Medical Journal of Babylon
Vol. 12- No. 3: 817-827, 2015
http://www.medicaljb.com
ISSN 2312-6760©2015 University of Babylon

Original Research Article

Breast Carcinoma in Young Woman :Pathological and Immunohistochemical Study

Sura Salman Ejam
College of Medicine , University of Babylon , Hilla , IRAQ

E-mail : ejamsura@yahoo.com

Accepted 1 June,2015

Abstract

The aim of study is to study the pathological types and the immunohistochemical properties of breast carcinoma young women and compared it with older women

This study included 80 paraffin embedded samples from female patients with breast carcinoma which were collected randomly from period of November 2010- November 2013 in AL-Hilla Teaching hospital. The clinical informations were collected including ages of the patients, histopathological types, & tumor grades from clinical reports of the hospital. A manual avidin biotin peroxidase complex procedure (ABC) system was used in the imunohistochemical analysis (Dako Cytomation Copenhagen, Denmark).

In this study, the histopathological examination of the 80 cases revealed that , 5 (6.25%) cases were of the pure in situ ductal type, 65 (81.25%) cases were of the invasive ductal carcinoma not otherwise specified, 7 (8.75%) cases were of the invasive lobular carcinoma, and 1 (1.25%) case was of each of the medullary, mucoid, squamous (metaplastic) types.

In the 80 cases of breast carcinoma that were studied, ER expression was positive in 8 cases (17.39%), PR expression was positive in 22 cases (27.5%), HER2/neu expression was positive in 20 cases (23.75%).

There were higher proportion of ER and Her2 negative breast carcinoma in early aged patients than the older patients so the early onset breast according to the immuno -histochemical parameters have the worse prognonsis than the late onset group.

Key words: estrogen, progesterone, Her2, breast carcinoma.

الخلاصة

سرطان الثدي يعتبر من أهم الامراض واشهرها التي تصيب النساء في العالم. ويعتبر من أهم الأمراض المؤديه الى الموت في النساء في عمر ٤٠-٥٠ سنة. سرطان الثدي غير شائع بين النساء بعمر مبكر أقل من ٣٥سنة لذلك فأن نسبة زيادة خطورة الأصابة بهذا المرض تزداد بزيادة التقدم في السن وتقل بعد سن اليأس. أن نسبة الأصابة بسرطان الثدي تزداد في الدول المتقدمة وذلك يرجع الى عدة أسباب منها الأختلاف الغذائي ,التغييرات الجنسية والهرمونية. أن الهدف من أجراء هذه الدراسة هو تقييم نسبة ظهور عوامل مثل الأستروجين والبروجسترون في سرطان الثدي وعلاقته بعمر المريضة , نوعية النسيج المرضي ودرجة التمايز بالنسبة لسرطان الثدي.

مفاتيح الكلمات :استروجين, بروجستيرون, هير ٢,سرطان الثدي .

Introduction

In females, one of the most common cancer is the breast cancer [1], In all industrialized countries the incidence of breast cancer is increasing [2]. young women (<35 years) have low incidence of breast carcinoma, constituting 5–7% of all breast cancers [3]. breast carcinoma of women in their 20s and 30s seem to have a poorer prognosis than women diagnosed in middle age, for unknown reason. breast cancer of young women is most likely to be presented with affected lymph nodes , with negative estrogen receptors

expression , and large tumors with a high grade of anaplasia. Thus, the poorer outcome could at least partly be due to differences in these important prognostic factors [4]. The presence or absence of various markers is associated with poorer prognosis, e.g. the over expression of Her2/neu (c-erbB-2) oncoprotein, and lack of estrogen and progesterone receptor [5].

After lung cancer breast cancer is the second most common cancer [6]. Breast cancer is the most common cause of death in females at age 29 to 59 [7], in the developed world breast cancer became the major health problem that affect as one in eight women during their lifetime [8].

Over the last ten years, there has been a three fold increase in the incidence of breast cancer in many countries [9], breast cancer is uncommon in women younger than age 30, thereafter, the risk steadily increases throughout life, but after menopause the upward slop of the curve almost plateaus [10].

The disease is the commonest cause of death among women aged 40-50, accounting for about a fifth of all death in this age group [11]. According to WHO records in 2006, the breast cancer constitutes the fifth most common cause of death from cancer in the world (after lung, stomach, liver , colon) [12].

Cancer is a multistep process in which normal healthy cells in the body go through stages that eventually change to abnormal cells that multiply out of control, in most cases; cancer takes many years to develop [13].

Breast carcinoma development can be triggered by mutations of the signals in the network that controls cell division , and this can be associated with genetic predisposition (e.g., mutation in BRCA1 and BRCA2 genes), exposure to some environmental factors (e.g., radiation exposure of the chest) , or both [14], so it is an interplay between genetic changes and environmental factors [15].

5%-10% of breast cancer is with autosomal dominant mutation which are usually diagnosed 5-15 years earlier than sporadic cases of breast cancer [16].

radiation and carcinogenic chemicals are the leading cause for acquired mutations, these mutations, in combination with certain life style and environmental factors are thought to contribute to cancer development.

Other factors associated with breast carcinoma development and progression are estrogen and progesterone exposure , normal breast cells have receptors that attach to circulating estrogen and progesterone, estrogen and progesterone bind to the receptors and may work with growth factors (e.g., oncogenes and tumor suppressor genes) to cause cancer cell growth and proliferation [17].

So part of the multistep process includes buildup of mutations to genes that normally regulate cell division, like oncogenes which code for protein signals that stimulate cell to enter or continue in the cell cycle, for example is the Her2/neu receptor gene. Other oncogenes include the tyrosine kinase family of growth factor receptors, the c-myc oncogenes, cyclin D1, and the cyclin regulator, CDK-1. Other genes involved are tumor suppressor genes that are when mutated lose control over their brakes , an example of a vital tumor suppressor gene is the p53, p53 recognized damaged DNA and tell the cell to "commit suicide" (apoptosis), a mutation in the p53 gene is the most common genetic change found in breast cancer [13].

Materials and Methods

This study was included 80 paraffin embedded samples from female patients with breast carcinoma were collected randomly from period of November 2010-November 2013 from Hilla Teaching hospital. female patients with breast carcinoma (as confirmed by H & E stained histopathological examination) were included in this study, their ages ranging between 21-79 years with a mean age of 45.6 years, All the specimens were from modified radical mastectomy

They are divided to:

1. Study Group: thirty seven cases who were < 35 years of age.
2. Comparative Group: fifty cases who were ≥ 35 years of age.

Control Group

Positive Control Slides: with each set of immunostaining , positive control sections were processed. Positive controls of breast carcinoma sections which are known to express ER, PR and Her2/neu, respectively were used with each run.

Negative Controls slides: Sections untreated with primary antibody (ER, PR and Her2/neu) were considered as negative controls for each set of slides.

The criterion for positive immunoreactions is dark brown precipitate in the cell nucleus for estrogen and progesterone, and at the cell membrane for Her2/neu.

The proportion of the staining was assessed by counting the percentage of positive cells in 100 malignant cells at objective 40 total magnifications, each sample was scanned for five fields randomly with a high power magnification [18].

Qualitative assessment: Faint staining pattern, whether membranous or nuclear, that only could be detected by using higher magnification (objective 40), while strong staining pattern, easily seen by low magnification (objective 4).

Her2/neu Scoring System

The Her2/neu scoring system is as in the table,below:

Her2/neu Scoring System [18]

Score	Staining pattern
Score 0	Completely negative
Score 1+	Faint perceptible staining of the membrane in >10% of the malignant cells.
Score 2+	Moderate staining of the partial membrane in >10% of the malignant cells.
Score 3+	Strong circumferential staining of the entire membrane creating a fishnet in >10% of the malignant cells.

Her2/neu protein overexpression assessment includes :[18]
Score 0 = negative
Score 1+ =negative
Score 2+ =equivocal (need FISH study)
Score 3+ =positive

Estrogen and Progesterone Receptors Scoring System [19]

Scoring is based on the examination of all tumor cells on the slide, the Altered scoring guideline is used and includes:

1-A proportion score (PS) =estimated proportion of tumor cells with positive nuclear staining and includes five grades:
0= no cell stained.
1= > 0 to 1/100 of cells stained.
2=> 1/100 to 1/10 of cells stained.
3=> 1/10 to 1/3 of cells stained.
4=> 1/3 to 2/3 of cells stained.
5=> 2/3 to 1/1 of cells stained.

2-An intensity score (IS) =estimated average staining intensity of all positive tumor cells and includes four grades:
0=negative
1=weak
2=intermediate

3=strong

3-A total score (TS) =sum of PS and IS.

A positive result for both ER and PR is defined as TS ≥ 3.

Statistical Analysis

Statistical analyses of all results were performed by the help of SPSS software statistical package (version 15) using Chi Square test, P value at level of significance < 0.05.

Results

Eighty cases of breast carcinoma were included in this study, and divided into two groups according to their age distribution; immunohistochemical parameters (ER, PR, and Her2/neu) were studied and histopathological types.

General Immunohistochemical Expression

In the 80 cases of breast carcinoma that were studied, ER expression was positive in 8 cases (17.39%), PR expression was positive in 22 cases (27.5%), HER2/neu expression was positive in 20 cases (23.75%).

The ER Expression

The ER expression was negative (0%) in the total 34 cases of the early age onset group, while it was positive in 8 cases (17.39%) out of total 46 cases in the late age onset group (Table 2).

The PR Expression

The PR expression was positive in 8 cases (23.53%) out of total 34 cases in the early age onset group, and it was positive in 14 cases (30.43%) out of total 46 cases in the late age onset group (Table 3).

The Her2/Neu Expression

The Her2/neu expression was positive in 12 cases (35.29%) out of total 34 cases in the early age onset group, and it was positive in 7 cases (15.22%) out of total 46 cases in the late age onset group (Table 4).

Histopathological Types

In this study, the histopathological examination of the 80 cases revealed that , 5 (6.25%) cases were of the pure *in situ* ductal type, 65 (81.25%) cases were of the invasive ductal carcinoma not otherwise specified, 7 (8.75%) cases were of the invasive lobular carcinoma, and 1 (1.25%) case was of each of the medullary, mucoid, squamous (metaplastic) types.

In the early age onset breast carcinoma group there were , 27 (97.41%) cases were of invasive ductal type not otherwise specified, 3 (8.82%) cases were of invasive lobular carcinoma, 2 (5.88%) case was of medullary type, 2 (5.88%)case was of mucoid type.

In the late age onset breast carcinoma group, 42 cases (88%) were of the invasive ductal carcinoma not otherwise specified, while the remaining 4 cases (8%) were of invasive lobular type.

Table 1: Histopathological types in the two patients groups

Age (years)	IDC NO. %	ILC NO. %	Medullay NO. %	Mucoid NO. %	Total	P value
<35	27 (79.41%)	3 (8.82 %)	2 (5.88%)	2 (5.88%)	34 (42.5%)	P>0.5
≥35	42 (91.3%)	4 (8.7%)	0 (0)	0 (0)	46 (57.5%	
Total	65 (81.25%)	7 (8.75)	1 (1.25%)	1 (1.25%)	1 (1.25%)	

Table 2: ER expression in the two patients group

Age (years)	ER Positive NO %	ER Negative NO. %	Total	P Value
<35	0	34(100%)	34(42.5%)	P < 0.5
≥35	8(17.39%)	38(82.6%)	46(57.5%)	
Total	8(10%)	72(90%)	80(100%)	

Table 3: PR expression in the two patients group

Age (years)	PR Positive NO %	PR Negative NO %	Total	P value
<35	8(23.53%)	26(76.47%)	34(42.5%)	P > 0.5
≥35	14(30.43%)	32(69.57%)	46(57.5%)	
TOTAL	22(27.5%)	58(72.5%)	80(100%)	

Table 4: Her2 expression in two patients groups

Age (years)	HER2 Positive NO %	HER2 Negative NO %	Total	P Value
<35	12(35.29%)	22(64.71%)	34(42.5%)	P <0.5
≥35	7(15.22%)	39(84.78%)	46(57.5%)	
Total	19(23.75%)	61(76.25%)	80(100%)	

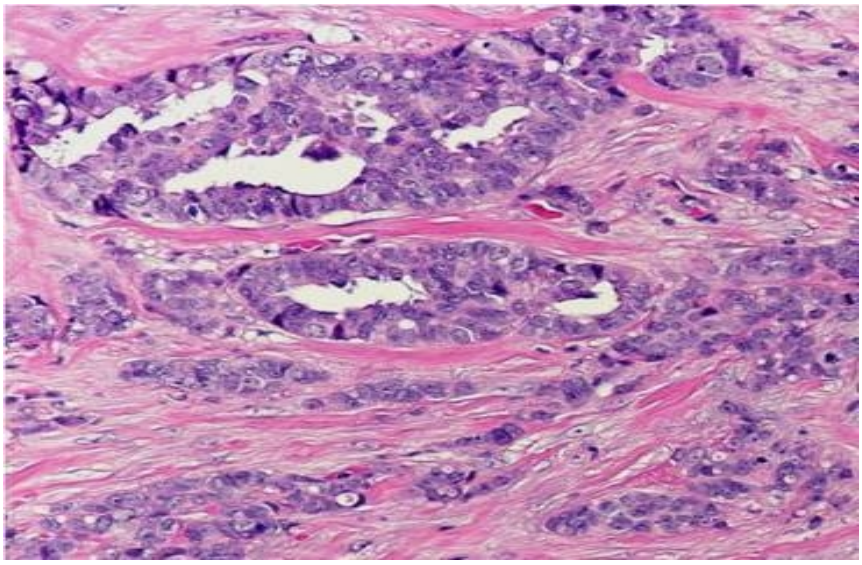

Figure 1: Invasive Ductal Carcinoma, Well Differentiated, (Hematoxylin and Eosin Staining, 40X).

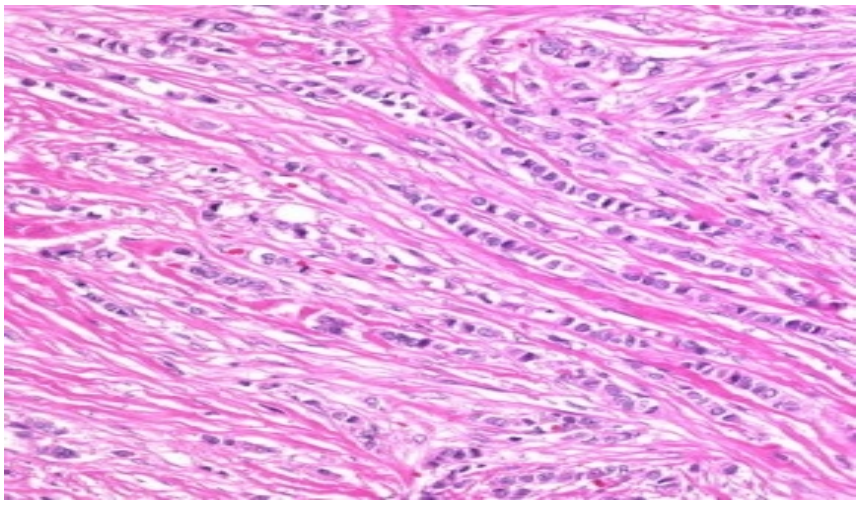

Figure 2: Invasive Lobular Carcinoma, (Hematoxylin and Eosin Staining (10X).

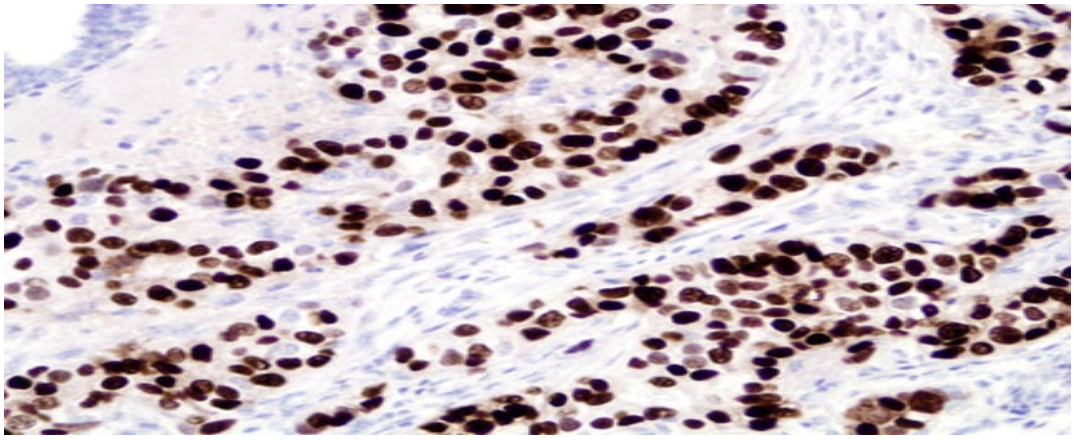

Figure 3: Invasive Ductal Carcinoma Moderately Differentiated Showing ER Nuclear Staining (Immunohistochemical Stain For ER, 10X).

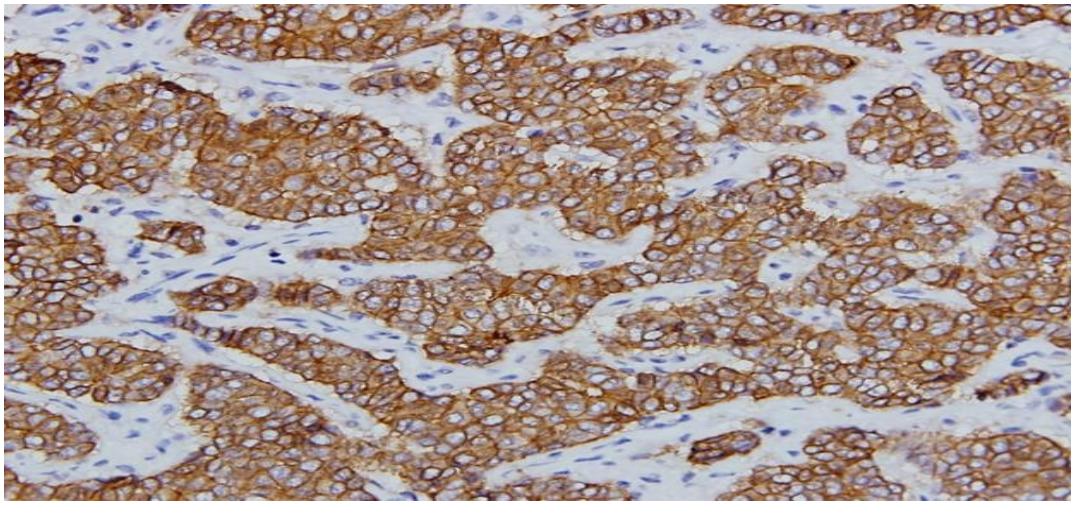

Figure 4: Invasive Ductal Carcinoma Moderately Differentiated Showing Strong Membranous Staining, With Fishnet Appearance. (Immunohistochemical Stain For Her2/Neu, 40X).

Discussion

1- Histopathological types

Regarding the histopathological there were no statistical differences between two patients groups $p > 0.5$.

The invasive ductal carcinomas, being the most common form of disease in both groups, and no significant difference in this histopathological type between the early and old age groups, these results were in agreement with Ava et al [21], Albektsen et al [22].

The proportion of invasive lobular carcinoma in both patients groups were approximately equal , these results were in consistent with Ellis et al., [23], Kollias et al., [24] who stated that the young age group were less likely to have lobular carcinomas, and this difference may be related to the way of randomized. selection of old age onset group of patients. There were 2 cases of medullary ca and 2 cases of mucoid carcinoma in early age groups and there were no cases of such histopathological types in old age groups,

These results were consistent with, Claus et al, [25] which have suggested that medullary ca is significantly more common in young age groups but inconsistent with Anderson et al which stated that the mucinous ca are more common in old age group all these differences in the results of these special types of breast carcinoma probably related to the process of randomized selection of old age onset patients.

2-Immunohistochemical properties

a-ER expression

In our study there were only 8 (17.39%) ER positive cases in all 80 case, this finding is lower than that obtained by (90%), Althuis et al. [25] (76%),
Liu et al [26] (68.9%), Alghanem et al., [27] (70.31%) [27], and this may reflect the racial variation in the percentage of ER positivity as suggested by Azam et al., [28] (28%) and Madhuri et al., [29] who showed high percentage of ER negative tumors in Asia, and or the effect of variation in immunohistochemical methods applied.
All 34 cases of early onset breast ca in our study were with negative estrogen expression while only 8 (17.39%) of late onset breast ca were with positive estrogen expression, the statistical difference between two groups were significant ($p<0.5$)
These results have been agreed with Han et al [30] which showed that younger patients have lower expression of estrogen receptors when we compared with older patients, and with Kroman et al [31] who stated that the risk of estrogen receptors negativity were more common in younger age groups.\

b-Progesteron expression

In our study there were 22(27.5%) cases with progesterone positive expression
this result was lower than that in the following two studies Althuis et al. [32] (65%), Liu et al., [33] (45.6%), this may refer to racial differences in the PR expression in the breast carcinoma .
The progesterone receptor (PR) expression in the two patients groups show that there were 8 (23.53%) cases of the early age onset group with PR positive receptor , and there were 14 (30.43%) of late onset breast carcinoma with positive PR receptor expression, there statistical differences in PR expression between these two groups were non significant (P value >0.5). These results have been not agreed with, Althuis et al., [32], Han et al., [30] who stated that there is significant differences in the expression of PR between the early age onset patients group and the older one.
These results can be explained by the facts that in this study there is high percentage of ER- patients who comprise, this subset of patients is more common in Asia [34] [35], and it occurs more frequently in premenopausal patients than postmeno-pausal patients, that may explain the relatively more ER- cases in early age onset breast carcinoma patients in this study , also those subset of patients (ER-) have worst prognosis than those who are positive for both ER and PR as shown by Rhodes et al., [35], and Rakha et al., [36], and this may explain part of the aggressive course seen in young patients with breast carcinoma.

c-Her2 expression

In our study there were 19 (23.75%) cases with Her2 positive expression. it is higher from results obtained in certain studies as Liu et al [33] (12.5%) and lower than the result from other studies as Azam et al [28] (44%) and it is included in the average percentage of breast carcinoma Her2/neu expression [37-38]. The immunohistochemical results of Her2/neu expression show that in the early age onset patients there were 12 cases (35.29%) positive for Her2/neu and in the late age onset patients there were 7 cases (15.22%) positive for Her2/neu, a significant difference was present between the two groups (P value <0.5), this was agreed with Maru et al., [38], Aksoy et al., [40] in the fact that Her2/neu is a marker of aggressive tumors. But inconsistent with Bertheau et al., [41], Colleoni et al., [42] which may reflect the variability in immunohistochemical methodology and interpretation.

Conclusion

There were higher proportion of ER and Her2 negative breast ca in early aged patients than the older patients so the early onset breast according to the immunp histochemical parameters have the worse prognonsis than the late onset group.

References

1- Singhal H, Gohel MS, Kaur K et al., Breast cancer evaluation. Medicine Journal Specialty 2008:2-10.

2- Sundquist M, Thorstenson S, Brudin L et al ., Incidence and prognosis in early onset breast cancer. The Breast (2002) 11, 30-35.

3- Hankey BF, Miller B, Curtis R et al., Trends in breast cancer in Younger women in contrast to older women. J Cancer Inst Monogr 1994; 16:7–14.

4- Niels K, Maj-Britt J, Jan W et al., Factors influencing the effect of age on prognosis in breast cancer: population based study. BMJ 2000; 320:474–9.

5- Walker RA, Lees E, Webb MB et al., Breast carcinomas occurring in young women (<35 years) are different. Br J Cancer 1996; 74: 1796–1800.

6- World health organization, international agency for research on cancer. World Cancer Report 2003:335.

7- Jemal A, Siegel R, Ward E et al., Cancer statistics, 2008. CA Cancer J Clin 2008, 58 (2):71-96.

8- Hayat MJ, Holader N, Reichman ME et al., Cancer statistics, trends, and multiple primary cancer analyses from the surveillance, epidemiology, and end Results (SEER) program. The Oncologist 2007,12: 20–37.

9- Al-Azzawi SN. Depleted uranium radioactive contamination in Iraq: an overview. Global Research 2006, 1:4.

10- Cotran RS, Kumar V, Fausto N et al., Robbins and Cotran pathologic basis of disease, Saunders Elsevier, Philadelphia 2007: 744.

11- Mc Pherson K, Steel M C, Dixon J M. ABC of breast disease: Breast cancer epidemiology, risk factors, and genetics. BMJ 2000, 321 (7261):624-628.

12- Parkin DM, Bray F, Ferlay J et al., Global cancer statistics, 2002. CA Cancer J Clin 2005, 55(2):74–108.

13- Rachel A.C, Roy L, Suzanne S. Program on breast cancer and environmental risk factors. The Biology of Breast Cancer. Fact Sheet #05, 1997 October: 62-70.

14- Underwood J.C, Hunter J. General and systemic pathology, Saunders Elsevier, Philadelphia, ed^{4th} 2004, 18:479-492.

15- Dickson R, Russo J. Biochemical control of breast development. Diseases of the breast, PA: Lippincott Williams & Wilkins, Philadelphia

16- Bethesda MD. Genetics of breast and ovarian cancer (PDQ), National Cancer Institute, 2005.

17- Chen Y.C, Hunter D.J. Molecular epidemiology of cancer. Cancer Journal for Clinicians 2005, 55 (1): 45-54.

18- Sophia K. A, Randolph J. H, David .N.L. IHC evaluation of K-ras, P53 and Her2/ neu expression in hyperplasic, dysplastic, and carcinomatous lesions of the pancreas: evidence for multistep carcinogenesis. Human Pathology 1999, 30(2): 123-129.

19- Truls G, Kennet W, Jörgen C et al., Analysis of Her2\ neu expression in primary bladder carcinoma and corresponding metastases. J. Pathol 2005, 95: 982- 986.

20- Dakocytomation catalog for products and services. Dako, Denmark 2011, 139-143.

21- Ava K, Cheung P, Chan S et al., Breast cancer in Chinese women younger than age 40: are they different from their older counterparts? World J Surg.2008.

22- Albrektsen G, Heuch I, Thoresen S. Histological type and grade of breast cancer tumors by parity, age at birth, and time since birth: a register-based study in Norway. BMC Cancer 2010, 10:226.

23- Ellis I. Galea M, Locker A et al., Pathological prognostic factors in breast cancer. II. Histological type. Relationship with survival in a large study with long-term follow-up. Histopathology 1992, 20: 479-489.

24- Claus E.B, Risch N, Thompson W.D et al., Relationship between breast histopathology and family history of breast cancer. Cantcer 1993, 71: 147-153.
25- Althuis M, Fergenbaum J,Garcia-Closas M et al., Etiology of hormone receptor–defined breast cancer: a systematic review of the literature. Cancer Epidemiol Biomarkers Prev 2004, 13(10):1558–68.
26- Liu S, Chia S, Mehl E et al., Progesterone receptor is a significant factor associated with clinical outcomes and effect of adjuvant tamoxifen therapy in breast cancer patients. Breast Cancer Res Treat 2010 119:53–61.
27- Alghanem A, Haussain S. The effect of age on estrogen and progesterone receptor in primary breast cancer. Journal of Surgical Oncology 1985, 30(1): 29–32.
28- Azam M, Qureshi A, Mansoor S. Comparison of estrogen receptors, progesterone receptors and HER-2/neu expression between primary and metastatic breast carcinoma. JPMA 2009, 59:736.
29- Madhuri K , Laura R, Michele C et al., Breast cancer histology and receptor status characterization in Asian Indian and Pakistani women in the U.S.- a SEER analysis. BMC Cancer 2010, 10:191.
30- Han W, Kang S. Relationship between age at diagnosis and outcome of premenopausal breast cancer: age less than 35 years is a reasonable cut-off for defining young age-onset breast cancer. Breast Cancer Res Treat 2010, 119:193–200.
31- Kroman N, Jensen M, Wohlfahrt J et al ., Factors influencing the effect of age on prognosis in breast cancer: population based study. BMJ 2000, 320:474–479.
32- Althuis M, Fergenbaum J, Garcia-Closas M et al., Etiology of hormone receptor– defined breast cancer: a systematic review of the literature . Cancer Epidemiol Biomarkers Prev 2004, 13 (10): 1558–68.
33- Liu S, Chia S, Mehl E et al., Progesterone receptor is a significant factor associated with clinical outcomes and effect of adjuvant tamoxifen therapy in breast cancer patients. Breast Cancer Res Treat 2010 119:53–61
34- Yip C. Breast cancer in Asia. Current Issues in Breast Cancer2010
35- Rhodes A, Jasani B. The estrogen receptor– negative/progesterone receptor-positive (ER-/PR+) breast tumor: A biological entity or a technical artifact? J Clin Pathol 2009, 62(1):95-96.
36- Rakha E.A, El-Sayed M.E, Green A.R et al., Biologic and clinical characteristics of breast cancer with single hormone receptor positive phenotype. J Clin Oncol 2007, 25 (30):4772-4778.
37- Dowsett M , Hanna W.M , Kockx M et al ., Standarization of her2 testing : results of an international proficiency-testing ring study. Modern Pathology 2007,20:584-591.
38- April D, Barbara A. H, Thomas L. B et al., Progression and treatment of Her2-positive breast cancer. Cancer Chemother Pharmacol 2010, 65:611–623.
39- Maru D, Middleton L, Wang S et al., Her2/neu and p53 overexpression as biomarkers of breast carcinoma in women age 30 years and younger. American Cancer Society 2005, 103(5): 900-905.
40- Aksoy A, Bulut N. Pathological characteristics of very young (<30 years) breast cancer patients: a single-institution experience from Turkey. Annals of Oncology Advance Access 2009, 20(4): 790.
41- Bertheau P, Steinberg S.M, Merino M.J et al., P53 and nm23 gene product expression in breast cancer in young women: immunohistochemical analysis and clinicopathologic correlation. Hum Pathol 1998, 29:323–329.
42- Colleoni M, Rotmensz N, Robertson C et al., Very young women (<35 years) with operable breast cancer: features of disease at presentation. Ann Oncol 2002, 13: 273–279.