Immunohistochemistry and histopathology study of breast cancer patients in Al-Najaf Governorate

Dr Musa Nima Mezher, Hayder Muhsin Khalfa,Ali Hassan Abood

Abstract—Breast Cancer occurs as a result of mutations, or abnormal changes, in the genes responsible for regulating the growth of cells and keeping them healthy. Cells that have been mutated gain the ability to keep dividing without control or order, producing more cells and forming a tumor.This study is designed to investigate the hormonal and histopathological changes that take place in breast cancer cells in patients who undergone surgery for breast tumour removal in Al sader hospital at Al najaf governate between the period of January 2012 and December 2013.The study examines the role of hormone Estrogen and progesterone in breast cancer patients to outline the involvement of such hormones and study their impact on cellular changes in breast cells. Furthermore the study investigates the histological changes that take place in breast cancer cells in patients suffering from breast cancer in Al najaf governate. A total of 60 female participants ageing between 30-90 years have been identified who attend the oncology clinic at alsader hospital in Al najaf who are suspected of breast cancer. Fine needle aspiration (FNA) smears were obtained from the hospital histology department and staining for progesterone and estrogen hormones were preformed. After undergoing breast tumour removal, tumours have been stained with Haematoxylin and eosin to examine their histopathological changes. Our results shows significant age pattern in patients with breast cancer and that woman aged 40-50 are those likely to get breast cancer in Al najaf governate. There is an apparent histological changes in their cell structure and their estrogen receptor expression was dominated by ER positive results which indicates its involvement in the tumor growth process. This study provided a specific age pattern in which patients in Alnajaf governate likely to acquire breast cancer and which hormone receptor there are likely to express in their immuno assay.

Index Terms—Breast cancer, histopathology, immunohistochemistry, FNA, Haematoxylin and eosin, Estrogen, Progesterone.

1 INTRODUCTION

Cancer is the term used for any cell that grows without any control and can invade other surrounding tissues. Most of the common cancers form a tumour which is a cluster of cells formed by replication with the human having no control over the replication process. It's known that most of the common cancers are caused by genetic abnormalities and other causes can include radiation, chemicals, carcinogens and tobacco smoking. Cancer is normally diagnosed through a histological examination of tissues and screening. Most cancers are treated with a combination of surgery, chemotherapy and radiotherapy. Cancer is caused by the exposure to Carcinogen which are particles that cause genetic abnormalities and they can be from radiation sources such as X-ray, UV-light and tobacco. There are two forms of cancerous cells that can be formed these are known as benign and malignant. Benign cancerous tissues non invasive and don't attack surrounding tissues were as malignant tissues are invasive and can attack surrounding tissues. Breast cancer remains one of the leading causes of death among women, and even if the death rates have been declining in the past few years, women at different ages should continuously test for breast cancer because detecting the disease at a very early stage can be very helpful. Breast cancer is diagnosed and treated through a series of stages, firstly a patient presents with symptoms these include lumps or swelling and weight loss. Tissues are obtained from the human body through a biopsy then Histology examination takes place to determine whether a cancerous cells are present in the tissues. If cancerous cells are present then a surgery is performed to remove the cells before they spread around and invade other surrounding tissues. The therapy choice depends on the location and grade of the tumour. Researchers have identified several kinds of breast cancers. This is not to mention the many cases in which a tumor in the breast is nothing more than a cyst or a harmless benign lump (1-9). The majority of breast cancers, almost 95% are cancer tumors that develop in the milk ducts (figure 1). Those cancers which remain inside the duct without spreading out are known as *in situ* cancers. On the other hand, if the cancer cells spread out and invade other areas, they are known as invasive cancers (figure 1). The other types of breast cancer, almost 5%, are known as lobular breast cancers because they develop in the breast lobes. A very rare type of cancer occurring in only 1% of all cases is known as inflammatory breast cancer. In inflammatory cases, the cancer cells spread very fast and invade other cells, but it can be identified very easily because it blocks the lymph vessels and the channels in the skin, turning the breast into a hard and warm surface with a clear red color (1-9).

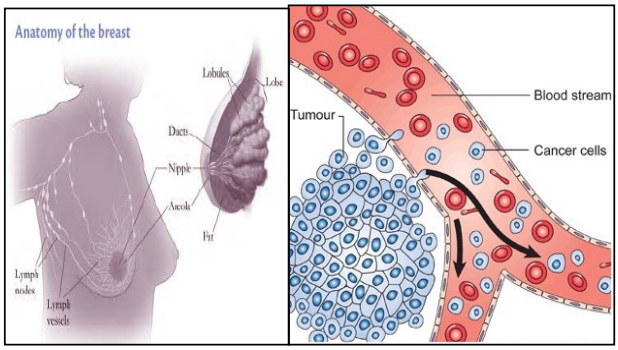

Fig. 1. A diagrammatic anatomy of the breast region illustrating the different tissues found in the breast.

Breast cancers can be caused by a variety of factors. Until today, many doctors believe that breast cancer has to do with heredity. Yet, only 15 to 20% of all women who have breast cancer histories in their families also have breast cancers. Furthermore, a gene known as p53 that is directly responsible for causing breast cancer has been found in extremely rare cases. In the families in which this gene existed, the risk of contracting breast cancer was 16 times more than average. Yet, the number of families in which this gene has been detected is only 100 all around the world, which leaves this cause to be very insignificant (6,7,8). However, there still seems to be some evidence relating breast cancer to genetic factors. Both the breast and the glands that produce wax in ears belong to the same family of glands. Researchers found that individuals with ear glands that produce wet wax have a double risk with respect to breast cancer in contrast to those whose ear glands produce dry wax. This is why, researchers believe in the US and Europe where most individuals have wet wax ear glands have much higher risks of developing breast cancers than in other continents or countries such as Asia (6,7,8).

Another risk related factor to breast cancer is menstruation. Doctors have discovered that risks of breast cancer are very high for women who menstruate before the age of 12 or who reach menopause after the age of fifty. Women who do not get pregnant at all or who become pregnant after the age of thirty also face very high risks. In relation to this risk factor, however, doctors believe that growing older is one factor that has to be considered because older age has been identified as having a high relation with breast cancer (1-10).

In many cases, the surgery requires removing a considerable part of the breast, leaving the woman with a great distress and a feeling of loss and disablement. In the past, women who underwent mastectomy suffered seriously because of the physical distortion of their bodies after the surgery. Breast cancer develops through a multistep process in which normal, healthy cells in the body go through stages that eventually change them to abnormal cells that multiply out of control. In most cases, cancer takes many years to develop.

Normal cells in the body communicate with each other and regulate each other's proliferation (division). Cells proliferate to replace worn-out cells. When cancer occurs, cells escape the normal controls on their growth and proliferation. This escape from control can happen through a variety of pathways. There is no specific age in which breast cancer develops however it has been shown through research that woman risk of getting breast cancer increases with age and this is due to changes in hormonal pattern as well as cellular changes and exposure to mutagens and carcinogenic substances (9).

1.2 Histopathology of breast cancer cells

Pathological analysis of breast cancer cells help provide a clear explanation into how aggressive the cancer is and whether it has traveled or is likely to travel outside its original location. The analysis of breast cancer cell based on histological features enables clinicians to select the best treatment method for the patient. Normal breast cells have an intact and a well defined basement membrane. No membrane or vascular penetration is seen in normal breast cells. Cellular structure is well defined and has a cellular shape.

1.3 Hormonal involvement in breast cancer

Involvement of hormone receptors has been used as a diagnostic technique. When a woman is diagnosed with breast cancer an estrogen or progesterone immunoassay is performed to determine their receptor status. Breast cancer is called an estrogen or progesterone positive cancer depending on the outcome of the immunohistochemistry examination. Research have shown that estrogen and progesterone receptor are involved in cellular growth and may receive signals from progesterone or estrogen hormones that can promote cellular growth (8). Testing for hormone receptors is important because the results helps clinicians decide whether the cancer is likely to respond to hormonal therapy or other treatments. Hormonal therapy includes medications that either lower the amount of estrogen in the body or block estrogen from supporting the growth and function of breast cells. If the breast cancer cells have hormone receptors, then these medications could help to slow or even stop their growth. If the cancer is **hormone-receptor-negative** (no receptors are present), then hormonal therapy is unlikely to work. You and your doctor will then choose other kinds of treatment (8). Estrogen receptors (ER) and progesterone receptors (PR; also called PgR) may be found in breast cancer cells. Cancer cells with these receptors depend on estrogen and related hormones, such as progesterone, to grow. Estrogen and progesterone influence many hormonal functions in women, such as breast development.If breast cancer cells have estrogen receptors, the cancer is called ER-positive breast cancer. If breast cancer cells have progesterone receptors, the cancer is called PR-positive breast cancer. If the cells do not have either of these two receptors, the cancer is called ER/PR-negative. About two-thirds of breast cancers are ER and/or PR positive.

2 METHDOLOGY

2.1 Participants:

A total of 60 participants aged between 25-90 years have been identified who attend the oncology clinic at Al-sader hospital in Al najaf for suspected breast cancer between the period of January 2012 and December 2013.

2.2 Estrogen and progesterone immunohistochemistry:

Fine-needle aspiration (FNA) smears from 60 patients suspect-

- *Hayder Muhsin: Assistant lecturer in Biotechnology, Department of biology, Faculty of science, Kufa university, Iraq E-mail:HaydarHussain@gmail.com*
- *Dr Musa Nima: Assistant professor in Microbiology, Department of biology, Faculty of science, Kufa university, Iraq*
- *Dr Ali Hassan Abood: Lecturer in Zoology/ Animal Physiology & histology/ physiology of hisological toxins , Department of biology, Faculty of science, Kufa university, Iraq*

ed of breast cancer were assessed by immunohistochemistry for estrogen receptor (ER; 1D5) and progesterone receptor (PR;636).

paraffin blocks were sectioned at 3.0 µm sections were collected on microscopic slides.Paraffin was allowed to melt by placing the slides in a 58°C oven for 30 minutes: dewax in xylene. Slides were rehydrated by decreasing ethanol grades.

Endogenous peroxidase blocking was achieved by using a 6% solution of hydrogen peroxide in water (3.0 minutes, room temperature). Slides were placed in a target retrieval solution (S1699, DakoCytomation, Carpinteria, CA) and heated at 90°C in a vegetable steamer for 10 minutes. Blocking endogenous biotin using biotin-blocking reagent (X0590, DakoCytomation). Slides were incubated with primary antibody ER-1D5 (dilution 1:25) and PR-636 (dilution 1:100), 22 minutes at room temperature (DakoCytomation). Linking solution was added to slides: biotinylated antimouse immunoglobulin and slides were incubated for 22 minutes (K0690, DakoCytomation). Streptavidin-peroxidase conjugate was added and slides were incubated for 22 minutes (K0690, DakoCytomation). Slides were placed in diaminobenzidine solution for 10 minutes (K3468, DakoCytomation). 1% cupric sulfate (1.0 minute, room temperature) was applied to intensify the signal; counterstain with 0.2% fast green (2.0 seconds). Slides were Dehydrated in increasing grades of ethanol and cleared in xylene and mount slides

2.3 Haematoxylin and eosin staining

Tissue processing

Tissue processing was concerned with the diffusion of various substances into and out of stabilizes porous tissues. The diffusion process results from the thermodynamic tendency of processing reagents to equalize concentrations inside and outside blocks of tissue.. The specimens were accessioned by giving them a number that will identify each specimen for each patient.

Preparation of tissue sections

Bancroft and Stevens (1982) had prepared tissue sections and by the way, which included the following steps:

Dehydration: progressive forms of ethanol (70, 80, 90, and 95,100%) are passed through the tissue for a period of (1.5 - 2) hours in each concentration in order to remove the water.

Clearing: Samples were cleared with xylene, twice for a period of (1.5-2) hours for each time in order to remove the clearing solution from the tissue.

Infiltration: samples were Infiltrated with molted paraffin wax (56-58 C˚) by placing the samples twice (1.5-2) hours each time.

Embedding: samples were buried in a container with specific templates to molten paraffin wax and left to harden.

Sectioning: Tissues were sectioned into (2-4µm) thickness using a rotary microtome and fixed models on the slides using the adhesive (Meyers albumin) . slides were then placed in the oven at a temperature of (56- 58c˚) for (20) minutes to remove excess wax.

2.4 Histological Staining –Haematoxylin & Eosin (H&E)

The staining process makes use of a variety of dyes that have been chosen for their ability to stain various cellular components of tissue. The routine stain is that of hematoxylin and eosion and the technique are showed in the table.

Step	Reagent	Time
1	Xylene	5min
2	Xylene	5min
3	Absolute Alcohol	2 min
4	Ethanol (90%)	2 min
5	Ethanol (70%)	2 min
6	D.w	2 min
7	Haematoxylin	3 min
8	Running tap water	10 min
9	0.1% Acid Alcohol	1 min
10	Running tap water	3 min
11	2% Eosin	1min
12	Ethanol (70%)	2 min
13	Ethanol (90%)	2 min
14	Absolute Alcohol	2min
15	Dry, Xylene and mounted in DPX	

2.5 Microscopic examinations

The stained section on the slide were examined using a light microscope (Olympus, Japan). Histopathological changes were reported by pathologist.

3 RESULTS

Cancer develops through a multistep process in which normal, healthy cells in the body go through stages that eventually change them to abnormal cells that multiply out of control. In most cases, cancer takes many years to develop.

Normal cells in the body communicate with each other and regulate each other's proliferation (division). Cells proliferate to replace worn-out cells. When cancer occurs, cells escape the normal control on their growth and proliferation. This escape from control can happen through a variety of pathways.

3.1 Histological changes

Tumor cellular changes is the overall process by which a small problem of cell behavior gradually turns into a fully advanced cancerous cell. Throughout this process, the cells of a tumor can change in size, shape, internal structure, and biochemistry. In order for an initial tumor to change and invade another area of the body, it must first undergoes several cellular and biochemical changes. A normal breast cell has an intact and a well defined basement membrane. No membrane or vascular penetration is seen in normal breast cells. Cellular structure is well defined and has a cellular shape. Our examination of breast cancer tissues of all participants showed various histopathlogical changes taking place on a cellular level.

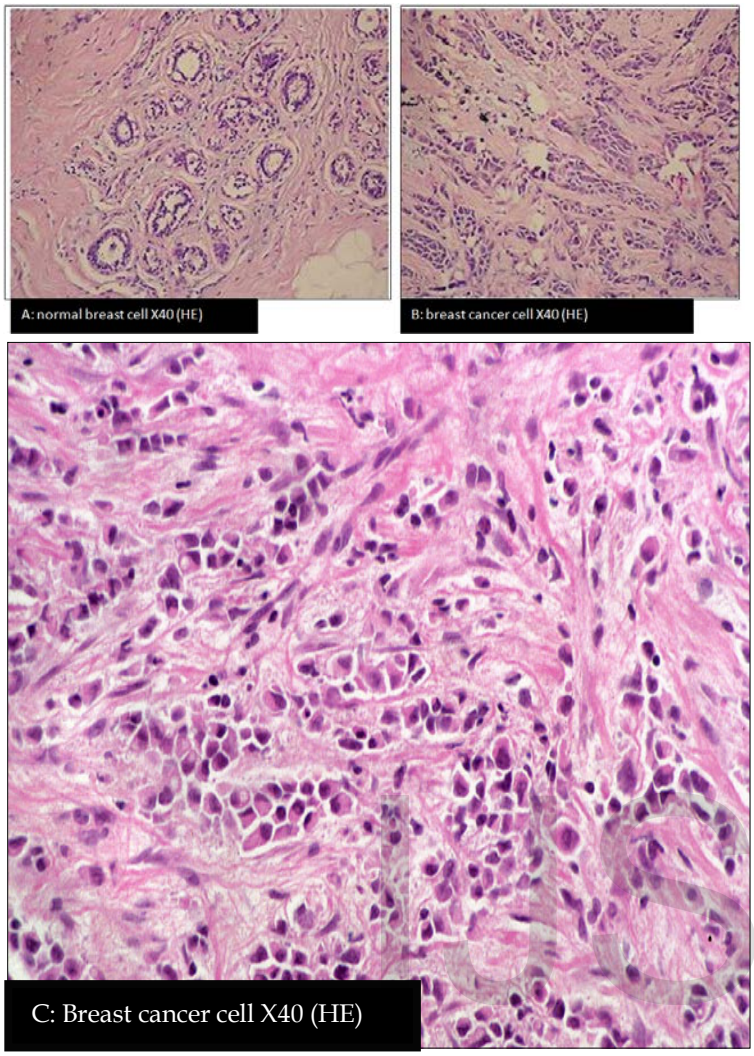

Figure 2 shows a breast cancer specimen compared to a normal tissue. We have observed that histological changes are seen in all tumors from all participants. A high degree of cell division was observed as well as cellular shapes were different compared to normal (figure 2B).

Breast cancer cell histology as seen in figure 2 have specific changes compared to normal breast tissues seen in figure 2. One of the main apparent histological changes seen is the cellular shapes which appears to be lobular and has a single file growth pattern. Cells of breast cancer tissues appear poorly differentiated in figure 3 which indicated that it's a low grade breast cancer. In general higher differentiation of breast cancer cells means the cancer has progressed to high level. In other slides basal lamina connections was observed proving that cells are ready to digest surrounding cells with enzymes and invade surrounding areas.

3.2 Progesterone and Estrogen receptors immunohistochemistry

Analysis of steroid receptor status has become the standard of care for patients with breast cancer. Estrogen receptor (ER) content, in particular, has been correlated with prolonged disease-free survival and increased likelihood of response to endocrine therapy.

Our results show that the majority of woman who attended the oncology clinic at al sader hospital were between (40-50) and (50-60) years of age as shown in table 1. There were 10 patients aged between (30-40) and 10 (60-70). The youngest participant in our study was aged less than 30 years old , on the other hand 5 patients were aged more than 70 years old.

Age groups	Number of participants
30 <	1
30_40	10
40_50	21
50_60	13
60_70	10
70 >	5

Table1: shows the different age groups of the participants that took part in this study.

The female estrogen hormone is one signal that tells certain kinds of breast cells to enter the cell cycle. This leads to increased cell division. In addition, researchers suspect an interaction between estrogen and certain cyclins which stimulates the cell cycle. The results of this study shows that most woman who presented at the oncology clinic in al sader hospital were Estrogen ER positive (table 2). Out of 60 participants 29 (48 %) patients had positive staining for estrogen receptors. progesterone PR positive nuclear staining participant were 26 (43 %), and 3 (5 %) participants had a combination of both progesterone and estrogen positive nuclear staining. More over a total of 20 (33%) participants had both PR and ER negative staining, and 11 (18 %) had a negative staining for ER but a positive PR receptor staining (table 2). To further establish more evidence that estrogen receptor is the more dominant in breast cancer tissues, immunohistochemistry images were recorded and examined as shown in figure 3. Normal breast tissue with normal cellular characteristics is compared to positive stained slides showing estrogen positive breast cancer tissues B (arrow) and progesterone positive staining C (arrow).

Hormonal status	Count	%
ER+	29	48
PR+	26	43
ER+/PR+	26	43
ER+/PR–	3	5
ER–/PR–	20	33
ER–/PR+	11	18

Table 2: shows hormonal status progesterone and estrogen of all participants.

Hormonal Receptor immunohistochemistry was confirmed by the presence of estrogen positive staining shown in figure 3 below B (arrow) compared to that of a normal breast tissue A. progesterone presence was also observed in some FNA samples belonging to some participant and positive staining was confirmed through immunohistochemistry for progesterone receptors C (arrow) compared to that of a normal breast tissue to establish the dominating receptor.

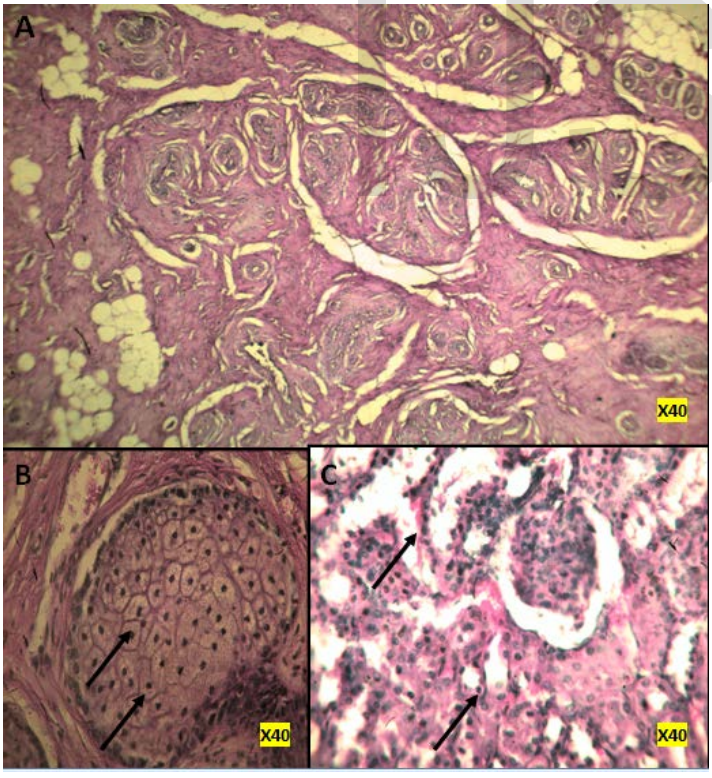

Figure 3: Immunohistochemistry of breast Tissues. A normal breast tissues, B Breast Tissues showing a positive staining for estrogen hormone receptors. Positive staining for progesterone hormone receptor is seen in C.

4 Discussion

In this study we have observed a certain age pattern which could provide a novel theory to raise awareness in Al najaf governate. Most female participant were aged between 40-50 years which could indicate that at this age they have reached the menopause stage which could have an influence on the behavior of breast through different hormone signals. Certain histological features were also observed in breast cancer cells compared to that of normal breast tissues. Cells from breast cancer tissues appeared to have cellular changes which include size and membrane changes. Normal breast cells have a defined intact membrane with no vascular or membrane penetration. Cells from breast cancer tissue appeared to be lobular and has a single file growth pattern. Cells of breast cancer tissues appear poorly differentiated in figure 3 which indicated that it's a low grade breast cancer. basal lamina connections was observed proving that cells are ready to digest surrounding cells with enzymes and invade surrounding areas in breast cancer specimens.

Hormonal changes plays an important role in cell division on a cellular level. This project agrees with most hormonal studies related to breast cancer on the basis that the majority of breast cancer specimens are estrogen positive. Estrogen and progesterone hormones regulate the cell cycle. Over expression of hormone receptors indicate that such cells are prone to cell division and that these cells can benefit from hormonal therapy. Our immunohistochemistry examination for ER and PR receptors showed a high level of expression most of the samples.

There were certain limiting factors affecting the progress of our study. We were unable to collect more information on participants which could provide further information that can support certain theories. Marital status and number of children are both risk factors that can increase the rate of acquiring breast cancer. Information such as family history and other medical history of patients were unavailable to show a relationship between consumption of certain medication and its influence on breast cancer as well as studying the family history for breast cancer and how this can influence the participant. Certain laboratory equipments were difficult to obtain and have slowed down this project , furthermore there was some difficulty in trying to gain samples from Al sader hospital due to the nature of hospitals in Iraq in general.

5 Conclusion

The results of this project shows that there is a significant age pattern in which breast cancer is diagnosed and treated in Al-najaf governate. Our results show specific histological changes within breast cancer cells. Hormonal activity shows that most woman are estrogen positive proving that such receptors plays an important role in the cell cycle and has a strong impact on cancer cellular growth furthermore it affects the histological appearance of breast tissues and in turn affects the grading system.

REFERENCES

[1] J.S. Bridle, "Probabilistic Interpretation of Feedforward Classification Network Outputs, with Relationships to Statistical Pattern Recognition," *Neurocomputing—Algorithms, Architectures and Applications*, F. Fogelman-Soulie and J. Herault, eds., NATO ASI Series F68, Berlin: Springer-Verlag, pp. 227-236, 1989. (Book style with paper title and editor)

[2] Andrew H. Beck, et al. Systematic Analysis of Breast Cancer Morphology Uncovers Stromal Features Associated with Survival Sci Transl Med 3, 108ra113 (2011).

[3] David G. Hicks, MD, Linda Schiffhauer, MD Standardized Assessment of the HER2 Status in Breast Cancer by Immunohistochemistry (Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY). Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY)

[4] Downs-Kelly E, Yoder BJ, Stoler M, et al. The influence of polysomy 17 on HER2 gene and protein expression in adenocarcinoma of the breast: A fluorescent in situ hybridization, immunohistochemical, and isotopic mRNA in situ hybridization study. Am J Surg Pathol. 2005;29:1221-1227.

[5] Hammond ME, Hayes DF, Dowsett M, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol. 2010;28:2784-2795.

[6] Hicks DG, Kulkarni S. HER2+ breast cancer: Review of biologic relevance and optimal use of diagnostic tools. Am J Clin Pathol. 2008;129:263-273.

[7] Hicks DG, Kulkarni S. Trastuzumab as adjuvant therapy for early breast cancer: The importance of accurate human epidermal growth factor receptor 2 testing. Arch Pathol Lab Med. 2008;132:1008-1015. 2. Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA. 2006;295:2492-2502.

[8] J. Chuck Harrell, Carol A. Sartorius, Wendy W. Dye, Britta M. Jacobsen,and Kathryn B. Horwitz. ZsGreen Labeling of Breast Cancer Cells to Visualize Metastasis. Program in Reproductive Sciences and Department of Medicine, University of Colorado Health Sciences Center, Aurora, Colorado, USA.

[9] Mehrdad Nadji, MD, Carmen Gomez-Fernandez, MD, Parvin Ganjei-Azar, MD, and Azorides R. Morales, MD. immunohistochemistry of Estrogen and Progesterone Receptors Reconsidered Experience With 5,993 Breast Cancers. Am J Clin Pathol 2005;123:21-27.

[10] Ramakrishnan R, Khan SA , Badve S . Morphological changes in breast tissue with menstrual cycle. Mod P athol 2002; 15(12):1348-56.

[11] Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med. 2005;353:1673-1684.

[12] Roskoski R Jr. The ErbB/HER receptor protein-tyrosine kinases and cancer. Biochem Biophys Res Commun. 2004;319:1-11.

[13] Ross JS, Slodkowska EA, Symmans WF, et al. The HER-2 receptor and breast cancer: Ten years of targeted anti-HER-2 therapy and personalized medicine. Oncologist. 2009;14:320-368.

[14] Sharon I King, Colin A Purdie, Susan E Bray, Philip R Quinlan, Lee B Jordan, Alastair M Thompson and David W Meek. Immunohistochemical detection of Polo-like kinase-1 (PLK1) in primary breast cancer is associated with TP53 mutation and poor clinical outcome. Breast Cancer Research 2012, 14:R40

[15] Sherman ME, Howatt W, Blows FM, et al. Molecular pathology in epidemiologic studies: A primer on key considerations. Cancer Epidemiol Biomarkers Prev. 2010;19:966-972.

[16] Sunil Badve, MBBS, MD (Path), FRCPath Studying Histological Changes in Breast Tissue with Menstrual Cycle using H&E Staining.

[17] Tien Yeh, MD; Carolyn Mies, MD.Application of Immunohistochemistry to Breast Lesions. Arch Pathol Lab Med—Vol 132, March 2008

[18] Vance GH, Barry TS, Bloom KJ, et al. Genetic heterogeneity in HER2 testing in breast cancer: Panel summary and guidelines. Arch Pathol Lab Med. 2009;133:611-612.

[19] Wolf G, Hildenbrand R, Schwar C, Grobholz R, Kaufmann M, Stutte HJ, Strebhardt K, Bleyl U: Polo-like kinase: a novel marker of proliferation: correlation with estrogen-receptor.

[20] Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med. 2007;131:18-43.

[21] Yaziji H, Taylor CR, Goldstein NS, et al. Consensus recommendations on estrogen receptor testing in breast cancer by immunohistochemistry. Appl Immunohistochem Mol Morphol. 2008;16:513-520.