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Original Article

Assessment as both pathologic, clinicopathologic and ımmunohistochemical of pregnancy-associated breast carcinoma

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Abstract: Introduction: Pregnancy-associated breast carcinoma (PABC) is stated as breast carcinoma (BC) diagnosed during pregnancy or within one or two years after delivery. It is rare during pregnancy. Materials and Methods: Thirteen cases of PABC occurring during the second and third trimester of pregnancy obtained from the computer database in hospital records during the year 2009/2016 are reported. The preparations stained with hematoxylin and eosin and stained as ımmunohistochemical for ER, PR, Ki-67 and HER2/neu expression were evaluated under light microscope. Discussion and Results: All patients were performed lumpectomy and sentinel lymphadenectomy. Surgical materials were sent to frozen. İn the frozen study 4 patients (31%) who had not tumors in sentinel lymph node were not performed axillary lymphadenectomy. 9 patients (69%) who had tumors in sentinel lymph node were performed axillary lymphadenectomy and breast-conserving surgery. All patients were identified as invasive ductal carcinoma with pathologicaly examination. The patients ranged in age from 26 to 42 years (mean age 35 and median age 34 years). The diagnosis was made in 5 cases during pregnancy and in the other 8 cases during lactation. Maternal age during pregnancy was mean 36 years and median 35 years. 2 patients were diagnosed in the third trimester and 3 patient was diagnosed in the second trimester, with mean gestational ages of 24.2 weeks and median gestational ages of 22 weeks. Maternal age during lactation was mean 34.4 years and median 33.5 years. According to Modified Scarff-Bloom-Richardson System 8 patients (61.5%) during pregnancy and lactation were grade 2 and 5 patients (38.5%) were grade 3. At presentation, 7 patients (54%) were classified as having American Joint Committee on Cancer clinical Stage II disease, 6 (46%) were classified as having Stage III disease in all patients. Conclusion: Late diagnosis of PABC in which advanced the stage of the disease is confirmed by my observation.

Keywords: Breast cancer, pregnancy, lactation

Introduction

Pregnancy-associated breast carcinoma (PABC) is defined as breast carcinoma (BC) diagnosed during pregnancy or within one or two years after delivery [1, 2]. PABC with an incidence from 1:3000 to 1:10000 is the second most common cancer type after cervical cancer, rare [3, 4] and is the most common malignancy occurring during pregnancy and lactation. The prevalence of PABC is increasing as women are delaying the onset of childbearing. During pregnancy and lactation, the breast undergoes dramatic changes in response to an increase in the circulating hormones estrogen, progesterone, and prolactin, which all have a proliferative effect on glandular and ductal tissue [5]. The risk of PABC is age related. Women who have their first term pregnancy after the age of 30 years old have a two to three times higher risk of developing BC than women who have their first pregnancy before the age of 20 years old. Most of women of PABC present with larger tumors and have a higher incidence of lymph node metastasis at diagnosis [6]. The reason might be due to a delay in diagnosis, secondary to the engorgement and physiologic hypertrophy of the pregnant. At any gestational age, ultrasound and mammography can be safely used to investigate any palpable mass in the breast or axilla [7], and biopsies can be safely performed. The concomitant occurrence of breast cancer and pregnancy remains a challenging clinical problem as treatments often

conflict with protecting the fetus [8]. Identifying high risk subgroups of premenopausal women are necessary for intervention and early detection.

The current study is to assessment as both pathologic, clinicopathologic and estrogen receptor (ER), progesterone receptor (PR), HER-2/neu, Ki-67 and cyclin D1 expression in BC coincident with pregnancy and lactation.

Materials and methods

I screened 521 cases registered from January 2009 to April 2016 and found 13 cases of PABC. Patient's informations were obtained from the computer database in hospital records. Paraffin blocks and slides were taken from pathology archives. Diagnosies of slides stained with hematoxylin and eosin were checked under light microscope by pathologist. All slides were identified as invasive ductal carcinoma with pathologicaly examination (**Figure 1A**). The 4 μm thickness paraffin sections obtained from paraffin blocks of primary tumors conventionally dehydrated and cleared. The slice was dewaxed and incubated in 3% H_2O_2 at room temperature for 10 minute to inactivate endogenous peroxidase. After three washes, the slices were repaired for 10 minute in antigen retrieval Citra HK0 86-5K Biogenex with a microwave. After three more washes, primary antibody diluted in PBS with 5% goat serum was added as follows: [Anti-Estrogen Receptor-Alpha (EP1), AN710-5ME, Biogenex], [Anti-Progesterone Receptor (PR88), AM328-5ME, Biogenex], [Anti-Ki-67 (K-2)-AM410-5M, Biogenex], [Anti-Human Her 2/ErbB2 (EP3), AN726-5ME, Biogenex] and [Anti-Cyclin DI-AN4-74-5M (EPR2241-32), Biogenex]. The following day, the slices were washed with PBS and then secondary antibody labeled with biotin was added. Streptavidin labeled with horseradish peroxidase was added and slices were incubated at room temperature for 30 min. The antigens were visualized with DAB. The sections were washed with double distilled water, air-dried, and fixed with neutral balsam.

The stained preparations as ımmunohistochemical were evaluated under light microscope by pathologist. Positive controls for ER, PR, Ki-67 and HER2/neu expression were obtained from positive invasive ductal carcinoma blocks, and positive controls for cyclin DI were sections of mantle cell lymphoma known to express high levels of cyclin DI in pathology archive. The intensity and and percentage of nuclear staining for ER, PR and percentage of nuclear staining for cyclin DI, Ki-67 was considered for the analyses. The percentage of immunoreactive nuclei for ER, PR, cyclin DI and Ki-67 was evaluated by scanning whole sections at medium and high magnification, and by counting at least 500 cells in the tumor areas with the highest intensity of staining.

Internal controls of nuclear staining for ER and PR were available for each slide. Nuclear staining for ER (**Figure 1B**), PR (**Figure 1C**), Ki-67 (**Figure 1D**), membranous staining for HER2/neu (**Figure 1E**) and nuclear staining for cyclin DI (**Figure 1F**) were evaluated.

The intensity of nuclear staining for ER, PR according to quick score; Score 0; no staining in the area of the lesion, Score 1; poor staining in the area of the lesion, Score 2; moderate staining in the area of the lesion, Score 3; strong staining in the area of the lesion.

The percentage of nuclear staining for ER, PR according to quick score; Score 0; no staining in the area of the lesion, Score 1; <1% staining in the area of the lesion, Score 2; 1-10% staining in the area of the lesion, Score 3; 11-33% staining in the area of the lesion, Score 4; 34-66% staining in the area of the lesion, Score 5; 67-100% staining in the area of the lesion.

The percentage of nuclear staining for Ki-67 was considered for the analyses according to St. Gallen consensus in 2009 as follows. The percentage of nuclear staining for Ki-67; Low; ≤% 15% staining in the area of the lesion, Intermediate; 16-30% staining in the area of the lesion, High; ≥31% staining in the area of the lesion.

Membranous staining for HER-2/neu was evaluated according to the quidelines of the American Society of Clinical Oncology and the college of American Pathologists as follows; Score 0-1; No membranous staining, membranous staining <10% in the area of the lesion or faint incomplete membranous staining >10% in the area of the lesion, Score 2; Weak to moderate complete membranous staining >10%, or strong complete membrane staining in <30% in the area of the lesion, Score 3; strong complete

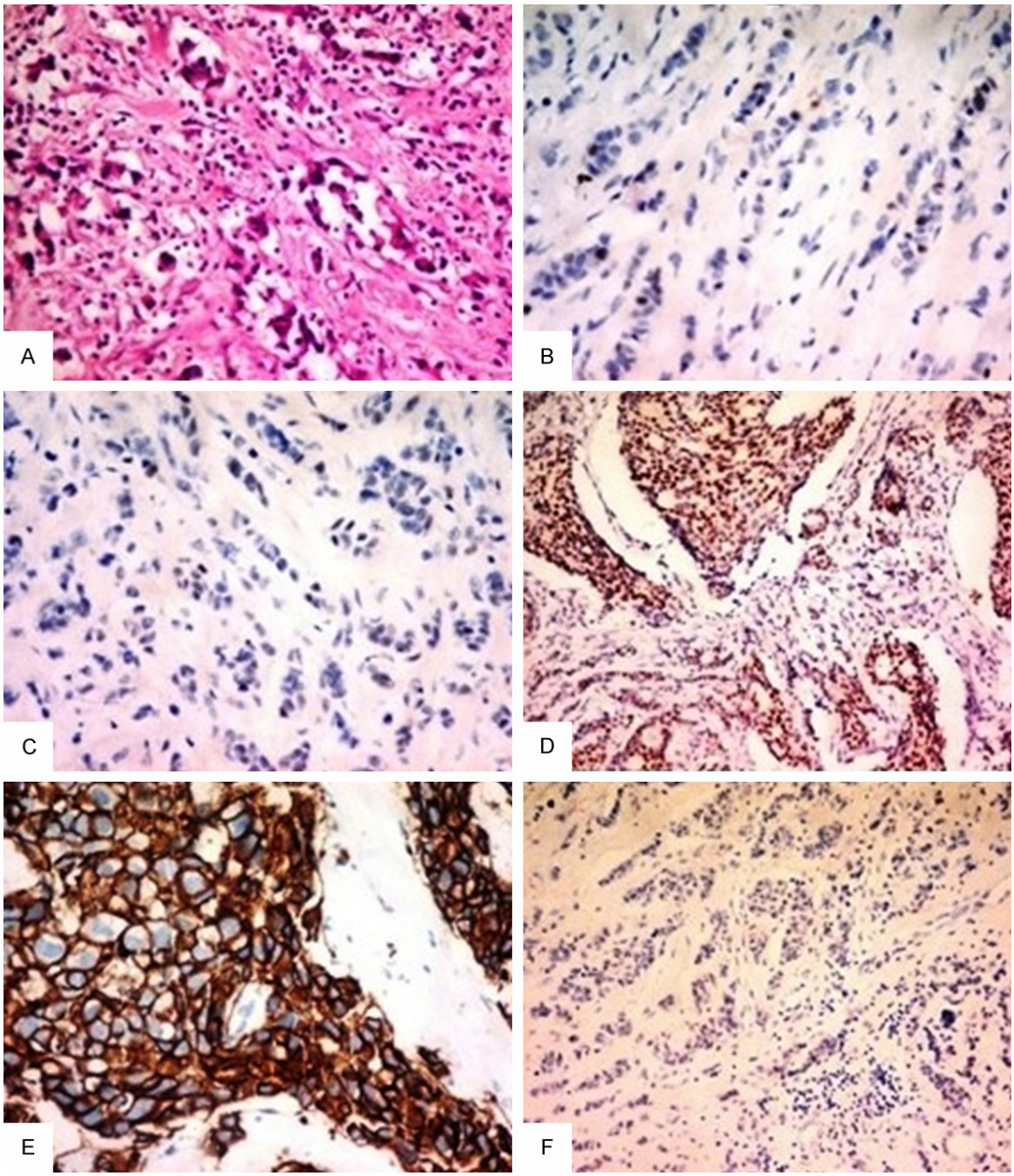

Figure 1. H&E and immunohistochemical stainings. A: Showing slides stained with hematoxylin and eosin of pregnancy-associated breast carcinoma (H&E ×400). B: ER positivity (Immunohistochemistry ×400). C. PR positivity (Immunohistochemistry ×400). D. Ki-67 positivity (Immunohistochemistry ×400). E. HER-2/neu positivity (Immunohistochemistry ×400). F. Cyclin D1 positivity (Immunohistochemistry ×400).

membranous staining >30% in the area of the lesion.

The stainability for cyclin D1 protein was scored as follows. Score 1; <10% positive cells (weakly), Score 2; 10-50% positive cells (moderate), Score 3; 50-100% positive cells (strong).

Results

In this study thirteen women with breast cancer were included. Twelve patients discovered the tumors as palpable masses themselves and one patient was admitted with a diffuse erythema (inflammatory carcinoma). Abnormalities

Table 1. Clinicopathological and obstetric characteristics during pregnancy of the patients with BC

	Case				
	1	2	3	4	5
Maternal age during pregnancy, years	40	33	35	32	40
Gestational age at diagnosis, weeks	22	29	18	20	32
Mode of deliver	Spontan.	C/S	C/S	C/S	C/S
Gestational age at delivery, weeks	39	36	36	37	37
Therapy during pregnancy	yes	no	yes	yes	no
Infant birth weight (g)	3120	2940	2945	2930	2990
Positive family history	no	no	no	yes	no

with mammography were observed for 10 patients and confirmed tumor masses with breast ultrasonography done following in all 13 patients. All patients were performed tru-cut biopsy. They were identified as invasive ductal carcinoma with pathologicaly examination. All patients were performed lumpectomy and sentinel lymphadenectomy. Surgical materials were sent to frozen. İn the frozen study 4 patients (31%) who had not tumors in sentinel lymph node were not performed axillary lymphadenectomy. 9 patients (69%) who had tumors in sentinel lymph node were performed axillary lymphadenectomy and breast-conserving surgery. The primary tumors ranged from 2 to 7 cm in diameter (mean, 4.3 cm; median, 4 cm). A solitary tumor mass presented in twelve patients. 1 patient had 2 separate foci of BC.

The patients ranged in age from 26 to 42 years (mean age 35 and median age 34 years). The diagnosis was made in 5 cases during pregnancy and in the other 8 cases during lactation. Maternal age during pregnancy was mean 36 years and median 35 years. 2 patients were diagnosed in the third trimester and 3 patient was diagnosed in the second trimester, with mean gestational ages of 24.2 weeks and median gestational ages of 22 weeks. Though one pregnant woman given birth vaginally, four pregnant women were carried out the cesarean section. Gestational age at delivery was mean 37 weeks and median 37 weeks. The three patients diagnosed around the second trimester normally received six courses of neoadjuvant chemotherapy with 5-fluorouracil, epirubicin and cyclophosphamide (FEC) during pregnancy, but locoregional radiotherapy and surgery were postponed until after delivery. The two patients who were diagnosed during the third trimester received therapy (surgery, chemotherapy, irradiation) after delivery. 5 patients after delivery were performed lumpectomy and sentinel lymphadenectomy. Surgical materials were sent to frozen. İn the frozen study 3 patients (60%) who had not tumors in sentinel lymph node were not performed axillary lymphadenectomy. 2 patients (40%) who had tumors in sentinel lymph node were performed axillary lymphadenectomy and breast-conserving surgery. An adjuvant hormoal therapy as well as locoregional radiotherapy was started after surgery. Inf-ant birth weight of mothers who was made the diagnosis during pregnancy was mean 2985 gram and median 2945 gram. One patient had positive family history (**Table 1**).

According to Modified Sc-arff Bloom-Richardson System three patients (60%) during pregnancy were grade 2 and two patients (40%) were grade 3. At presentation, 3 patients (60%) were classified as having American Joint Committee on Cancer clinical Stage II disease, 2 (40%) were classified as having Stage III disease. Tumor size during pregnancy was mean 4.6 cm and median 5 cm. The intensity and the percentage of nuclear staining for PR and ER of all patients was negative or too low. ER immunoreactivity was noted in one patient (20%) and PR immunoreactivity was observed in one patient (20%) during pregnancy. Both ER and PR positivity were identified in any a patient. Membranous staining for HER-2/neu of patients during pregnancy was high. Membranous staining for the Her-2/neu oncoprotein in 3 of the 5 patients (60%) revealed skor 2 and in 2 (40%) showed skor 3. Nuclear staining for Ki-67 of 5 patients was high. In 3 of 5 cases (60%), the proliferation rate as determined by Ki-67 staining was high, the proliferation rate was intermediate in 2 cases (40%) during pregnancy. Nuclear staining for cyclin D1 was too low during pregnancy. Nuclear staining for cyclin D1 in 4 of the 5 patients (80%) revealed skor 1 and in 1 (20%) showed skor 2 (**Table 2**).

Maternal age during lactation was mean 34.4 years and median 33.5 years. Postnatal age at diagnosis in 6 patients was one months and in

Table 2. Histopathology, pathology and immunohistochemistry during pregnancy of the patients with BC. (AMERICAN JOINT COMMITTEE ON CANCER-AJCC)

	Case				
	1	2	3	4	5
Histopathology	IDC	IDC	IDC	IDC	IDC
According to Modified Scarff-Bloom-Richardson System	Grade 3	Grade 2	Grade 2	Grade 2	Grade 3
According to AJCC Stage	T2, N2b, M0	T2, N1, M0	T2, N0, M0	T2, N0, M0	T3, N2a, M0
Tumor Size (cm)	5	5	3.5	3.5	6
Comedo Necrosis	-	-	-	-	+
Intraductal Carcinoma	+	+	+	+	+
Perineural Invasion	+	-	+	-	+
Lymphovascular Invasion	+	+	-	-	+
Microcalcifications	-	-	-	-	+
The intensity of nuclear staining for ER	Score 0	Score 0	Score 1	Score 0	Score 0
The percentage of nuclear staining for ER	Score 0	Score 0	Score 1	Score 0	Score 0
The intensity of nuclear staining for PR	Score 0	Score 0	Score 0	Score 1	Score 0
The percentage of nuclear staining for PR	Score 0	Score 0	Score 0	Score 1	Score 0
Membranous staining for HER-2/neu	Score 3	Score 2	Score 2	Score 2	Score 3
Nuclear staining for Ki-67	High	High	Inter-mediate	Inter-mediate	High
Nuclear staining for cyclin D1	Score 1	Score 1	Score 2	Score 1	Score 1

Table 3. Clinicopathological and obstetric characteristics during lactation of the patients with BC

	Case							
	6	7	8	9	10	11	12	13
Maternal age during lactation, years	26	41	32	34	33	35	32	42
Postnatal age at diagnosis (Months)	1	2	1	1	1	2	1	1
Mode of deliver	C/S	C/S	C/S	C/S	C/S	C/S	C/S	Spontan
Gestational age at delivery, weeks	41	40	40	41	39	39	40	41
Therapy during lactation	yes	yes	yes	yes	yes	yes	yes	yes
Infant birth weight (g)	2990	3025	2950	3010	3100	3200	2925	3350
Postpartum ablactation (Month)	1	1	1	1	1	2	1	1
Positive family history	yes	no	no	no	no	no	no	no

2 patients was two months. Though one pregnant woman given birth vaginally, 7 pregnant women were carried out the cesarean section. Gestational age at delivery was mean 40.1 weeks and median 40 weeks. All patients were provided chemotherapy, irradiation and surgery treatment during lactation. 8 patients during lactation were performed lumpectomy and sentinel lymphadenectomy. Surgical materials were sent to frozen. İn the frozen study 2 patients (25%) who had not tumors in sentinel lymph node were not performed axillary lymphadenectomy. 6 patients (75%) who had tumors in sentinel lymph node were performed axillary lymphadenectomy and breast-conserving surgery. An adjuvant hormonal therapy as well as locoregional radiotherapy was started after surgery. Infant birth weight of mothers was mean 3068.1 gram and median 3017.5 gram. Infant birth weight of mothers giving birth during lactation was higher than infant birth weight of mothers giving birth during pregnancy. The postpartum ablactation in 7 patients was first month, in one patient was second month. One patient had positive family history (**Table 3**).

According to Modified Scarff-Bloom-Richardson System five patients (62.5%) were grade 2 and three patients (37.5 %) were grade 3. At presentation, 4 patients (50%) were classified as having American Joint Committee on Cancer clinical Stage II disease, 4 (50%) were classified as having Stage III disease. Tumor size during lactation was mean 4.6 cm and median 4 cm. The intensity and the percentage of nuclear staining for PR and ER of all patients was negative

Table 4. Histopathology, pathology and immunohistochemistry during lactation of the patients with BC

	Case							
	6	7	8	9	10	11	12	13
Histopathology	IDC	IDC	IDC	IDC	IDC	IDC	IDC	IDC
According to Modified Scarff-Bloom-Richardson System	Grade 2	Grade 2	Grade 3	Grade 2	Grade 3	Grade 2	Grade 2	Grade 3
According to AJCC Stage	T2, N2b, M0	T2, N0, M0	T3, N2a, M0	T2, N0, M0	T2, N2b, M0	T2, N0, M0	T2, N0, M0	T4d, N0, M0
Tumor Size (cm)	4	2	6.5	3	4	2.5	4	7
Comedo Necrosis	-	-	-	-	+	-	-	+
Intraductal Carcinoma	+	+	+	+	+	+	+	+
Perineural Invasion	+	-	+	-	+	-	+	+
Lymphovascular Invasion	+	-	+	+	+	-	+	+
Microcalcifications	-	-	-	-	+	-	-	+
The intensity of nuclear staining for ER	Score 0	Score 1	Score 0	Score 1	Score 0	Score 1	Score 0	Score 0
The percentage of nuclear staining for ER	Score 0	Score 2	Score 0	Score 2	Score 0	Score 1	Score 0	Score 0
The intensity of nuclear staining for PR	Score 0	Score 1	Score 0	Score 0	Score 0	Score 0	Score 1	Score 0
The percentage of nuclear staining for PR	Score 0	Score 1	Score 0	Score 0	Score 0	Score 0	Score 1	Score 0
Membranous staining for HER-2/neu	Score 2	Score 2	Score 3	Score 2	Score 3	Score 2	Score 2	Score 3
Nuclear staining for Ki-67	Inter-mediate	Low	High	High	High	Inter-mediate	High	High
Nuclear staining for cyclin D1	Score 1	Score 2	Score 1	Score 1	Score 1	Score 2	Score 1	Score 1

and too low. ER immunoreactivity was noted in three patient (37.5%) and PR immunoreactivity was observed in two patient (25%) during lactation. Both ER and PR positivity were identified in one patient. Membranous staining for HER-2/neu of patients during lactation was high. Membranous staining for the Her-2/neu oncoprotein in 3 of the 8 patients (37.5%) revealed skor 3 and in 5 (62.5%) showed skor 2. The nuclear staining for Ki-67 of 8 patients was significantly high. In 5 of 8 cases (62.5%), the proliferation rate as determined by Ki-67 staining was high, the proliferation rate was intermediate in 2 cases (25%), and the proliferation rate was low in the remaining 1 cases (12.5%) during lactation. Nuclear staining for cyclin D1 was too low. Nuclear staining for cyclin D1 in 6 of the 8 patients (75%) displayed skor 1 and in 2 (25%) revealed skor 2 (**Table 4**).

In all patients in 8 of 13 cases (61.5%), the proliferation rate as determined by Ki-67 staining was high, the proliferation rate was intermediate in 4 cases (30.8%), and the proliferation rate was low in the remaining 1 cases (7.7%) during pregnancy and lactation.

Discussion

PABC is one the most common tumor of reproductive age group. It is relatively rare and its incidence is 0.2-3.8% [9]. In my study, PABC constitutes 2.5% of total cases.

In previous studies PABC has been associated with a genetic predisposition and a strong family history [10, 11]. In my study, I found only two patients have a positive family history in 13 patients.

The incidence of breast cancer in pregnancy is likely to increase due to the trend of women, mainly in modern society,to postpone pregnancy to older age, between 35 and 45 years, a period of fertile age in which there is an increasing incidence of breast cancer. In my study as compatible with earlier mentioned, the median age of the patients during pregnancy and lactation was 35 years (range 26-42 years). Maternal age during pregnancy was mean 36 years and maternal age during lactation was mean 34.4 years.

PABC has been associated with a poor prognos. Physiological changes during pregnancy and lactation, due to increased hormone levels, result in an increase in breast volume and firmness. These changes make clinical and radiological detection and evaluation of breast masses difficult [12]. It is possible that the increased hormone levels during pregnancy also accelerate the growth of any existing tumors, after transformation from premalignant to malignant breast cells has been triggered [13]. Because of these reasons, the management of patients with cancer during pregnancy requires the effort of a multidisciplinary team.

The Cancer and Pregnancy Registry has data on the largest cohort of pregnant patients diagnosed with breast cancer, the majority followed up prospectively, of whom 104 were treated with chemotherapy. Consistent with the literature, the majority of pregnant women in this series were diagnosed with stage II or III disease. In my study as compatible with earlier mentioned, 3 patients were classified as having Stage II disease, 2 were classified as having Stage III disease during pregnancy and 4 patients were classified as having Stage II disease, 4 were classified as having Stage III disease during lactation. Similar to nonpregnant women, pregnant women with breast cancer often present with a palpable mass. A delay in diagnosis occurs in pregnant women because the changes that occur in the breast during pregnancy, masses or lumps, may be ascribed to the “normal” changes of pregnancy. To avoid a delay in diagnosis, any solitary mass found during pregnancy or postpartum period should be evaluated promptly.

ER and PR are usually less positive in PABC than non-PABC. This is explained by the interference of circulating steroids with used the assays used to determine hormonal receptor status [14] or by downregulation of the receptors as a negative feedback effect of estrogen and progesterone upon hormonal receptor expresion. Although there is a significant trend in the literature that support this observation, [6] several reports claim that this phenomenon may not be entirely representative since most studies have not used age-matched controls [15]. An issue to be discussed whether hormonal receptor status is sicnificantly different in non-pregnant breast cancer patients of the same age, since breast cancer occuring at an age below 40 years usually has a negative ER/

PR profile [16]. As consistent with the literature, in my study ER and PR receptor expressions of all patients were negative or too low positive both during pregnancy and lactation.

Over-expression of the HER-2/neu oncogene, which occurs in 20 to 30% of invasive breast cancers, is associated with poorer prognosis [17]. HER-2/neu is synthesized in many embryonic tissues during pregnancy [15], and thus HER-2/neu over-expression was observed in 47.6% of the PABC patients, which is higher than that reported by Middleton (28%) [18], Garcia-Manero (31.6%) [19] and Halaska (33.3%) [20]. The rate of HER-2/neu oncogene over-expression in PABC patients was also higher than that among non-PABC group, which is similar to previously reported studies of 44% versus 28% of the control group [21]. In my study the HER-2/neu expressions of all PABC patients were high both during pregnancy and lactation.

Ki-67 protein (also known as MKI67) is a cellular marker for proliferation. This nuclear protein is expressed in proliferating cells during G1 through M phases of the cell cycle, but is not detected in resting cells. The Ki-67 expression as detected by immunohistochemistry is one of the most reliable indicators of the proliferative status of cancer cells [22] and is referred to as Ki-67 hence forward. In 2009, at the St-Gallen breast cancer conference, Ki-67 was recommended as a biomarker for prognosis and sensitivity of cancer cells to endocrine therapy or chemotherapy [23]. In 2011, Ki-67 was regarded as one of the factors influencing molecular subtypes [24]. Ki-67 is an important factor affecting the recurrence of early breast cancer and the survival of breast cancer patients [25, 26]. Ki-67 expression is closely associated with the growth and invasion of breast cancer: Ki-67-positive breast cancers are more active in growth, more aggressive in invasion, and more metastatic. The cut-off level for Ki-67 positive staining has varied from 5% to 30% [25], which complicates the comparison of the findings. In my study Ki-67 expression of all patients were usually high (≥31%) both during pregnancy and lactation. As with previous immunohistochemical studies, the localization of the cyclin D1 protein was predominantly confined to the nucleus of the breast cancer cells [27, 28]. Cyclin Dl is mainly overexpressed in the well differentiated and lobular types of invasive breast cancer and is strongly associated with estrogen receptor positivity. In the present study, I found a significant positive correlation between ER and cyclin D1 expression.

It is negatively correlated with the proliferation marker mitoses count and with the differentiation markers nuclear area and nuclear volume. As consistent with the literature, in my study I revealed a significant contrary correlation between Ki-67 and cyclin D1 expression.

Conclusion

Late diagnosis of PABC both during pregnancy and lactation is confirmed by my observation in which advanced the stage of the disease at the time of diagnosis was also common. PABC constituted 2.5% of all breast cancer patients. I observed low expression of ER, PR and cyclin D1, and high expression of Ki-67 and HER-2/neu in women with BC occurring during pregnancy and lactation. PABC is rare and challenging entity and more prospective randomized studies are needed.

Disclosure of conflict of interest

None.

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References

[1] Petrek JA, Dukoff R, Rogatko A. Prognosis of pregnancy-associated breast cancer. Cancer 1991; 67: 869-872.

[2] Woo JC, Yu T, Hurd TC. Breast cancer in pregnancy: a literature review. Arch Surg 2003; 138: 91-98.

[3] Anderson JM: Mammary cancers and pregnancy. B Med J 1979; 1: 1124-1127.

[4] White TT. Prognosis of breast cancer for pregnant and nursing women: analysis of 1413 cases. Surg Gynecol Obstet 1955; 100: 661-666.

[5] Hogge JP, De Paredes ES, Magnant CM, Lage J. Imaging and management of breast masses during pregnancy andlactation. Breast J 1999; 5: 272-283.

[6] Ishida T, Yokoe T, Kasumi F, Sakamoto G, Makita M, Tominaga T, Simozuma K, Enomoto K, Fujiwara K, Nanasawa T. Clinicopathologic characteristics and prognosis of breast cancer patients associated with pregnancy and lacta-

tion: Analysis of case-control study in Japan. Jpn J Cancer Res 1992; 83: 1143-1149.
[7] Yang WT, Dryden MJ, Gwyn K, Whitman GJ, Theriault R. Imaging of breast cancer diagnosed and treated with chemotherapy during pregnancy. Radiology 2006; 239: 52-60.
[8] Loibl S, von Minckwitz G, Gwyn K, Ellis P, Blohmer JU, Schlegelberger B, Keller M, Harder S, Theriault RL, Crivellari D, Klingebiel T, Louwen F, Kaufmann M. Breast carcinoma during pregnancy. International recommendations from an expert meeting. Cancer 2006; 106: 237-246.
[9] Wallack MK, Wolf JA Jr, Bedwinek J, Denes AE, Glasgow G, Kumar B, Meyer JS, Rigg LA, Wilson-Krechel S. Gestational carcinoma of the female breast. Curr Probl Cancer 1983; 7: 1-58.
[10] Beadle BM, Woodward WA, Middleton LP, Tereffe W, Strom EA, Litton JK, Meric-Bernstam F, Theriault RL, Buchholz TA, Perkins GH. The impact of pregnancy on breast cancer outcomes in women <or=35 years. Cancer 2009; 115: 1174-1184.
[11] Shen T, Vortmeyer AO, Zhuang Z, Tavassoli FA. High frequency of allelic loss of BRCA2 gene in pregnancy-associated breast carcinoma. J Natl Cancer Inst 1999; 91: 1686-1687.
[12] Yang WT, Dryden MJ, Gwyn K, Whitman GJ and Theriault R. Imaging of breast cancer diagnosed and treated with chemotherapy during pregnancy. Radiology 2006; 10: 1148.
[13] Albrektsen G, Heuch I, Thoresen S and Kvale G: Clinical stage of breast cancer by parity, age at birth, and time since birth: a progressive effect of pregnancy hormones? Cancer Epidemiol Biomarkers Prev 2006; 15: 65-69.
[14] Elledge RM, Ciocca DR, Langone G, McGuire WL. Estrogen receptor, progesterone receptor, and HER-2/neu protein in breast cancers from pregnant patients. Cancer 1993; 71: 2499-2506.
[15] Middletone LP, Amin M, Gwyn K, Theriault R, Sahin A. Breast carcinoma in pregnant women: assessment of clinicopathologic and immunohistochemical fetaures. Cancer 2003; 98: 1055-1060.
[16] Kathori AS, Fentiman IS. Breast cancer in young women. Int J Clin Pract 2002; 56: 184-187.
[17] Hayes DF, Thor AD. C-erbB-2 in breast cancer: development of a clinically useful marker. Semin Oncol 2002; 29: 231-245.
[18] Daling JR, Malone KE, Doody DR, Anderson BO, Porter PL. The relation of reproductive factors to mortality from breast cancer. Cancer Epidemiol Biomarkers Prev 2002; 11: 235-241.
[19] Garcia-Manero M, Royo MP, Espinos J, Pina L, Alcazar JL, López G. Pregnancy associated breast cancer. EJSO 2009; 35: 215-218.
[20] Halaska MJ, Pentheroudakis G, Strnad P, Stankusova H, Chod J, Robova H, Petruzelka L, Rob L, Pavlidis N. Presentation, management and outcomes of 32 patients with pregnancy-associated breast cancer: a matched controlled study. Breast J 2009; 15: 461-467.
[21] Reed W, Hannisdal E, Skovlund E, Thoresen S, Lilleng P, Nesland JM. Pregnancy and breast cancer: a population-based study. Virchows Arch 2003; 443: 44-50.
[22] Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol 2000; 182: 311-322.
[23] Goldhirsch A, Ingle JN, Gelber RD, Coates AS, Thurlimann B, Senn HJ; Panel members. Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer. Ann Oncol 2009; 20: 1319-1329.
[24] Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B, Senn HJ; Panel members. Strategies for subtypes-dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer. Ann Oncol 2011; 22: 1736-1747.
[25] De Azambuja E, Cardoso F, de Castro G Jr, Colozza M, Mano MS, Durbecq V, Sotiriou C, Larsimont D, Piccart-Gebhart MJ, Paesmans M. Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. Br J Cancer 2007; 96: 1504-1513.
[26] Stuart-Harris R, Caldas C, Pinder SE, Pharoah P. Proliferation markers and survival in early breast cancer: a systematic review and meta-analysis of 85 studies in 32,825 patients. Breast 2008; 17: 323-334.
[27] Gillett C, Fantl V, Smith R, Fisher C, Bartek J, Dickson C, Barnes D, Peters G. Amplification and overexpression of cyclin D1 in breast cancer detected by immunohistochemical staining. Cancer Res 1994; 54: 1812-1817.
[28] Bartkova J, Lukas J, Strauss M, Bartek J. Cell cycle-related variation and tissue-restricted expression of human cyclin D1 protein. J Pathol 1994; 172: 237-245.