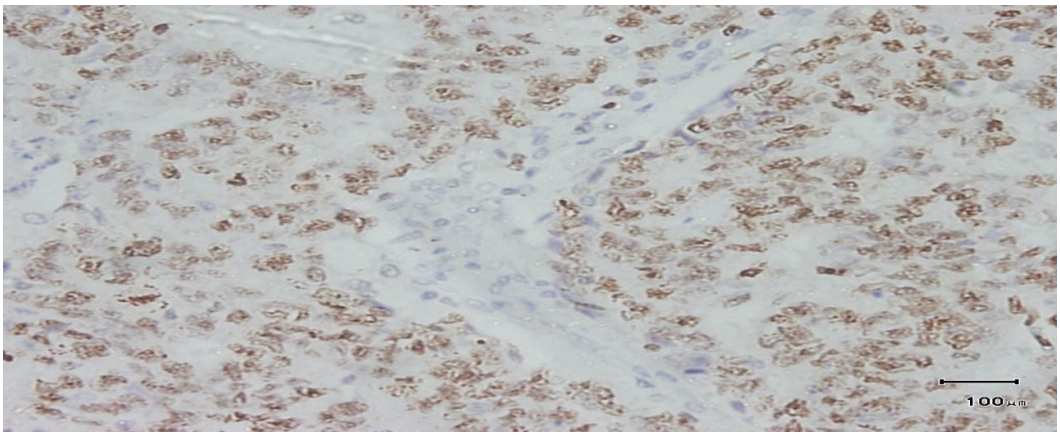
Grade	KI67		Total
	Positive	Negative	
Grade I	2	18	20
Grade II	13	7	20
Grade III	60	0	60
Total	75	25	100

P.value: 0.00

Table4.12: Relation between BRCA-1 andgradingof tumor:

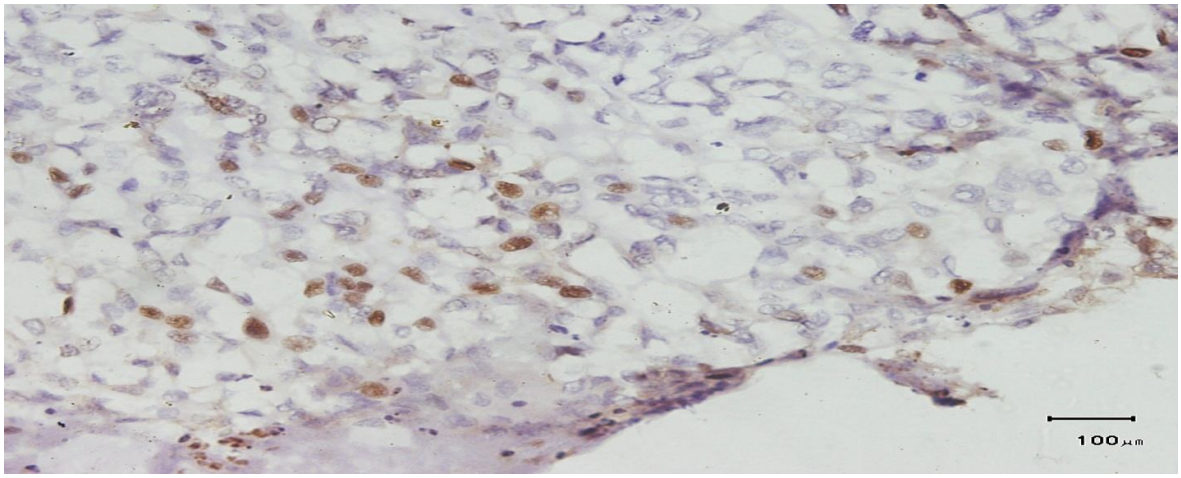
Grade	BRCA-1mutation		Total
	Positive	Negative	
Grade I	0	20	20
Grade II	0	20	20
Grade III	16	44	60
Total	16	84	100

P.value: 0.002

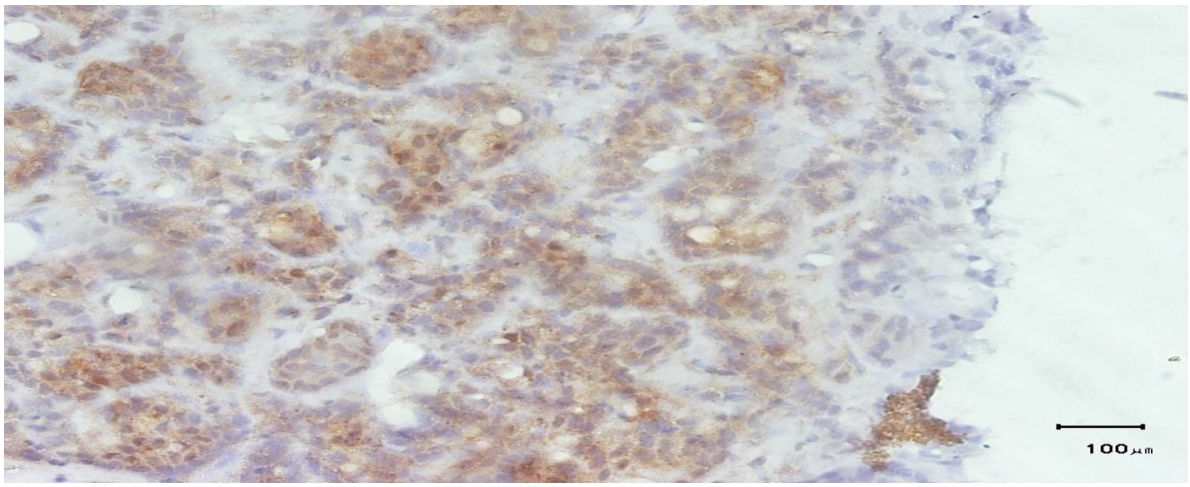


Microphotograph 4.1: positive expression of Ki67 marker (neuclear stain) in TNBC invasive ductal carcinoma grad III

(40x)

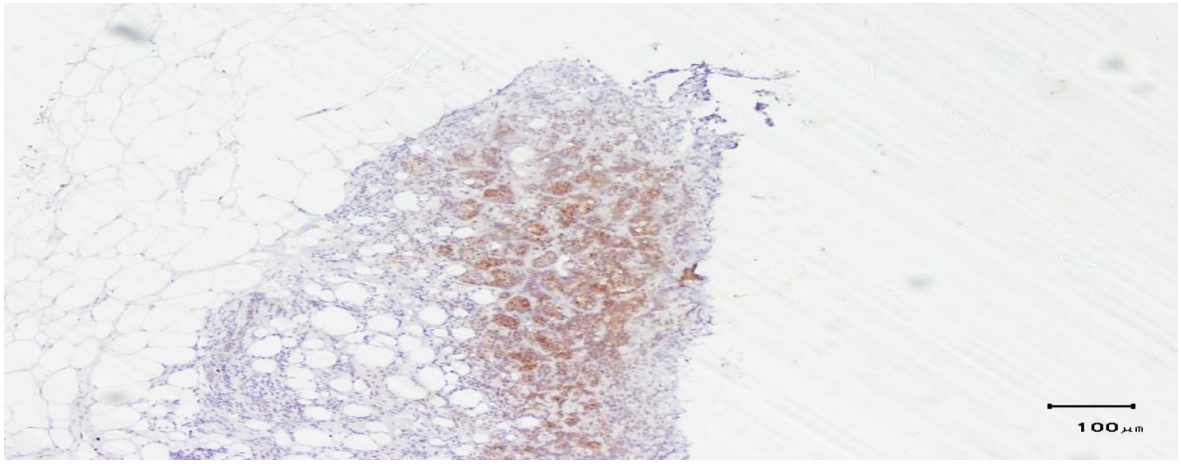


Microphotograph 4.2: positive expression of BRCA1 marker (neuclear stain) in TNBC invasive ductal carcinoma grad III (40x)



Microphotograph 4.3:positive expression EBV marker(cell memberane and cytoplasmic stain) in TNB invasive ductal carcinoma grad III

(40x)



Microphotograph 4.4:positive expression EBV marker(cell memberane and cytoplasmic stain) in TNB invasive ductal carcinoma grad III

(10x)

Chapter Five

Discussion

Chapter Five

Discussion

In this study the patient age above 20yearswhich was the age of early one set of breast cancer which was reported worldwide and with high significant relation to triple negative breast cancer these finding was similar to that study reported byLara, *et al*.2011 that younger age were associated independently with TNBC.

Another similar study in an Asian series of TNBC They found that among women diagnosed with breast cancer aged 36 to 50 years. Bosch, *et al* .2010

TNBC in this study show high cancer grade, most cases were grad III 60/100. These finding were in similarity with the study reported by Lara, *et al*.2011, who reported that triple-negative breast cancer in had high histological grade.,

Invasive ductal carcinoma showed high frequency in this study comparing withother type of breast cancer diagnosis, similar finding were reported by Carey, *et al* .2006.

In the study the evaluation of Ki67 as a factors that are involved in the management of breast cancer ki67 marker in TNBC was positive in all samples of case group (100%) and 16%(5/30) of control group, with significant relation between ki67 expression and breast cancer status in similarity with many studies Ki-67 is an excellent marker to determine the growth fraction of a given cell population. The fraction of Ki-67-positive tumor cells (the Ki-67 labeling index) is often correlated with the clinical course of breast cancer (Kametaka,*et al*. 2002).

In the present study breast cancer gene1 is useful marker for diagnosis of TNBC . 70 samples were tested for BRCA1 mutation in of case group against 30 samples of control group, 16 case showed positive reaction from70 (22%) and these finding were similar with frequency of BRCA1mutations in Sudan

reported for BRCA1 by Awadelkarim, *et al.* (2007), who found BRCA1mutations detected in the breast cancer series were highly diverse. Other study done in north Sudan for BRCA1detection, reported a similar result of BRCA mutation, which was 20% in the sample group Elnour, *et al* (2012).

Another similar study reported worldwide in an Asian series of TNBC patients demonstratedthat 24.5% of patients have germ line mutations in *BRCA1***(**Bosch, *et al* .2010).

Immunohistochemistry targets and localizes viral proteins, thus distinguishing EBV in tumor cells from EBV in lymphocytes. HIC targeting LMP1 is a widely employed assay that is sensitive. In the present study noassociation between EBV with TNBC .However ,several studies explained that the latent membrane protein (LMP)of EBV may play arole in the development of breast cancer(Curran,*et al.*2011).Expression of LMP in theepidermis of transgenic by LMP mice induces hyperplasia an early step in the carcinogenic process(Wilson,*et al.*1990).In this study we detect EBV in TNBC , 24.3% of samples were gave positive and 13.3% of samples from non –TNBC were gave positive resultwith no significant relation with TNBC ,these results similar to many immunohistochemical studies targeting LMP1 foundno relation between LMP and TNBC (Luqmani, *et al.* 1995. Chu, *et al.* 1998).

However A number of studies explained that the latent membrane protein (LMP) of EBV may play a role in the development of breast cancer (Murray, *et al*. 2003; Preciado, *et al*. 2005; Fawzy, *et al*. 2008).

Chapter six

Conclusion and Recommendations

Chapter Six

6. Conclusion and recommendations

6.1 Conclusion:

On the bases of this study we conclude the following:

- The most common type of TNBC histological diagnosis was invasive ductal carcinoma with high grade cancer.
- No relation between EBV infection and TNBC
- BRCA1gene mutation and ki67associated with TNBC,
- BRCA1gene associated with early one set of breast cancer.

6.2 Recommendations:

On the bases of this study we recommended that:

- BRCA1 should be applied forgenetic screening for younger women for early detection to follow family history.
- Further studies should be done with large sample size in considering of other risk factors.
- BRCA1should be applied immunohistochemically for diagnosis. Because is less expensive and not time consuming than genetic.
- EBV detection should be assessment with advance technique such as PCR or FISH

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Appendixes

Appendix1

Solutions and stains

Hematoxylin Solution (Mayer):

Potassium or ammonium (alum) -------- 50 g

Hematoxylin ---------- 1 g

Sodium iodate ---------- 0.2 g

Citric acid ---------- 1 g

Distilled water ---------- 1000 ml

Stir to dissolve the chemicals in the order listed above. For example, dissolve alum in 1000 ml distilled water first. When alum is completely dissolved, add hematoxylin. When hematoxylin is completely dissolved, add sodium iodate, etc.

Citrate Buffer Antigen Retrieval Protocol

Description: Formalin or other aldehyde fixation forms protein cross-links that mask the antigenic sites in tissue specimens, thereby giving weak or false negative staining for immunohistochemical detection of certain proteins. The citrate based solution is designed to break the protein cross-links, therefore unmask the antigens and epitopes in formalin-fixed and paraffin embedded tissue sections, thus enhancing staining intensity of antibodies.

Sodium Citrate Buffer (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0):

Tri-sodium citrate (dihydrate) --------- 2.94 g

Distilled water ---------- 1000 ml

Mix to dissolve. Adjust pH to 6.0 with 1N HCl and then add 0.5 ml of Tween 20 and mix well. Store this solution at room temperature for 3 months or at 4 C for longer storage.

Note: this buffer is commonly used and works perfectly with many antibodies. It gives very nice intense staining with very low background.

Citrate Buffer (10mM Citric Acid, 0.05% Tween 20, pH 6.0):

Citric acid (anhydrous) ---------- 1.92 g

Distilled water ---------- 1000 ml

Mix to dissolve. Adjust pH to 6.0 with 1N NaOH and then add 0.5 ml of Tween 20 and mix well. Store this solution at room temperature for 3 months or at 4 C for longer storage

Phosphate Buffered Saline (PBS)

10X PBS (0.1M PBS, pH 7.4):

Na2HPO4 (anhydrous) ---------- 10.9 g

NaH2PO4 (anhydrous) ---------- 3.2 g

NaCl ---------- 90 g

Distilled water ---------- 1000 ml

Mix to dissolve and adjust pH to 7.4

Store this solution at room temperature. Dilute 1:10 with distilled water before use and adjust pH if necessary.

Appendix 2

MICROBIOLOGICAL STATE

Product(s) not sterile.

MATERIALS REQUIRED BUT NOT PROVIDED

Primary antibody. Diluent.

SPECIMEN & REAGENT PREPARATION

Refer to Procedure.

PROCEDURE

STAINING PROTOCOL (kit components in bold):

NOTE: The appropriate controls, especially negative controls, must be included with every manual or automated slide run. The inclusion of negative controls will aid in accurate interpretation of the staining results and help in determining false positives. Refer to the warnings and precaution section for details.

1. Deparaffinize and rehydrate tissue section.
2. Wash 2 times in buffer.
3. If required, incubate tissue in digestive enzyme (or appropriate pretreatment).
4. Wash 4 times in buffer.
5. To reduce nonspecific background staining due to endogenous peroxidase, incubate slide in **Hydrogen Peroxide Block** for 10-15 minutes.
6. Wash 4 times in buffer.
7. Optional: Apply **Ultra V Block** and incubate for 5 minutes at room temperature to block nonspecific background staining. **NOTE:** Do not exceed 10 minutes or there may be a reduction in desired stain. (*May be omitted if primary antibodies are diluted in buffers containing 5-10% normal goat serum.*)
8. Wash (*Optional*).
9. Apply primary antibody and incubate according to manufacturer's recommended protocol.
10. Wash 4 times in buffer.
11. Apply **UltraVision ONE HRP Polymer** and incubate for 30 minutes at room temperature. (**NOTE:** HRP Polymer is light sensitive. Please avoid unnecessary light exposure and store in opaque vial.)
12. Wash 4 times in buffer.
13. Add 1 drop (40 µl) **DAB Plus Chromogen** to 2 ml of **DAB Plus Substrate**, mix by swirling and apply to tissue. Incubate for 5-15 minutes, depending on the desired stain intensity.
14. Wash 4 times in DI water.
15. Counterstain and coverslip using a permanent mounting media.

The specificity and sensitivity of antigen detection is dependent on the specific primary antibody used.

REFERENCES

1. Shan-Rong Shi, James Guo, Richard J. Cote, Lillian Young, Debra Hawes, Yan Shi, Sandra Thu, and Clive R. Taylor, Applied Immunohistochemistry & Molecular Morphology, vol 7, 201-208, 1999.
2. Karen Petrosyan, Rosalba Tamayo, and Daisy Joseph, "Sensitivity of a Novel Biotin-free Detection Reagent (PowerVision+) for Immunohistochemistry" J. Histotechnology, vol 25, 247-250, 2002.

TROUBLESHOOTING

Please contact Thermo Fisher Scientific Technical Support by phone (1-510-991-2800 or 1-800-828-1628) or by email (lab.reagents@thermofisher.com).

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Thermo SCIENTIFIC

UltraVision ONE Detection System
HRP Polymer & DAB Plus Chromogen

Please note this data sheet has been changed effective December 19, 2011

INTENDED USE

For In Vitro Diagnostic Use

AVAILABILITY:

Catalog #	Slide Volume
TL-015-HDJ	75-150 slides

SPECIFICITY: Anti-Mouse IgG (H+L), Anti-Rabbit IgG (H+L)
ENZYME: Peroxidase
CHROMOGEN/SUBSTRATE: Diaminobenzidine (DAB)

REAGENTS

Qty.	Component	TL-015-HD
1	Ultra V block	TA-015-UB
1	Hydrogen Peroxide Block	TA-015-HP
1	UltraVision ONE HRP Polymer	TL-015-PHJ
1	DAB Plus Substrate	TA-015-HSX
1	DAB Plus Chromogen	TA-001-HCX

(The three-digit number in the middle of each Catalog # designates the reagent volume in mL or number of tablets.)

DESCRIPTION

UltraVision ONE is a robust ONE-step polymer system that provides increased sensitivity, time savings and detection simplicity. The UltraVision ONE HRP polymer is an innovative, patented technology. It consists of smaller amino acid polymer subunits that minimize conflicts in binding the target protein. Decreased binding conflicts result in more consistent staining and better signal amplification. Ultimately, this gives the user higher sensitivity and antibody efficiency. UltraVision ONE HRP polymer allows the use of less antibody to obtain better signal-to-noise ratios. This system is also biotin-free, which eliminates background staining found with traditional biotin-based detection methods. For optimal interpretation of results, appropriate positive and negative controls must be included.

PRINCIPLE OF THE PROCEDURE

This UltraVision ONE HRP polymer detection system detects rabbit and mouse immunoglobulins bound to an antigen in tissue sections. The specific primary antibody is located by a universal secondary antibody polymer formulation. The amino acid polymer is conjugated to horseradish peroxidase and the Fab fragments of goat anti-rabbit and goat anti-mouse. The polymer complex is then visualized with an appropriate substrate/chromogen.

WARNINGS & PRECAUTIONS

Clinical cases should be evaluated within the context of the performance of appropriate controls. Inclusion of a negative control fixed and processed in the same manner as the patient specimen placed on every slide run in addition to the case tissue is strongly recommended. For the test to be considered valid, the negative control should be clean. In some instances, very faint staining may be observed and is deemed acceptable. In addition, it is recommended that a negative tissue control slide be included for every batch of samples processed and run on the Lab Vision Autostainer. This negative tissue control should be included to ensure that the other treatment procedures did not create false positive staining.

Refer to MSDS for material safety instructions.

STORAGE & SHELF LIFE

Store at 2-8°C. Each component is stable for 18 months.

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Appendix 3

Thermo SCIENTIFIC

RESEARCH USE ONLY DATA SHEET
Rev 102414G

Ki67

Rabbit Polyclonal Antibody

Cat. #RB-9043-P0, -P1, or -P (0.1ml, 0.5ml, or 1.0ml at 200µg/ml) (Purified Ab with BSA and Azide)
Cat. #RB-9043-R7 (7.0ml) (Ready-to-Use for Immunohistochemical Staining)

Please note this data sheet has been changed effective October 24, 2014

Description:
Ki-67 is a nuclear protein, which is expressed in the proliferating cells. Ki-67 is preferentially expressed during late G1-, S-, M-, and G2-phases of the cell cycle, while cells in the G0 (quiescent) phase are negative for this protein.

Comments: This antibody is excellent for staining of formalin-fixed, paraffin-embedded tissues.

Mol. Wt. of Antigen:

Epitope: C-terminal

Species Reactivity: Human and Rat. Others not tested.

Immunogen: 345kDa
A synthetic peptide from C-terminus of human Ki67

Applications and Suggested Dilutions:

- Immunohistology (Formalin/paraffin)

(Ab 1:300 to 1:600 for 20 min with LV's LP and 30min with UV ONE or UV)

- [Staining of formalin-fixed tissues requires boiling tissue sections in 10mM citrate buffer, pH 6.0 for 10-20 min followed by cooling at RT for 20 min.]

The optimal dilution for a specific application should be determined by the investigator.

Positive Control: Breast carcinoma and lymph node

Cellular Localization: Nuclear

Storage and Stability:
Store vial at 4°C. When stored at 2-8°C, this antibody is stable for 24 months.

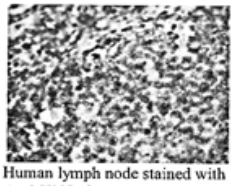
Supplied As: Antibody fraction purified from rabbit anti-serum. Prepared in 10mM PBS, pH 7.6, with 0.2% BSA and 15mM sodium azide.

or

Prediluted antibody which is ready-to-use for immunohistochemical staining.

Limitations and Warranty:
Our products are intended FOR RESEARCH USE ONLY and are not approved for clinical diagnosis, drug use or therapeutic procedures. No products are to be construed as a recommendation for use in violation of any patents. We make no representations, warranties or assurances as to the accuracy or completeness of information provided on our data sheets and website. Our warranty is limited to the actual price paid for the product. NeoMarkers is not liable for any property damage, personal injury, time or effort or economic loss caused by our products.

Material Safety Data:
This product is not licensed or approved for administration to humans or to animals other than the experimental animals. Standard Laboratory Practices should be followed when handling this material. The chemical, physical, and toxicological properties of this material have not been thoroughly investigated. Appropriate measures should be taken to avoid skin and eye contact, inhalation, and ingestion. The material contains 0.09% sodium azide as a preservative. Although the quantity of azide is very small, appropriate care should be taken when handling this material as indicated above. The National Institute of Occupational Safety and Health has issued a bulletin citing the potential explosion hazard due to the reaction of sodium azide with copper, lead, brass, or solder in the plumbing systems. Sodium azide forms hydrazoic acid in acidic conditions and should be discarded in a large volume of running water to avoid deposits forming in metal drainage pipes.



Human lymph node stained with Anti-Ki67, Cat# RB-9043-P

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Thermo SCIENTIFIC

RESEARCH USE ONLY DATA SHEET

Rev 100305C

Clinical customers please refer to IVD / ASR Data Sheet

Epstein-Barr Virus / LMP Ab-1 (Clone CS1+CS2+CS3+CS4)

Mouse Monoclonal Antibody

Cat. #MS-1458-S0, -S1, or -S (0.1ml, 0.5ml, or 1.0ml Supernatant)

Cat. #MS-1458-R7 (7.0ml) (Ready-to-Use for Immunohistochemical Staining)

Comments: Ab-1 reacts strongly with EBV-positive lymphoblastoid cell lines and EBV infected B cell immunoblasts in infectious mononucleosis. It also reacts with 25 to 50 per cent of EBV-associated undifferentiated nasopharyngeal carcinomas and with Reed Sternberg cells in approximately 90% of EBV-associated Hodgkin's disease cases. The cocktail recognizes distinct epitopes on the hydrophilic carboxyl region of LMP which is exposed to the cytosol.

Mol. Wt. of Antigen: 60kDa (LMP)

Epitope: Located in the internal part of the membrane-associated viral LMP.

Species Reactivity: EBV-positive cells.

Clone Designation: CS1+CS2+CS3+CS4

Ig Isotype / Light Chain: IgG_1 / κ

Immunogen: EBV-encoded recombinant latent membrane protein.

Applications and Suggested Dilutions:

- Immunohistology (Formalin/paraffin)
  (Use Ab at 1:25 to 1:50 for 60 min at RT)
- [Staining of formalin-fixed tissues REQUIRES boiling tissue sections in 10mM citrate buffer, pH 6.0, (**NEOMARKERS'** Cat. #AP-9003), for 10-20 min followed by cooling at RT for 20 min.]

The optimal dilution for a specific application should be determined by the investigator.

Positive Control: EBV-positive lymphoblastoma.

Cellular Localization: Cytoplasmic

Supplied As: Tissue culture supernatant with 0.09% sodium azide, **or** Prediluted antibody which is ready-to-use for staining of formalin-fixed, paraffin-embedded tissues.

Storage and Stability:
Store vial at 4°C. When stored at 2-8°C, this antibody is stable for 24 months.

Suggested References:

1. Rowe M et al. J Gen Virol 1987; 68:1575-86.
2. Liebowitz D et al. J Virol 1986; 58:233-7.

Limitations and Warranty:
Our products are intended FOR RESEARCH USE ONLY and are not approved for clinical diagnosis, drug use or therapeutic procedures. No products are to be construed as a recommendation for use in violation of any patents. We make no representations, warranties or assurances as to the accuracy or completeness of information provided on our data sheets and website. Our warranty is limited to the actual price paid for the product. NeoMarkers is not liable for any property damage, personal injury, time or effort or economic loss caused by our products.

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'roduct Data Sheet

{CA1 Antibody (8F7)

Tested Species Reactivity
nan (Hu)
use (Ms)

Tested Applications	Dilution *
stern Blot (WB)	1-10 µg/ml
nmunohistochemistry (Paraffin) ·C (P))	Assay dependent
nunoprecipitation (IP)	Assay dependent

…ested working dilutions are given as a guide only. It is recommended that the user titrates the product for use in their own …riment using appropriate negative and positive controls.

Details	
Catalog Number:	MA1-23162
Size:	100 µg
Class:	Monoclonal
Type:	Antibody
Clone:	8F7
Host / Isotype:	Mouse / IgG1, k
Immunogen:	Protein fragment expressed in E. coli corresponding to amino acids 341-748.

Form Information	
Form:	Liquid
Concentration:	1mg/ml
Purification:	Protein G
Storage Buffer:	PBS, pH 7.2
Preservative:	no preservative
Storage Conditions:	Store at 4°C short term. For long term storage, store at -20°C avoiding freeze/thaw cycles

Product Specific Information

1-23162 detects BRCA1 in Human and Mouse samples.

1-23162 has been successfully used in Immunohistochemistry, nunoprecipitation and Western Blot procedures. For nunohistochemistry (paraffin), perform heat mediated antigen retrieval ore commencing with IHC staining protocol (0.1M citrate buffer at 95°C vater bath for 20 minutes). Avoid nonspecific binding by incubation in rmal rabbit anti-mouse serum. Incubate primary antibody overnight and biotin-streptavidin amplification kit. Block endogenous peroxidase with % H2O2 for 30 minutes.

A1-23162 immunogen corresponds to Protein fragment expressed in E. corresponding to amino acids 341-748..

General Information

BRCA1-8F7 recognizes full-length BRCA1, a 220kDa nuclear phosphoprotein. In a high proportion of breast and ovarian cancer cell lines, BRCA1 aberrantly mislocates to the cytoplasm. Its usefulness to monitor functional inactivation of BRCA1 in sporadic breast cancers is under active investigation.

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