Aim and Scope

Archives of Breast Cancer (ABC) is an open access, peer-reviewed journal that publishes articles on all aspects of breast cancer research, including the pathophysiology, prevention, early detection, diagnosis, treatment, molecular and cellular biology, genetics, epidemiology, psychological issues, rehabilitation and quality of life. Although the main focus of the journal is breast cancer, some important topics among benign breast diseases and breast health such as breastfeeding will be considered for publication.

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Dear colleagues,

We are proud to announce the publication of 2018 year book of Archives of Breast Cancer. This year book includes papers from diverse as well as relevant areas including surgical aspects, medical ethics, chemoprevention, genetics, neoadjuvants, pathology, risk assessment, nano-medicine, quality of life, patient satisfaction, medical decision making, legal aspects and prognosis. All these papers have valuable messages to share in one direction: contributing to optimum prevention, diagnosis, treatment and rehabilitation of breast cancer. In 2019, we intend to expand this diversity not only in the contents but also in our audience around the world. Moreover, we are going to endorse the COPE (Committee on Publication Ethics) guidelines; therefore, all researchers are kindly requested to consult these guidelines to avoid cases of suspected research or publication misconduct (e.g. falsification, fabrication, plagiarism, inappropriate image manipulation, and redundant publication). For more information about COPE, please visit http://www.publicationethics.org.

Thank you in advance for being with us in 2019 and sharing your valuable research work with ABC. Your feedback on this year book would encourage us to work with more passion and determination in Archives of Breast cancer.

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Breast cancer is one of the most common forms of cancer that threatens women and needs more attention ethically. Your valuable editorial titled “ABC of Medical Ethics and Professionalism in the Breast Clinic” was published in the recent issue. Because of my interest in medical ethics, and in line with your claim, I would like to contribute the results of my PhD dissertation in this area. The study was conducted in a public hospital in Iran based on critical ethnography and included 156 recorded clinical consultations, 920 hours of participant observation, and six focus groups consisting of patients and their families. The theme that was explored was violation of patients’ privacy. This theme was about the concerns of female patients of urology and breast cancer clinics. Their main concern was about being visited by male physicians. Exposure during examination, operation, and changing dressing were their main concerns. One of my participants who was very upset was a 52-year-old woman with breast cancer who had mastectomy. Despite the success of the operation, she was very upset that her breasts had to be examined in the presence of 6 or 7 male medical students at operation room. She said this was one of the worst experiences she ever had. She believed that although her physician was quite expert at his job, he did not acknowledge her privacy concerns. That seems to be a problem in medical departments of breast cancer. Other evidence also confirms that privacy is a major issue. Mamdouh et al showed that 71.4% of Egyptian women complained about the lack of privacy as a barrier to participation in breast cancer screening. Also, Brown et al found that a small percentage (3%) of women were dissatisfied with privacy arrangements in screening units; however, when privacy was discussed in an ethical context, the percentage increased significantly. I believe that this problem lies in doctor-patient interaction (DPI). Study showed that, in the context of Iranian health system, DPI has unequal, unprofessional, instrumental, and non-cooperative characteristics, which can lead to “patient’s distrust, patient’s dissatisfaction, lack of mutual understanding, patient suppression, and patient deception.” Theoretically, it is due to doctor-centeredness that doctors determine everything and do not pay attention to patients’ lifeworld. If physicians want to become great physicians, they must strengthen their human dimensions and communication skills alongside their medical skills. In this view, doctors look at the patients from a holistic point of view, treating them while taking into consideration every dimension of their problems. As for women with breast cancer, as you observed, breast is a feminine organ and is considered a private organ for many women; and paying attention to all concerns of these people is the first principle of medical ethics. Thus, all physicians, specialists, and nurses who provide care for these people should appreciate the fact that privacy is an important issue for women with breast cancer. It is the women’s right as well as an ethical obligation that their privacy not be violated in the clinic, operation room, recovery room, and ward. Finally, based on our Islamic views, patient privacy is a main responsibility of the medical team in all situations, which should be considered for women with breast cancer.

Conflict of Interest
None to declare.

References


Breast cancer is the most frequently occurring cause of cancer-related mortality in women all around the world. However, the risk of cardiovascular diseases increases in parallel with dramatic improvements in target-specific treatment for breast cancer. The aim of this review was to show the importance of cardiovascular involvement in patients with breast cancer.

Methods: Published literature, regarding breast cancer and cardiovascular involvements, as well as cardiovascular complications of current treatments for breast cancer, including chemotherapy and radiotherapy, was reviewed.

Results: Review of our data revealed that there are extensive direct and indirect impacts of breast cancer on the cardiovascular system. Cardiovascular complications of breast cancer are common and range from cardiomyopathy, pericardial involvement, venous thromboembolism, and arterial thrombosis to some uncommon problems.

Conclusion: Early detection of cardiovascular damages from breast cancer is strongly recommended. Considering the significant cardiovascular complications of both breast cancer and its treatment, early recognition, prevention, and management of these complications, even the minor ones, improve prognosis and survival of patients with breast cancer.
of breast cancer irrespective of chemotherapy or radiotherapy. In the second section, we will talk about cardiovascular complications of radiotherapy or chemotherapy in patients treated for breast cancer.

**Methods**

A literature search was performed for the years 1989 through 2017. We searched PubMed, Elsevier, MEDLINE, and Google Scholar. We considered scientific publications relevant to breast cancer for inclusion in our work.

First of all, we searched with “breast cancer,” and again with the “breast cancer and cardiovascular.” Then we select some of the articles obtained from the first search strategy, but most of them were related to the second search strategy.

Published literature regarding breast cancer and cardiovascular involvements, as well as cardiovascular complications of the current treatments for breast cancer, including chemotherapy and radiotherapy, were reviewed.

We used review articles (n = 22), cohort studies (n = 5), cross-sectional studies (n = 27), population-based case-control studies (n = 1), case reports (n = 6), guidelines (n = 2), case-control studies (n = 2), experimental studies (n = 3), and RCTs (n = 5).

Papers without full text, presented at conferences, or published in languages other than English were excluded.

**Results**

**Cardiovascular complications of metastatic breast cancer**

Cardiovascular complications of breast cancer included metastasis to the heart, superior vena cava syndrome, pericardial involvement, vascular problems, and cardiac function deterioration in a background of paraneoplastic syndrome.

**Cardiac metastases**

Cardiac metastases are detected in 6–20% of autopsies of patients with malignant neoplasms such as lung cancer, mediastinal tumors, breast cancer, melanoma, and esophageal cancer. Although intracavitary growth of secondary heart tumors is rare, the important point is that symptomatic heart metastasis can occur even many years after diagnosis. According to Bussani et al., cardiac metastasis (more commonly pericardial metastasis, and rarely direct myocardial and endocardial metastasis) could be as high as 15.5% in patients with breast cancer.

From pathophysiological viewpoint, multiple mechanisms have been proposed for breast cancers that are able to induce heart metastasis. For instance, breast-tumor cells in brain and heart metastases express high levels of endoglin, a cell-surface disulfide-linked homodimeric glycoprotein which binds to integrins and is a co-receptor for TGF-β. These ligands and mediators participate in heart metastasis. Two-dimensional transthoracic and transesophageal echocardiography are easy, quick, and sensitive techniques for detection of cardiac metastasis. Computed tomography (CT) scan, magnetic resonance imaging (MRI), positron emission tomography with 2-[fluorine-18]-fluoro-2-deoxy-D-glucose integrated with computed tomography (18F-FDG PET/CT), and PET-MRI as confirming methods could be helpful when there is any suspicion as to excluding cardiac involvement during clinical follow-up of these patients.

**Superior vena cava syndrome (SVCS)**

Approximately 87% to 97% of SVCS cases are caused by primary intrathoracic malignancies. Breast cancer is one of the solid tumors causing SVCS, but not as much as other tumors like non-Hodgkin lymphoma, lung, or testicular cancer. The most common nonmalignant cause of SVCS in cancer patients is thrombosis associated with venous access devices, especially patients with breast cancer who have long term central venous port catheter. There are some noninvasive therapeutic measures for SVCS, but endovascular revascularization of complete occlusion of the SVC is considered the therapy of choice.

**Pericardial involvement**

Breast cancer could cause significant pericardial effusion or tamponade. This kind of pericardial involvements impairs quality of life. Recurrent pericardial effusion along with the development of dyspnea or tachycardia may necessitate repetitive hospitalization. Percutaneous pericardiocentesis with extended catheter drainage can be safely and effectively implemented as the primary treatment for pericardial effusion in cancer patients, including those with thrombocytopenia.

**Venous thromboembolism (VTE)**

Patients constitute about 20% of all cases of VTE, and are also 4- to 7-fold more likely to develop VTE compared with patients without cancer. Patients with cancer often show abnormalities in each component of Virchow’s triad, leading to hypercoagulability. Breast cancer is not a common cause of primary VTE per se, but patients with multiple metastasis or immobile ones, or who are under hormone therapy with tamoxifen or aromatase inhibitors, have higher risk for VTE and should receive VTE prophylaxis.

**Arterial thrombosis**

Arterial thrombosis and its resultant clinical syndromes, such as cerebrovascular events or peripheral ischemia, could happen in cancer patients, and the most common reported malignancy in this setting is metastatic breast cancer.
Thrombosis may be related to cancer itself or its treatment. The paraneoplastic process leads to hypercoagulability with changes in levels of factor VII and proteins C and S. Tissue factor and cancer procoagulant levels may rise. Thrombocytosis, increased fibrinogen levels and reduced fibrinolysis, endothelial damage, and stasis may also contribute to thrombosis. Premenopausal breast cancer patients who receive both chemotherapy and tamoxifen are more likely to present with arterial thrombosis compared with those who receive chemotherapy alone.6,27

Heart failure (HF) or cardiac function deterioration also occurs in a background of paraneoplastic syndrome of breast cancer.28

Complications of therapy for breast cancer
Here we explain more about anti-human epidermal growth factor receptor 2 (HER2) agents, especially trastuzumab.

Novel chemotherapy agents and related cardiac toxicity
The overexpression of HER2 in breast cancer is associated with more aggressive disease with a poor prognosis.2,6,30 Trastuzumab, pertuzumab, and other anti-HER2 agents are monoclonal antibodies against the extracellular domain of HER2 that have been shown to be effective in metastatic breast cancer as monotherapy or combining with other chemotherapy agents.31,32 These agents have proved to be effective in both metastatic and early-stage breast cancers when combined with chemotherapy, and reduce relapse rates by 50% irrespective of age and other relevant prognostic factors.31,33

As mentioned by the American Society of Clinical Oncology, in contrast to anthracycline-related cardiac toxicity (type 2/irreversible), trastuzumab does not result in myocyte loss. In trastuzumab-induced cardiac dysfunction, myocytes appear normal histologically and alterations may be noticed only by using electron microscopy.33

In a study by Swain et al., the addition of pertuzumab to trastuzumab and docetaxel in patients with HER2-positive metastatic breast cancer significantly increased the median overall survival to 56.5 months—an improvement of 15.7 months over survival in the control group.30

As mentioned before, these novel biologic therapies improve disease-free and overall survival, but increase the risk of cardiotoxicity,6,33 as has been shown in many studies.30,39 The cardiotoxicity of these agents could involve any part of the heart’s structure, and the ongoing studies are assessing these damages to heart using various methods and parameters.

Recently, we compared cardiomyopathy-related findings before and after trastuzumab therapy in patients with breast cancer and found that diastolic function was significantly impaired after treatment (25.9% versus 43.6%).30 After the therapy, left ventricular ejection fraction (LVEF) was reduced significantly, and troponin levels were increased remarkably (0% versus 6.7%); however, no significant differences were observed for CRP and NT-pro-BNP levels. This study showed the importance of troponin for trastuzumab-induced cardiomyopathy.

In a study by Rossner and colleagues38 in 28 female patients with metastatic HER2-positive breast cancer, blood samples were drawn before and 30 min after intravenous trastuzumab infusion, and EF and NT-pro-BNP levels before and after the initiation of trastuzumab were compared. According to higher median 3-month mortality in cases with elevated levels of NT-pro-BNP, this marker could be considered as a prognostic factor in these patients.

In one study by Cardinale et al.,7 the incidence rate of trastuzumab-induced cardiotoxicity (TIC) in breast cancer patients was reported to be 17%, and TIC was significantly associated with elevated levels of troponin I (TNI) (62% in TNI+ vs 5%; P < .001). They suggested that increased TNI levels can identify trastuzumab-treated patients who are at risk for cardiotoxicity and who are unlikely to recover from cardiac dysfunction despite HF therapy.

In another study by Goel et al., serum troponin I and NT-pro-BNP were assayed immediately before and 24 hours after trastuzumab infusion in patients with breast cancer. A significant proportion of the patients with normal LVEF who received trastuzumab experienced elevated levels of NT-pro-BNP, but that was not the case for troponin I levels.40

Lamot et al. reported evaluated trastuzumab treatment–induced cardiac toxicity in 30 breast cancer patients.41 Cardiac toxicity was assessed based on LV function. LVEF showed a significant decrease after trastuzumab adjuvant therapy.

In a large cohort study by Chavez-MacGregor et al.,42 2203 older breast cancer patients under trastuzumab therapy were evaluated. They observed a chronic heart failure rate of 29.4% among trastuzumab users, compared with 18.9% in non-trastuzumab users (P < 0.001).

In addition to ejection fraction (EF) and cardiac biomarkers, a few studies have also shown adverse effect of this drug on left atrium. A recently published observational study revealed changes in atrial diameter and geometry during the early periods of trastuzumab treatment.43 Ongoing studies are evaluating precisely the effect of these monoclonal antibodies on atrial structure and function.

Finally, regarding the research on cardiotoxicity of trastuzumab, most recent and valuable studies some of which have been mentioned above, have demonstrated the adverse effect of newer biologic therapies on heart system, but their cardiotoxicity is significantly lower than the older agents such as
anthracyclines.4

Contemporary radiotherapy and related cardiac toxicity

Like chemotherapy, radiation causes mainly a series of toxic effects and hemodynamic and structural damages to the cardiovascular system, affecting the long-term survival.44, 45 Radiation to the chest can damage the pericardium, myocardium, heart valves, and coronary vessels.46, 47 According to available evidence, the damage to the cardiovascular system is directly related to the dose of radiation and the volume of heart irradiated, especially if combined with chemotherapy.48 The extent of damage will be doubled in patients with preexisting cardiac diseases. In radiation-induced vascular damage, endothelial dysfunction is the first sign.49 After starting radiation therapy, large myocardial perfusion defects were detected in single-photon emission computerized tomography scans (even in 55% of asymptomatic patients),50, 51 and radiotherapy has even been accompanied by HF in these patients; overall, however, HF is not common in patients undergoing radiation therapy and is usually a late effect of radiation therapy. The mechanism responsible for radiation-induced injury to heart could be myocardial fibrosis resulting from collagen disruption.52 Radiation can cause injury to the intima of the coronary arteries and initiate a cascade of atherosclerotic events. The left anterior descending and the right coronary arteries are most often involved in patients undergoing mediastinal radiation for Hodgkin’s disease.53

Progressive fibrosis following radiation-induced myocardial collagen synthesis results in valvular heart disease by increasing the valvular thickness, and in left ventricular dysfunction by increasing ventricular wall thickness.54 Ongoing studies are evaluating radiotherapy-induced LV dysfunction by new imaging methods and parameters like (18F-FDG PET/CT) and PET-MRI, as mentioned.

In spite of all the mentioned complications, the absolute risk of radiation therapy is small and seems to be cancelled out by the advantages for patients receiving radiation therapy.

Discussion

Approach to cardiac toxicity of chemotherapeutic agents in patients with breast cancer

Risk factors of trastuzumab-induced cardiomyopathy

Epidemiologic evidence indicates that, even without a clear LVEF at the time of treatment, early treatment of breast cancer with trastuzumab presents a substantial long-term risk of HF, especially for women older than 65 years.55

Diagnosis of trastuzumab-induced cardiomyopathy

Echocardiographic evolution of EF using Simpson’s method is recommended for assessment of left ventricular function.56, 57 Abnormalities of right ventricular contractility, ventricular dilation, and abnormal left ventricular contractility are the earliest presentations of myocardial damage diagnosed by echocardiography.

Strain rate imaging (SRI) is a new echocardiographic modality that enables accurate measurement of regional myocardial function and is recommended especially for chemotherapy- and radiotherapy-induced cardiotoxicity in breast cancer patients.58

There are some recommendations by experts and in reported articles from tertiary centers, and almost all of them agree that treatment with trastuzumab must be stopped if clinical symptoms of HF are present.53, 60, 61

In the event of an asymptomatic decrease in LVEF by 15% or more, discontinuation of trastuzumab therapy is mandatory.62, 63 Patients receiving chemotherapy may be considered to be at elevated risk of developing cardiac dysfunction — Stage A heart failure in ACC/AHA guidelines.64

Baseline cardiac evaluation through history taking, physical examination, and electrocardiography should be done for all patients before they are given trastuzumab.65 Five main randomized trials have demonstrated the survival advantages of adjuvant trastuzumab in early breast cancer patients.35, 66 Recently, guidelines were developed for cardiac monitoring of metastatic breast cancers receiving trastuzumab treatment.67 Since trastuzumab-associated cardiac toxicity is a great concern in making all the adjuvant trials, strict cardiac evaluation is necessary in these trials prior designing and monitoring at regular intervals during therapy. In National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31, trastuzumab-induced symptoms were monitored and patients who developed clinically significant cardiac symptoms while receiving anthracycline treatment were excluded from subsequent trastuzumab therapy. The initiation or continuation of trastuzumab treatment in asymptomatic patients required an LVEF equal to or exceeding the lower limit of normal range.68 In National Central Cancer Treatment Group (NCCTG) N9831, pooled with NSABP B-31, 6.7% of the enrolled patients were not allowed to start trastuzumab treatment because their LVEF had declined to a subnormal level or had been decreased by ≥ 16% from baseline after completion of anthracycline treatment.69, 70 Jones et al., published cardiology assessment and monitoring methods in British Journal of Cancer,71 and Saad et al. presented the second recommendations and their related demonstrations.72

The newest accepted guidelines are developed by the American Society of Echocardiography and the European Association of Cardiovascular Imaging.64
Baseline evaluation of LVEF by 2D or 3D echocardiography, global longitudinal strain (GLS), and troponin I should be determined at the initiation of any regimen potentially associated with type I toxicity.

At the initiation of trastuzumab, baseline evaluation of LVEF should be done by 2D or 3D echocardiography, GLS, and troponin I assessment. If LVEF is less than 53%, GLS is near the lower limit of normal, and troponin test is positive, cardiology consultation should be recommended, and if these three parameters are in normal range, follow-up by measurement of LVEF, GLS, and troponin every 3 months is recommended.

At the initiation of trastuzumab after a regimen associated with type I toxicity, such as anthracycline (cell apoptosis and irreversible cell damage) the assessment is similar to above guidelines. Follow-up by measurement of LVEF, GLS, and troponin every 3 months during therapy, and 6 months after therapy, is recommended. As in trastuzumab monitoring, if parameters are normal, cardiology consultation would be necessary; and if parameters are within normal range, follow-up at the completion of therapy and 6 months later should be considered. For early detection of subclinical LV dysfunction GLS is the optimal parameter: a relative percentage reduction of < 8% from baseline is not significant, but those >15% are probably abnormal.

Today, cardiovascular magnetic resonance (CMR) imaging is widely used in patients with breast cancer for detecting both the acute and chronic complications of cardiotoxic chemotherapeutic agents. CMR is recommended when the quality of the echocardiogram is suboptimal. With the introduction of late gadolinium enhancement (LGE), CMR is considered the gold standard for myocardial viability imaging accompanied by positron emission tomography.

Aerobic training (AT) is a non-pharmacological strategy to attenuate or even counteract acute and chronic cardiovascular abnormalities in the context of early breast cancer. It can improve systolic and diastolic function and reduce pathologic cardiac remodeling. This may lead to enhanced exercise tolerance and resistance to fatigue during exertion in patients with known cardiovascular disease. Cardioprotective properties of AT in the context of early breast cancer has been well explained in an important study by Scott et al. They proposed an exercise paradigm based on the principles of AT to facilitate a personalized medicine approach that may optimize prevention or attenuation of breast cancer therapy-associated cardiovascular disease.

Echocardiography can be utilized as a routine method for monitoring cardiac side effects. It helps in assessment of parameters for systolic and diastolic function and anatomical cardiac dimensions as well.

The remarkable improvements in screening and adjuvant therapy for breast cancer, combined with close surveillance of cancer survivors, have led to a significant decrease in recurrence rate. Currently, cardiovascular disease is one of the leading causes of death in many patients who have been treated for breast cancer.

We recommend screening and surveillance for early detection of subclinical cardiovascular complications of breast cancer itself, or cardiotoxicity associated with its treatment. Early detection and treatment of even the smallest cardiac damages will improve prognosis and life expectancy of patients with breast cancer.

**Conflict of Interest**
None to declare.

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A Comparative Study of Multilayer Neural Network and C4.5 Decision Tree Models for Predicting the Risk of Breast Cancer


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ABSTRACT

Background: Diagnosing breast cancer at an early stage can have a great impact on cancer mortality. One of the fundamental problems in cancer treatment is the lack of a proper method for early detection, which may lead to diagnostic errors. Using data analysis techniques can significantly help in early diagnosis of the disease. The purpose of this study was to evaluate and compare the efficacy of two data mining techniques, i.e., multilayer neural network and C4.5, in early diagnosis of breast cancer.

Methods: A data set from Motamed Cancer Institute's breast cancer research clinic, Tehran, containing 2860 records related to breast cancer risk factors were used. Of the records, 1141 (40%) were related to malignant changes and breast cancer and 1719 (60%) to benign tumors. The data set was analyzed using perceptron neural network and decision tree algorithms, and was split into two a training data set (70%) and a testing data set (30%) using Rapid Miner 5.2.

Results: For neural networks, accuracy was 80.52%, precision 88.91%, and sensitivity 90.88%; and for decision tree, accuracy was 80.98%, precision 80.97%, and sensitivity 89.32%. Results indicated that both algorithms have acceptable capabilities for analyzing breast cancer data.

Conclusion: Although both models provided good results, neural network showed more reliable diagnosis for positive cases. Data set type and analysis method affect results. On the other hand, information about more powerful risk factors of breast cancer, such as genetic mutations, can provide models with high coverage.
Researchers have been interested in artificial intelligence for developing prediction models in various scientific fields such as medical engineering. Medical prediction models help physicians in overcoming health care problems and decreasing medical errors. Furthermore, data analysis models may result in better and more accurate diagnosis of conditions in clinical settings by detecting hidden patterns. Classification is one of the main functions of data analysis. Neural networks are the most applicable models of artificial intelligence in medicine because they provide accurate responses and decision trees and the process is simple to follow. Since the classification of medical problems is inherently non-linear, prediction models based on linear statistical methods would not be precise. Furthermore, conventional statistical techniques are not suitable for analyzing large data sets. Data analysis and its techniques, if used properly, can be more efficient in this regard. Considering that the use of modern technologies and software knowledge have increased in medicine during the last two decades, and given the fact that early diagnosis has a significant role in decreasing cancer mortality, applying data analysis techniques to breast cancer data sets and extracting useful results for improvement of accuracy in medical diagnosis is crucial.

Given the importance of breast cancer and its early diagnosis as well as understanding the effective role of different data analysis methods in development of prediction models, it seems essential that the accuracy of these techniques be evaluated practically in various sites and the most efficient and effective models be identified. Thus, the objective of the present study was to compare the accuracy of two different models, namely, neural network data analysis and decision tree, in predicting the risk of breast cancer.

**Methods**

In this retrospective study, a data set from Breast Cancer Research Center of Motamed Cancer Institute, Tehran, Iran, were used, which contained information related to patients admitted to ACECR breast diseases clinic in Tehran from March 2007 to September 2015. Every record consisted of 14 fields of information on breast cancer risk factors and 1 field on the type of main tumor (malignant or benign). The data set consisted of 2860 records, of which 1141 (40%) were related to breast cancer patients and 1719 (60%) to benign breast tumors.

Table 1 presents the evaluated risk factors.

In preprocessing stage, columns unrelated to disease risk factors or related to patients’ demographic information were omitted. Then, for the purpose of increasing validity, efforts were made to omit records with more than 20% missed information and records having irrelevant information, although no such record was identified. Finally, missing values were replaced by the mean of that variable for 25 adjacent cases in SPSS 21 so that the number of the remaining records were 2860 (unchanged). Then, by random sampling, 70% and 30% of the data set were used for model training and model testing, respectively. In order to design a multilayer perceptron neural network with Rapid Miner 5.2, the number of nodes was considered 14 with a learning rate of 0.3 and 1 hidden layer. The number of nodes in hidden layer was 10, and the number of iteration was considered 1000. To design the tree with Rapid Miner 5.2, data productivity criteria, minimum branch size of 4, minimum leaf size of 2, minimum productivity of 0.1, and confidence of 0.25 were used, and models were evaluated using 70% of the training data and 30% of the test data.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The age at the time of diagnosis</td>
<td>Quantitative – discrete</td>
</tr>
<tr>
<td>2. The age of the first menstruation</td>
<td>Quantitative – discrete</td>
</tr>
<tr>
<td>3. Menopausal age</td>
<td>Qualitative – classified</td>
</tr>
<tr>
<td>4. The age of the first pregnancy</td>
<td>Qualitative – classified</td>
</tr>
<tr>
<td>5. History of breastfeeding</td>
<td>Qualitative – classified</td>
</tr>
<tr>
<td>6. History of taking OCP</td>
<td>Qualitative – classified</td>
</tr>
<tr>
<td>7. History of hormone therapy after menopause</td>
<td>Qualitative – classified</td>
</tr>
<tr>
<td>8. History of breast cancer</td>
<td>Qualitative – classified</td>
</tr>
<tr>
<td>9. Family history of breast cancer</td>
<td>Qualitative – classified</td>
</tr>
<tr>
<td>10. History of infertility</td>
<td>Qualitative – classified</td>
</tr>
<tr>
<td>11. Tobacco use</td>
<td>Qualitative – classified</td>
</tr>
<tr>
<td>12. Marital status</td>
<td>Qualitative – classified</td>
</tr>
<tr>
<td>13. Education</td>
<td>Qualitative – classified</td>
</tr>
<tr>
<td>14. Traumatic events in life</td>
<td>Qualitative – classified</td>
</tr>
<tr>
<td>15. Type of disease (malignant or benign)</td>
<td>Qualitative – classified</td>
</tr>
</tbody>
</table>
For investigation of the success rate and efficacy of these models, we used confusion matrix and ROC diagram as common techniques in diagnosis classification models.\textsuperscript{12} For interpretation of classification and diagnosis of diseases and breast cancer patients using confusion matrix, there exists four states including true positive, true negative, false positive, and false negative, with each one having a special meaning in confusion matrix as follows:

True Positive (TP): the number of records that are positive and the algorithm has truly identified their class.

False Positive (FP): the number of records that are negative but the algorithm has falsely identified their class as positive.

True Negative (TN): the number of records that are negative and the algorithm has truly identified their class.

False Negative (FN): the number of records that are positive but the algorithm has falsely identified their class as positive.\textsuperscript{13}

In this paper, the function of confusion matrix was developed using concepts above, and, for analyzing their functions, three main criteria of sensitivity, specificity, and accuracy in classification were used. Definitions and characteristics of these indices have been described in all resources for data analysis.\textsuperscript{13}

As mentioned earlier, after being entered in Rapid Miner software, the data set was split into two sets (70% for training and 30% for testing models), and the multi-layer perceptron neural network (MLP) and the decision tree (C4.5) were trained and tested using those data sets. Results were provided by three criteria of accuracy, sensitivity and specificity.

Results

As described in previous section, after training and testing the models, the software reported results by three indices of sensitivity, specificity, and accuracy. Table 2 presents the results of the evaluation of models.

It can be seen from the table that there was no significant difference between sensitivity and accuracy indices. However, regarding specificity, neural network is significantly effective than decision tree.

Discussion

In this study, we analyzed a data set from ACECR Breast Cancer Research Center for diagnosis of breast cancer using multilayer perceptron neural network and decision tree algorithms. In comparison, the neural network was significantly effective in diagnosing negative cases. Early diagnosis of breast cancer is important from different aspects and can improve patients’ survival. Considering the importance of the risk factors in breast cancer incidence, the efficacy of data analysis techniques in development of effective models for prediction and diagnosis is undeniable.\textsuperscript{7} It is worth mentioning that the use of the general terms “neural network” and “decision tree” do not seem appropriate for other algorithms like C4.5 and multilayer neural networks. In other words, “neural network” and “decision tree” are general terms for techniques which contain various algorithms.

Researchers have used neural network and decision tree algorithms with other breast cancer data sets, and the results are different from the present study. For instance, in a work by Senturk and Kara using neural network and decision tree algorithms for analysis of Wisconsin sampling data set, the accuracy of both models was greater than that of the present study. The reason for this difference can be attributed to the difference in databases, methods, and missing data management.\textsuperscript{14}

In another work, Rajesh and Anand used C4.5 algorithm for analysis of SEER data set for diagnosing breast cancer, which displayed greater accuracy compared with the present study. Again, the difference can be attributed to differences in data sets, data selection, and data classification methods.\textsuperscript{15}

In a work by Lakshmi et al evaluating the efficacy of data analysis algorithms, Wisconsin sampling data set was analyzed using C4.5 algorithm. The accuracy of this model was significantly higher than our study because of the difference in the evaluation method. Therefore, differences in data sets can produce different results in data analysis.\textsuperscript{16} Kiani and Atashi used decision tree algorithm for prediction of breast cancer recurrence. Similarities of these two studies are using decision tree, using a real sample of patients, and using similar outcomes for evaluation of the models. The researchers showed 75% accuracy for decision tree model, which is lower than that for our study. The most important reason for this difference may be that Kiani and Atashi used a lower number of records and different set of dependent variables.\textsuperscript{17} Also, it is possible that increasing the amount of training data to a specific level may improve model accuracy.\textsuperscript{18} Furthermore, Tolooi et al used C5 decision tree for analysis of the same data set used in this study and obtained an accuracy of 95%.

Table 2 . The results of model testing by sensitivity, specificity, and accuracy of models

<table>
<thead>
<tr>
<th>Model</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multilayer perceptron neural network</td>
<td>90.88%</td>
<td>88.91%</td>
<td>80.52%</td>
</tr>
<tr>
<td>C4.5 decision tree</td>
<td>89.32%</td>
<td>80.97%</td>
<td>80.98%</td>
</tr>
</tbody>
</table>

This shows that significant differences in data modeling can be found among decision tree algorithms.

Among limitation of this study, we can mention the missing data. Because of independency among variables and the lack of specific order in them, missing data were estimated using replacement methods, which may have affected the results. Another limitation was the use of only one of the various available methods in neural network and decision tree, so it was not possible to identify the most effective algorithm among these algorithms. However, the main strength of this study was using a real-world data set of patients consisting of a large number of records, which improves system training and is relatively better than other regional studies in this context.

Considering one of the main purposes of medical data analysis, which is to produce the best algorithm for data description, the results of analyses of data sets are unique based on the method applied in every study, so the results are only valid for that specific method. On the other hand, a more complete list of risk factors can provide a model with more extensive coverage. Moreover, results of the models may be affected by data preprocessing and missing data handling, and the method used for data evaluation. Researchers can use the results of the present study for future analyses of breast cancer risk factor data sets to generate models with higher efficacy and accuracy. It is suggested that future studies compare separate modeling results in decision tree with different numbers of iterations, investigate results with different neural network indices, and compare more algorithms-specially SVM, due to its promising results in medicine.

**Conflict of Interest**
The authors have none to declare.

**References**

**Background:** Physicians’ beliefs about disclosure manner and their ethical attitude for telling the truth is an important issue in patient-physician interaction. The aim of this study was to examine clinicians’ practice and perception of disclosure models for giving bad news to breast cancer patients.

**Methods:** Participants (n = 207, age 21–61 years, mean work experience = 4.03 ± 6 years) working in different medical centers in Tehran, Iran, were recruited by purposive sampling method. They completed clinicians’ attitude and practice of Breaking Bad News (BBN) scales. Psychometric properties (reliability and validity) of these scales were approved.

**Results:** Clinicians’ practice differed significantly by their perception of disclosure model for giving bad news. Furthermore, difference in clinicians’ practice and perception of disclosure model for BBN was observed for age, gender, medical work experience in oncology setting, and receiving special training. Finally, clinicians’ perception of disclosure model for BBN (Adj. R2 = 0.32), age (Adj. R2 = 0.17), gender (Adj. R2 = 0.11), and receiving special training for giving bad news (Adj. R2 = 0.09) positively predicted their practice of BBN.

**Conclusion:** Findings of the study point to the importance of the clinicians’ perception of disclosure model for giving bad news and transcultural variables as factors affecting their practice. Therefore, it seems necessary to incorporate special BBN trainings and protocols culturally adapted to the Iranian society in educational curricula of medical specialties and medical ethics in breast cancer setting.

**Introduction**

The recent Greek *New Code of Medical Ethics and Deontology* declared that according to the principle of patients’ autonomy, physicians should provide the patients with the appropriate information about their health status.¹ Based on the International Code of Medical Ethics, physicians must respect patient’s right to select different methods of treatment and inform patients about their decisions.²

Informing patients about the diagnosis of a serious health-threatening disease or failure in treatment is called a clinically bad news.³,⁴ In other words, “bad news” is any information that negatively impacts one’s expectations for the future.⁵ Breaking bad news (BBN) has been studied widely in oncology setting.⁶,⁷ Disclosing the diagnosis of cancer or its prognosis is a stressful task for doctors.⁸ Oncologists may have to break bad news to patients with an average of 20000 times over the course of their career.⁹
Breast cancer is the most prevalent cancer among women worldwide. Disclosure of breast cancer to the patients is of utmost importance and affects all aspects of the patients’ life as well as their decisions on different types of treatment. It can also affect the patient’s hope and trust in surgeon’s expertise, and have a considerable effect on the communication with the surgeon and the other physicians in the team. A scientific, stepwise disclosure method has a positive effect on patients’ quality of life, reducing patient’s stress and maintaining hope, increasing satisfaction with and confidence in the health care team, and improving patient cooperation in treatment process. Hence, clinicians’ attitude toward and practice of BBN is an essential part of patient care in breast cancer.

Clinicians have different attitudes toward disclosure of bad news. A model suggested by the World Health Organization (WHO) distinguishes three disclosure models regarding clinician-patient communication and decision-making style: full disclosure, where the physician tells all the truth to the patients, and clinical decision-making is considered the patient’s responsibility; non-disclosure, where the physician has a dominant role, and the patient will be dependent on the clinicians’ decision-making and play a passive role; and individual disclosure, where delivering of information will be done based on the patients’ preference.

Prior studies showed that physicians’ tendency to each disclosure model is under the influence of the following factors: institutional norms, previous specific training for BBN, preference of patients’ families, patients’ and clinicians’ characteristics, time considerations, organizational considerations, and clinicians’ work experience. Cultural and social norms are important factors influencing clinicians’ attitudes toward BBN, eg clinicians’ inclination to family-centered decision-making style in non-Western nations, vs. the higher tendency toward patients’ autonomy in Western countries. Thus, telling the truth is not a simple task; instead, it is a dynamic process that concerns not only the disclosure of the truth to patients, but also communication between clinician and the patients as well as their families according to social norms.

BBN is a complex practice which requires a variety of skills such as communication, understanding, and empathy. From this point of view, some studies offer various models and protocols that guide clinicians on how to disclose bad news to their patients. One of the most important guidelines is SPIKES, which is designed in six phases. In this acronym “S” stands for setting up the interview, “P” assessing the patient’s perception; “I” obtaining the patient’s invitation, “K” giving knowledge and information to the patient, “E” addressing the patient’s emotions with empathic responses, and “S” strategy and summary. ABCDE is another guideline for BBN five steps of which are as follows: Advance preparation, Build a therapeutic environment/relationship, Communicate well, Deal with the patient and family reactions, and Encourage and validate emotions.

Previous studies usually highlighted that “giving bad news” includes several steps: (a) the pre-delivery phase including preparation of the appropriate space and time, paying attention to patient’s cultural background, considering patient’s needs, assessing what patients know, and the amount of information he needs to know; (b) the delivery phase, which is dedicated to providing the patients with all necessary information and clarifying any misunderstanding; and (c) the post-delivery phase consisting of patient follow-up, addressing the next step, and closing the session.

The existing literature on the physicians’ perception and practice of BBN shows a global trend toward full disclosure of bad news to cancer patients.

However, as stated above, there are many other parameters affecting the truth-telling and physicians’ perception of disclosure method for BBN. The physicians’ personal characteristics are among the most important factors in this regard. In this context, attitude and belief will be among the best predictors for the future behavior when they are easy to recall and stable over time. Henderson et al. indicated that knowledge was not significantly related to nursing practice and that attitude moderated the relation between knowledge and practice; so attitudes and beliefs have direct effects on nursing practice. Beach et al. showed that physician’s attitude has an impact on healthcare quality and communication with patients. One study suggested that the physicians’ attitudes toward BBN might affect their behavior, although it has not been examined despite its implications. Apparently, another element that affects disclosure manner is a transcultural factor. Locatelli et al. observed that Italian physicians’ age and gender influenced telling bad news to the older cancer patients and managing emotional reactions. Special BBN training is another element that impacts physicians’ disclosure manner. Therefore, it is essential to consider beliefs and transcultural factors in analyzing physicians’ BBN approaches.

Considering the high prevalence of breast cancer in all countries, including Iran, there are insufficient studies on the clinicians’ attitudes toward, perception, and practice of disclosing bad news to breast cancer patients. The purpose of this study was to assess Iranian clinicians’ perception and
practice of telling bad news to breast cancer patients according to disclosure models. Besides, the influence of transcultural factors on the physicians’ perception and practice of disclosing bad news to breast cancer patients were evaluated.

Methods
A cross-sectional design was used to examine physicians’ practice of delivering bad news to breast cancer patients based on the perception of disclosure models (full-disclosure, non-disclosure, and individual disclosure) and two protocols for BBN, like SPIKES and ABCDE.

The sample of the present study included 207 Iranian clinicians (surgeons, hematology-oncology, radiologists, radiation oncologists, nurses, and midwives) working in medical centers of Tehran, particularly at wards treating breast cancer patients. They were recruited via purposive sampling. The sample size was calculated according to previous studies that showed the percentage of physicians who informed patients about their cancer diagnosis was about 45%, 40, 41 a confidence level of 95%, and margin of error of %0.675 (15% prevalence). From December 2015 until March 2016, all eligible participants were informed about the aim of the study, and those who were willing to participate were included. They had full cooperation in the survey and completed the questionnaire.

Ethical approval
The study was approved by the Research Ethics Committee of Tehran University of Medical Sciences.

Measurement tools
Attitude Toward Breaking Bad News scale
Physicians’ perception of disclosure model for BBN was measured by using the Attitude Toward Breaking Bad News scale developed by Borjalilu and colleagues. According to WHO disclosure model. The scale comprises three factors with 12 items rated on 5-point Likert-type scale ranging from 1 (never) to 5 (always). The subscales were shown to have acceptable internal consistency (Preparation: $\alpha = 0.765$, Setting of the interaction: $\alpha = 0.63$, Communication: $\alpha = 0.65$, Using the word “cancer”: $\alpha = 0.793$, Patient’s right to know: $\alpha = 0.759$, and Closing the interview and summarizing: $\alpha = 0.7$).

Data regarding physicians’ demographic and career characteristics were collected, which included the following: age, gender, specialty, medical work experience in the oncology setting, job title, frequency of giving bad news within the past three months, minimum and maximum time (minutes) spent on disclosing bad news to patients, the need for special BBN training, and the need for guidelines on how to deliver bad news.

Statistical analysis
We used Statistical Package for Social Sciences (SPSS) version 22 for data analysis. Descriptive statistics (mean, standard deviation, minimum, and maximum) were used to describe variables, and independent $t$ test was performed to compare the physicians’ perception and practice of BBN between genders. For comparison of physicians’ BBN practice based on their perceptions of BBN models and transcultural factors (age, work experience, and receiving special BBN training), the one-way analysis of variance (ANOVA) along with the Tukey post hoc test was used. Finally, linear regression analysis was used to examine the association of physicians’ practice with their perception of BBN and transcultural factors. Cronbach’s $\alpha$ was calculated to assess the reliability of physician’s attitude and practice scales for BBN to breast cancer patients. Level of statistical significance was set at 0.05.

Results
A total of 207 participants completed the questionnaires. There were 119 females (57%) and 88 males (43%) clinicians (age: 38.1 ± 9.1 years, range: 21–61) practicing in different fields (surgery: 52 (25%), radiation oncology: 38 (18.5%), nursing: 42 (20%), hematology-oncology: 31(15%), radiology: 30 (14.5%), and midwifery: 14 (7%).

Of 207 participants, 123 (59.56%) worked in educational hospitals (faculty members = 51 (24.5%), clinical fellowship members = 19 (9.1%), specialists = 14 (6.7%), and residents = 39 (18.8%)), and 84 (40.44%) were employed in general hospitals. The average work experience of physicians in the field of oncology was 4.03 ± 6 years.

Fifty-eight percent of participants had disclosed bad news to less than 5 breast cancer patients within the past three months. The minimum and maximum amount of time participants would spend on giving bad news to patients was 5 and 15 minutes. The results showed that only 24 of the clinicians (11.5%)
The mean scores for disclosure models were 21±2 (full disclosure), 6±1 (individual disclosure), and 15±4 (non-disclosure). Descriptive analysis of the study variables is shown separately for physicians’ practice and three disclosure models in Table 2.

Physicians’ practice and perception of disclosure model for BBN

The mean scores for disclosure models were 21±2 (full disclosure), 6±1 (individual disclosure), and 15±4 (non-disclosure). Descriptive analysis of the study variables is shown separately for physicians’ practice and three disclosure models in Table 2.

Difference in clinicians’ practice by their perception of disclosure model for BBN

For the comparison of groups, we checked the assumptions (normality and homogeneity) of one-way ANOVA and independent t tests. The results indicated that the assumption was met. Afterwards, one-way ANOVA was done to compare the clinicians’ practice of BBN to breast cancer patients by their perception of disclosure models (full disclosure, non-disclosure and individual disclosure) (Table 3).

As revealed by ANOVA analysis, there was a significant difference in total mean scores of clinicians’ practice for the three perception of BBN disclosure models \([F (4, 202)= 7.0391, P= 0.005]\). Post hoc analysis showed that clinicians with a full disclosure attitude had significantly higher scores compared with clinicians with other two attitudes. In subscale comparisons, there was a significant effect of disclosure attitude on clinicians’ using the word “cancer” \([F (4, 202)= 9.745, P= 0.001]\). Post hoc comparison showed that clinicians with a full disclosure attitude towards BBN tended to use the word “cancer” more frequently than those with other two attitudes. Also, there was a significant effect of disclosure attitude on “Patients’ right to know” subscale for the three attitudes \([F (4, 202) = 8.026, \ldots\]
Post hoc analysis revealed that clinicians with the full disclosure attitude scored significantly higher than those with other two attitudes, implying that they respected the patients' right to be informed about their medical conditions more than did their colleagues with other two attitudes. Finally, there was no significant difference in the mean scores for preparation \[F (4, 202) = 4.356, P= 0.09\], setting the interaction \[F (4, 202) = 7, P= 0.087\], communication \[F (4, 202) = 7.065, P= 0.325\], and closing the interview and summarizing \[F (4, 202) = 6.128, P= 0.531\] for the three disclosure attitudes.

The comparison of the mean scores for the different disclosure models by age demonstrated a significant effect of age on non-disclosure attitude \[F (3, 203)= 3.26, P= 0.002\]. Post hoc analysis showed that clinicians under 30 years of age had significantly higher scores (13.01± 4.14) on non-disclosure attitude compared with that of other two groups. Similarly, a significant effect of age was observed for the clinicians’ practice scores \[F (3, 203)= 1.12, P= 0.001\] and closing the interview and summarizing \[F (4, 202) = 6.128, P= 0.531\] for the three disclosure attitudes.

**Differences in clinicians’ perception of disclosure model for BBN and practice by age**

The comparison of the mean scores for the different disclosure models by age demonstrated a significant effect of age on non-disclosure attitude \[F (3, 203)= 3.26, P= 0.002\]. Post hoc analysis showed that clinicians under 30 years of age had significantly higher scores (13.01± 4.14) on non-disclosure attitude compared with that of other two groups. Similarly, a significant effect of age was observed for the clinicians’ practice scores \[F (3, 203)= 8.71, P= 0.001\]. Post hoc analysis indicated that the mean score for practice of BBN in 30–50-year age group was significantly higher (79± 7.41) than that of other age groups.

**The effect of clinicians’ gender on their perception of disclosure model for BBN and practice**

The results of this study showed significant gender difference in the scores for non-disclosure attitude \(t= 2.169, P= 0.03\). Female clinicians had higher mean score for the non-disclosure attitude compared with the males (15± 4.1). Also, there was a significant gender difference in the total mean score for the clinicians’ practice \(t = 2.11, P= 0.036\), with females having higher mean score compared with males (79.1± 6.1).

**Table 2. Descriptive analysis of variables**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perception of Disclosure model</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full disclosure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-disclosure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Individual disclosure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscale and total scores for Practice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preparation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Setting of the interaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Communication</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Using the “cancer” word</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients’ right to know</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Closing the interview and summarizing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total score for practice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Difference in clinicians’ practice by perception of disclosure model for BBN**

<table>
<thead>
<tr>
<th>Practice</th>
<th>Full disclosure Mean (SD)</th>
<th>Non-disclosure Mean (SD)</th>
<th>Individual disclosure Mean (SD)</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation</td>
<td>18 (2)</td>
<td>15 (3)</td>
<td>16 (2)</td>
<td>4.35</td>
<td>0.09</td>
</tr>
<tr>
<td>Setting of the interaction</td>
<td>13 (1)</td>
<td>10 (2)</td>
<td>14 (2)</td>
<td>7</td>
<td>0.087</td>
</tr>
<tr>
<td>Communication</td>
<td>16 (1)</td>
<td>14 (1)</td>
<td>15 (1)</td>
<td>7.06</td>
<td>0.325</td>
</tr>
<tr>
<td>Using the “cancer” word</td>
<td>9 (1)</td>
<td>7 (2)</td>
<td>7 (1)</td>
<td>9.74</td>
<td>0.001</td>
</tr>
<tr>
<td>Patient’s right to know</td>
<td>7 (2)</td>
<td>6 (1)</td>
<td>7 (1)</td>
<td>8.02</td>
<td>0.002</td>
</tr>
<tr>
<td>Closing the interview and summarizing</td>
<td>22 (2)</td>
<td>20.03 (3)</td>
<td>21 (3)</td>
<td>6.12</td>
<td>0.531</td>
</tr>
<tr>
<td>Total score for practice</td>
<td>87 (6)</td>
<td>75 (9)</td>
<td>83 (7)</td>
<td>7.03</td>
<td>0.005</td>
</tr>
</tbody>
</table>

P= 0.002. Post hoc analysis revealed that clinicians with the full disclosure attitude scored significantly higher than those with other two attitudes, implying that they respected the patients’ right to be informed about their medical conditions more than did their colleagues with other two attitudes. Finally, there was no significant difference in the mean scores for preparation \[F (4, 202)= 4.356, P= 0.09\], setting the interaction \[F (4, 202)= 7, P= 0.087\], communication \[F (4, 202) = 7.065, P= 0.325\], and closing the interview and summarizing \[F (4, 202) = 6.128, P= 0.531\] for the three disclosure attitudes.

**Differences in clinicians’ perception of disclosure model for BBN and practice by work experience**

There was a significant effect of job experience on perception of disclosure models for non-disclosure \[F (3, 203)= 2.045, P= 0.001\] and individual disclosure attitudes \[F (3, 203)= 1.12, P= 0.002\]. Post hoc analyses indicated that the mean score for non-disclosure attitude was significantly higher in the group with under 10 years of work experience (14± 3) compared with that of other two groups, and the mean score for individual disclosure for the group with 10–20 years of work experience (6.41± 0.1) was significantly higher than those for the other groups. Finally, there was no significant difference in the total mean score for the clinicians’ practice of BBN for the three work experience groups.

**Differences in clinicians’ perception of disclosure model for BBN and practice by receiving special training**

There was a significant difference in full-disclosure attitude scores by “receiving special BBN training” \[F (3, 203)= 2.09, P= 0.001\]. Post hoc analysis indicated that the mean score for full-disclosure attitude was significantly higher for the group who had received training (22 ± 2.1) compared with the other two groups. Receiving BBN training
also had significant effect on non-disclosure attitude [F (3, 203)= 1.01, P= 0.001]. Post hoc analysis indicated that the mean score for non-disclosure was significantly higher for the group with training (15± 3.04) compared with the other two groups. The scores for individual disclosure attitude were also significantly different for different BBN training groups [F (3, 203)= 0.89, P= 0.001], with the mean score for individual-disclosure attitude for the group with BBN training being significantly higher (6.05± 1.25) than that for the other groups. Finally, there was a significant difference in the total mean scores for clinicians’ BBN practice by special BBN training [F (3, 203)= 2.028, P= 0.001], and post hoc analysis indicated that the mean overall BBN practice score for the group with BBN training (81± 7.1) was significantly higher than that for the groups who had not received any training or had received little training.

Prediction of clinicians’ practice from their perception of disclosure model for BBN and transcultural factors

The regression model for prediction of clinicians’ practice from their perception of disclosure model for BBN was significant [F (3, 203)= 17.11, P= 0.001] and was able to explain 32% of the variance in clinicians’ practice. Also, the regression models for prediction of clinicians’ practice from their age [F (3, 203)= 15.81, P= 0.001], gender [F (3, 203)= 4.45, P= 0.001], and receiving BBN training [F (3, 203)= 7.15, P= 0.001] were significant and explained 17%, 11%, 9% of the variance in clinicians’ practice, respectively. However, medical experience in the field of oncology was not a significant predictor of clinicians’ practice.

Discussion

The aim of the study was to determine clinicians’ perception of disclosure models and practice of delivering bad news to breast cancer patients in Iran and to explore differences in practice according to perception of disclosure models.

Our results showed that the majority of clinicians who participated in this study used full-disclosure and individual disclosure models for BBN. From the total mean scores for practice and their subscales, which mostly fall above the minimum scores, it seems that clinicians had acceptable practice for giving bad news to breast cancer patients. Previous studies support this finding. It seems that clinicians have a tendency toward full disclosure of the bad news to cancer patients in Iran, like Western countries. We found that a large majority of clinicians (91%) believed that “most of the patients want to know the truth about their illness” or “disclosure of the diagnosis of breast cancer to patients is necessary” (87%). According to prior studies, most clinicians (90%) believed that disclosure of bad news would enable patients to cooperate in the treatment process and decrease confusion and ambiguity for the patients and their families (81%). Regarding disclosure of breast cancer, clinicians reported that they spent a minimum of 5 minutes and a maximum of 15 minutes per case. It may be due to organization barriers (lack of enough time and private place), engagement in different activities (clinical education and research activities in the educational hospital), and/or individual barriers (insufficient skills or knowledge and lacking a sense of responsibility). Thus, it is highly recommended that these potential barriers to delivering bad news to patients be explored through further research.

In our study, a significant difference was observed for clinicians’ BBN practice by their perception of disclosure models for BBN (P=0.001), and their perception of disclosure models explained 32% of the variance in their practice. Post hoc analysis showed that clinicians with attitude toward full disclosure reported better practice for BBN to breast cancer patients than clinicians with different disclosure models (P= 0.001). As mentioned, attitude directly affects health care practice, as shown in previous research. It seems that attitude is the most effective element in the practice of the clinicians who participated in the study. Clinicians who preferred full disclosure model for BBN had better disclosure manners and made appropriate arrangements for this task. But other clinicians with tendency for non-disclosure and individual disclosure models had lower scores on the professional practice of BBN scale, probably due to their beliefs and attitudes. Regarding the relationship between attitude and behavior, research indicates that attitude accessibility (easy to recall) and stability (stable information) are the main factors affecting attitude retrieval and reconstruction. Therefore, it is important to work on clinicians’ attitude in medical schools and educational hospitals. Also, one of the significant differences in clinicians’ practice was related to using the word “cancer” (P< 0.05). Post hoc analysis showed that clinicians with tendency toward full disclosure model used “cancer” more frequently than those with tendency for other models (P< 0.05). We also found that the majority of clinicians (77%) did not agree that the use of the word “cancer” would lead to panic in patients, which is consistent with the protocols for BBN. This finding is similar to the reports on the physicians’ practice in countries like Australia and Canada where truth disclosure is an established routine. However, in Asia and the Middle East, physicians, patients and their families usually try to avoid using the word “cancer,” because in these cultures “cancer” is equivalent to death or incurable illnesses; therefore it produces such negative emotions as fear, stress, and helplessness in cancer patients. Hence,
future research is necessary to determine the preference of Iranian women with breast cancer and their families regarding the use of the term “cancer,” or other words such as “tumor” or “mass,” when the diagnosis is disclosed to them.

Our results showed a significant difference in clinicians’ practice of respecting “patients’ right to know” (P< 0.05). Post hoc analysis showed that physicians with tendency for full disclosure model believed that patients had the right to be informed about their medical conditions and breast cancer (P< 0.05). Respecting patients’ right to be informed of their conditions, which is asserted in medical ethics, has also been observed in previous studies. However, research has demonstrated that in some Asian countries, such as China and Japan, as well as some European countries, for example Spain and Italy, the patients’ families tend to ask clinicians to refrain from disclosing the truth about diagnosis or prognosis to patients. Accordingly, it is necessary to develop an appropriate strategy according to breast cancer patients’ right to know and their families’ preferences for disclosure of bad news.

In this study, we observed a significant difference between clinicians’ perception of disclosure model and practice of BBN by age and medical experience in oncology setting (P< 0.05). Post hoc analyses showed that younger clinicians preferred to use non-disclosure model for delivering bad news (P< 0.005). Locatelli et al. and Baile also reported similar findings. It may be because they lack the skills to deal with the difficult situation of telling the truth and they cannot manage the process of BBN. We also found that the majority of older clinicians (30–50 year age group) with 10–20 years of work experience preferred individual disclosure model and that their practice of giving bad news was better than those of the younger ones (P< 0.005). There are several explanations for this finding. Studies have shown that some breast cancer patients do not want to hear everything from the surgeons and would prefer information that gave them hope and maintained a personal clinical relationship with the surgeon. Azu showed that breast cancer patients in the United States believed that physicians were supposed to know about the appropriate amount of information that should be delivered to each patient. Also, Lobb et al. reported that half of the women preferred hearing positive information, e.g. chance of cure. For Iranian women with breast cancer, creating hope and building trust are the preferences for clinicians’ manner of disclosure. Against this background, other studies indicated that women undergoing screening mammography wanted truthful results of imaging from radiologists and agreed that the radiologist should communicate the results directly to them. As the evidence is not conclusive toward one specific approach, it may be reasonable that physicians with longer work experience in cancer treatment practice the individual disclosure model for giving bad news. It is likely that they would be aware of the patients’ needs and socio-demographic status due to their extensive experience and would select the appropriate information and frame it to be disclosed to their patients. In our survey, middle-aged clinicians with enough work experience and appropriate skills reported better practice of BBN. Moreover, regression analysis showed that age explained 17% of the variance in clinicians’ practice, but work experience (in years) did not prove to be a significant predictor; so clinicians’ age is an important factors in their practice of BBN.

The results of this study confirmed that clinicians’ gender affected their perception of disclosure model for BBN and practice for informing breast cancer patients, explaining 11% of the variance in the clinicians’ practice. In the disclosure model we observed a significant gender difference for non-disclosure model (P< 0.05), with the females having higher mean score for the non-disclosure than the males. Further, there was a significant gender difference in the total mean score for the clinicians’ practice (P< 0.05), and the female clinicians had better practice for giving bad news to breast cancer patients than males physicians. Our results are consistent with other studies about doctor-patient relationship. Compared with male physicians, female clinicians tend to spend more time with each patient, display better communication skills, and be more effective in triggering positive emotions in their patients. They usually disclose more information to patients than their male counterparts do, and their communication style is more commonly a patient-centered approach. Furthermore, male clinicians tend to favor a direct manner of giving information to patients, compared with indirect approaches preferred by female clinicians. It is plausible that female clinicians are better able to avoid disclosure dilemma (attention to patients’ autonomy vs paternalism) or deal with negative emotions of breast cancer and lack of skills for managing this situation. For an in-depth description, we suggest that male and female physicians’ experiences regarding delivering bad news to breast cancer patients be explored in future studies.

The result of our survey showed that there was a significant difference in perception of disclosure models and practice of delivering bad news between clinicians who had received special BBN training and those who had not received training (P< 0.005), and that receiving special training for BBN explained 9% of the variance the clinicians’ practice. Post hoc analysis showed that participants with special BBN training had higher mean scores for the full-disclosure and individual disclosure, and those who had not received training had higher mean scores for the non-disclosure (P< 0.005). Physicians who had received special BBN training had better practice...

than the group without any training (P< 0.005). Novack et al reported that medical school and hospital training impact clinicians’ attitude toward truth-telling in favor of full disclosure of cancer diagnosis to patients.  Prior research has indicated that communication skills training can improve clinicians’ interviewing skills and affect their attitude. Also, BBN training courses can improve communication skills, increase self-efficacy for giving bad news, elicit appropriate levels of physiological arousal, and promote competence for patient care. Results from our study reflect an essential role of BBN training. However, we observed a lack of sufficient training among the clinicians who participated in this study. Only 11.5% of the clinicians reported that they had received specific training in BBN, similar to other studies in this regard. Therefore, the lack of training is one of the reasons that giving bad news to breast cancer patients is a difficult task for physicians.

It seems that lack of a context-based protocol for delivering bad news is a barrier to clinicians’ practice. A considerable majority of the clinicians in our survey (82%) expressed the need for appropriate culturally-adjusted guideline regarding giving bad news to breast cancer patients in Iran. Therefore, it is necessary to design a BBN protocol compatible with Iranian breast cancer patients and their family member preference, considering both clinicians’ attitude regarding disclosing bad news and the culture of Iranian society. For example, study shows that resorting to religious and spiritual sources can help some patients to better cope with their cancer situation, particularly in an Islamic country. In that study, 86% of clinicians endorsed that after informing breast cancer patients, they reminded patient as they believed that everything was in the hands of God, and he is the healer. Hence, it seems necessary to develop a guideline for delivering bad news according to Iranian cultural context.

We have attempted to describe clinicians’ preferences for and practice of BBN in breast cancer setting and discuss the main factors affecting this process. But our study has some limitations. First, generalization of the results must be done with caution due to the cross-sectional nature of the surveys. Second, the results of this study were produced by self-report measurement method. We recommend that future studies use observational method for assessing clinicians’ practice.

Despite these limitations, our results highlighted some evidence that would be useful regarding its practical implication. It emphasized the need for special BBN training to change the clinicians’ attitude toward truth disclosure and improve their BBN skills. Our survey indicated that physicians show interest in training courses; therefore, the training programs should be designed according to the needs of patients and physicians with respect to Iranian socio-culture environment. Furthermore, developing a context-based protocol seems essential.

Moreover, the results of this study have implications for designing curriculum in medical education and ethics so that appropriate protocols according to Iranian culture based on clinicians’ preferences and practice can be developed. To achieve this, however, we need to know about Iranian women’s as well as their families’ preferences.

Conflict of Interest
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Breaking bad news to breast cancer patients


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The Relationship Between Breast Cancer and VDR Gene Polymorphisms

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ABSTRACT

Background: Vitamin D serves several cancer protective roles within the human body. Vitamin D functions through binding with the VDR encoded by VDR gene. It has been demonstrated that polymorphism in VDR gene would influence expression and/or function of the VDR protein. The researchers found that the most important VDR gene polymorphisms that are associated with tumorigenesis include Fok1 (rs2228570), Bsm1 (rs1544410), Taq1 (rs771236), and Apa1 (rs7975232). The purpose of this study was to assess the association between Fok1, Bsm1, and Taq1 polymorphisms and breast cancer development.

Methods: In this study, 50 patients suffering from breast cancer with less than 6 months after the diagnosis of breast cancer and 50 healthy control individuals were included. Restriction fragment length polymorphism PCR (RFLP-PCR) was used to determine the genotype of polymorphisms.

Results: Statistical results showed that among the studied polymorphisms, Tt genotypes of Taq1 polymorphism have correlations with breast cancer development (P<0.001, OR=5.51, 0.95 CI=2.30-13.21).

Conclusion: The results of the present clearly demonstrated that there is a relationship between Taq1 polymorphism in VDR gene and development of breast cancer.

Introduction

Breast cancer is the most common cancer among women worldwide and, after lung cancer, it is the second main cause of death among them. There are various risk factors in breast cancer including age, gender, benign tumors of the breast, early menopause, late menarche, hormone therapy, chest exposure to radiation, alcohol consumption, combined use of estrogen and progesterone, diethylstilbestrol consumption, genetic factors, postmenopausal obesity, first pregnancy after the age of 30, lack of breastfeeding, and environmental factors. Genetic factors in breast cancer include changing the level of gene expression, epigenetic modifications, and polymorphisms (DNA Sequence alterations).

The role of vitamin D among the various environmental factors that play a key role in cancer progression is noticeable. Vitamin D is available in 2 forms: vitamin D2 in plants and Vitamin D3 in the human skin. Vitamin D in body comes from sun exposure (up to 90%) and food supplements. Several enzymatic steps and genes are involved in vitamin D metabolism. The first occurs in the liver and the second in the kidneys, which construct the most common biologically active form.
important role of vitamin D is the regulation of bone metabolism. Also, it has a protective function on cancer through the regulation of gene expression, the reduction of invasiveness and angiogenesis, proliferation, differentiation, and the apoptosis of several cancer cell lines.\textsuperscript{10} This process occurs through binding vitamin D to its receptor. Vitamin D receptor (VDR) is expressed in more than 30 human tissues.\textsuperscript{10}

The VDR gene is on the long arm of chromosome 12 with at least 5 promoters and 11 exons.\textsuperscript{11} Previous studies have demonstrated the impact of polymorphisms in VDR gene on the expression and/or function of the VD protein.\textsuperscript{12} Among 200 polymorphisms described in VDR, Fok1, Bsm1, Taq1, Apa1, EcoRV, and Cdx2 are more frequently associated with tumorigenesis, although data are yet controversial in this filed.\textsuperscript{11} Epidemiological studies and laboratory investigations have proposed the increased cancer risk would be related with the level of vitamin D and its expressed receptor (VDR).\textsuperscript{12}

**Polymorphism Fok1**

This polymorphism is located in exon 2, next to the 5'UTR region of VDR gene and causes the transition of C to T at this position. The Fok1 polymorphism has 2 alleles; the shorter VDR (C allele) has higher transcription activity compared to the longer type (T allele).\textsuperscript{12} Despite no significant difference in DNA binding, ligand affinity, and transactivation affinity between 2 VDR forms, the shortened VDR variant showed higher potency than the longer one.

**Polymorphism Bsm1**

Polymorphism Bsm1 is in intron 8, where guanosine converts to adenosine and it may be associated with poly-A sequence in 3'-UTR region; hence, it may affect VDR gene expression via the regulation of mRNA stability.\textsuperscript{13}

**Polymorphism Taq1**

This polymorphism is located in exon 9 and, instead of T nucleotide, has a C nucleotide. The polymorphisms in the 3'-UTR region of the gene are associated with linkage disequilibrium (LD) and allele frequencies of these polymorphisms seem to differ within populations.\textsuperscript{13}

In this study, we have concentrated on the distribution of VDR Fok1, Bsm1, and Taq1 polymorphisms in patients with breast cancer in Isfahan, compared with a healthy population.

**Methods**

**Study population**

The present study includes case and control groups. In this study, 50 patients suffering from breast cancer with less that 6 months after the diagnosis of breast cancer were referred to two breast cancer centers in Isfahan, Iran. Also, 50 healthy control individuals were included. Healthy women without breast cancer were randomly selected among the women who, visited Alzahra hospital for routine mother and child healthcare. The study was approved by the Ethics Committee of Iran National Science Foundation and the questionnaire and informed consent forms were completed by the study subjects. Demographic data such as age, familial, or sporadic status and types of breast tumors were obtained (Table 1).

**DNA extraction, SNP selection, and genotyping**

In this survey, 3-5cc peripheral blood was taken from participants. Genomic DNA was extracted from samples by the salting-out method. The quality and concentration of extracted DNA were measured, using NanoDrop® ND-1000 spectrophotometer at 260nm and 280nm wavelengths. Specific PCR primers were designed through BLAST website (http:// blast.ncbi.nlm.nih.gov/Blast.cgi) and single nucleotide polymorphisms (SNPs) database (dbSNP 129; http:// www.ncbi.nlm.nih.gov/projects/SNP/) and Genomic DNA was amplified by PCR protocol as follow (Table 2).

We focused on 3 well-characterized polymorphisms. PCR (RFLP) was used to determine the genotype of polymorphisms. PCR-RFLP analysis was performed in 10μL of reaction volume, containing 1x PCR buffer (75 mM Tris-HCl, pH8.8, 20 mM (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, 0.01% (v/v) Tween 20), 2 mM MgCl\textsubscript{2}, 0.2 mM dNTP, 0.2 mM of each primer (Table 2), 40 ng of DNA, and 0.3 U of Taq DNA polymerase. The touchdown PCR program was performed for Fok1 (cycle: 30 s, initial denaturation:95 °C for 4 min, Denaturation: 95 °C for 1 min, Anneling: 70 °C for 1 min), Bsm1 (cycle: 30s, initial denaturation: 94 °C for 4 min, Denaturation: 94 °C for 1 min, Anneling: 66 °C for 30 min), and Taq1 (cycle: 30 s, initial denaturation: 95 °C for 4 min, Denaturation: 95 °C for 1 min, Anneling: 65 °C for 1 min, and 72 °C for 5 min for each primer [final extension]).

The PCR products (5 μL) of VDR gene were digested in a 20 μL reaction volume for 7h with 1.5 U of Fok1, Bsm1, and Taq1 restriction endonucleases (Fermentas, St. Leon-Rot, Germany) at 37 °C. The digested PCR product was separated, using electrophoresis on 3% agarose gel by ethidium bromide and it was analyzed, using UV light after staining.

The product alleles of the VDR gene at the restriction enzyme site Fok1, Bsm1, and Taq1 involved a T→C (Fok1), a C→T (Bsm1), and a C→T (Taq1) transition. The PCR products were digested with appropriate restriction endonuclease per manufacturer’s instruction (Macrogen, South Korea). Digested products of the product alleles of cutting by Fok1, Bsm1, and Taq1 enzymes were indicated by f, b, and t, while undigested alleles were...
To investigate the VDR gene polymorphisms, we randomly selected 50 patients with breast cancer, with the mean age of 47.18 ± 14.36 years and 50 healthy subjects, with the mean age of 43.70 ± 14.70 years. Independent t test showed that the average age assigned as F, B, and T, respectively. In order to confirm the RFLP results, randomly selected PCR products were sequenced.

### Statistical analysis

The data were analyzed by SPSS (Version.18), using Chi-square, independent sample t test, and logistic regression with 95% confidence intervals (CIs). Chi-square test and independent t test were performed. P value less than 0.05 was considered statistically significant.

### Results

To investigate the VDR gene polymorphisms, we randomly selected 50 patients with breast cancer, with the mean age of 47.18 ± 14.36 years and 50 healthy subjects, with the mean age of 43.70 ± 14.70 years. Independent t test showed that the average age of the two groups did not have any difference (P = 0.23). Moreover, Chi-square test did not show any differences between the two groups regarding menopausal status (P=0.17).

The histopathology reports of the patients' tumors showed 62% Positive and 38% Negative estrogen receptor expression. Progesterone receptor (PR) expression was positive in 62% of patients. The Her-2 over expression was detected in 30% of the breast tumors.

The frequencies of each allele for both groups are shown in Table 3.

### Table 3. Fok1, Bsm1, and Taq1 polymorphisms and breast cancer

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Breast cancer (N=50)</th>
<th>Control (N= 50)</th>
<th>P value</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative Frequency (%)</td>
<td>Relative Frequency (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fok1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>34(68)</td>
<td>F 0.94</td>
<td>0.14</td>
<td>0.67(0.11-4.17)</td>
</tr>
<tr>
<td>Ff</td>
<td>13(26)</td>
<td>f 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ff</td>
<td>3(6)</td>
<td>f 0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bsm1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB</td>
<td>12(24)</td>
<td>B 0.66</td>
<td>0.50</td>
<td>0.91(0.40-2.10)</td>
</tr>
<tr>
<td>Bb</td>
<td>21(42)</td>
<td>b 0.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bb</td>
<td>17(34)</td>
<td>b 0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Taq1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>2(4)</td>
<td>T 0.74</td>
<td>&lt;0.001</td>
<td>5.51(2.30-13.21)</td>
</tr>
<tr>
<td>Tt</td>
<td>35(70)</td>
<td>t 0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tt</td>
<td>31(62)</td>
<td>t 0.66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Breast cancer is a common cancer with major public health implications. In 1919, vitamin D was originally discovered by Edward Mellanby and in 1969, the vitamin D receptor (VDR) was discovered by Norman. Recent studies demonstrate that not only vitamin D has the main function in bones, but also it significantly affects differentiation and cell proliferation. The metabolite of vitamin D, 1,25-dihydroxycholecalciferol (1,25[OH]2D3) can suppress cell proliferation in cancer cells. One study suggested that vitamin D effects, for the most part, are correlated with nuclear VDR.

Many human tissues express VDR like breast, prostate, bone, etc. In recent years, many molecular epidemiological studies (case-control studies and nested case-control studies) were conducted on women to investigate the associations of different VDR polymorphisms with breast cancer. The association of all mentioned polymorphisms (Fok1, Bsm1, and Taq1) and breast cancer were investigated in different studies. Taq1 was associated with breast cancer.

In a study, Abbas et al. showed a significant association between the Taq1 polymorphism and increased breast cancer risk in estrogen receptor positive patients. They emphasized concerning the strong linkage disequilibrium of 3 polymorphisms; the combinations of 3 variants may be more discriminating as risk factors than a single one. Perna et al. demonstrated a significant prognostic value of Taq1 in patients with breast cancer without any risk for women both heterozygous and homozygous for t allele. In a Swedish study in the field of breast cancer, a trend was found towards a higher survival rate, especially among those tamoxifen-treated estrogen receptor positive patients and homozygous for the rare Taq1 allele. However, Perna et al. reported increased death rate in rare homozygous carriers Taq1 homozygous genotype compared with homozygous carriers with the common allele. This study confirms our findings. In the present study, the statistical results showed that among the studied polymorphisms, Tt genotypes of Taq1 polymorphism correlate with breast cancer (P<0.001, OR=5.51, 0.95 CI= 2.30-13.21). In the mentioned polymorphism, Fok1 is the one that has most frequently been studied regarding its association with different types of cancer.

Several lines of studies have reported a significant association between the Fok1 polymorphism and different types of cancer including multiple myeloma, breast, prostate, and ovarian cancer, while others did not observe any significant associations. For example, Sinotte et al., Gapska et al., and McKay et al. reported increased risk among ff carriers on Fok1, whereas, Anderson et al. reported decreased risk among ff carriers. Curran et al., Guy et al., Abbas et al., Engel et al., Rollison et al., Fuhrman et al., Mishra et al., and Shahbazi et al. reported no association between ff carriers and breast cancer risk. As the last polymorphism, Guy et al. reported an increased risk of breast cancer for bb genotype. Another study reported Bsm1 polymorphism in LD with the poly-A tail sequence in the 3` UTR with a higher risk of breast cancer and bb genotype. Ruggiero et al. showed no statistically different distribution of Bsm1 polymorphism in case and control groups, while two-fold higher prevalence of the bb genotype was found in metastatic cancer group and percentage of BB women with metastases was half in control group. On the other hand, no significant association between the Bsm1 polymorphism and breast cancer risk made a different study, but we should note that this study included only a limited number of Turkish cases. Three other studies referring to Taiwanese women and Caucasian women were associated with BB genotype with increased breast cancer risk. Hou et al. showed an association between Bsm1 B allele and increased breast cancer risk. As the results of studies in various cancers are inconsistent, the role of vitamin D in the development and progression of cancer is still unknown and further studies are required. In conclusion, as the result of the present study showed, VDR Taq1 RFLP seems to be associated with breast cancer. T allele could be considered as risk allele and t allele as the protective allele. On the other hand, we found a higher prevalence of Tt genotype among patients with breast cancer in comparison with health controls. The VDR may represent an important target for breast cancer prevention.

Conflict of Interest
The authors declare that they have no conflict of interests.

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The authors would like to thank Mahdieh Behnam, Majid Hosseinzadeh for their sincere support.

References


Neoadjuvant Metformin Added to Systemic Therapy Increases Pathological Complete Response in Breast Cancer: A Cross-sectional Study, Mexico Hospital, Costa Rica

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ABSTRACT

Background: Metformin shows anti-proliferative effect on tumor cells. We studied the effect of metformin on achieving complete pathological response (pCR) in breast cancer patients receiving neoadjuvant therapy in a Latin American population.

Methods: We conducted a cross-sectional study in Mexico Hospital, Costa Rica, from January 2007 to December 2015. Women with early-stage or locally advanced breast cancer receiving neoadjuvant systemic treatment were recruited for the study. Univariate and multivariate models were used to compare the pCR rate with metformin plus standard therapy versus standard treatment alone.

Results: Of 53 included women with early-stage or locally advanced breast cancer were included, 14 received metformin with systemic therapy, and 39 had systemic therapy alone. Only 15% of the patients had diabetes mellitus. The pCR rate was in the metformin group was 64.3% compared with 23.1% in the systemic therapy-alone group (OR: 6.0, 95% CI: 1.60–22.53, P= 0.008). This finding was confirmed after adjustment for potential confounders, suggesting that the use of metformin increased the pCR likelihood regardless of breast cancer subtype (adjusted OR: 5.56, 95% CI: 1.27–24.3, P = 0.02). There was a trend of achieving pCR in patients with Ki-67 > 55%. However, it did not reach statistical significance when metformin was added, suggesting that probably a high Ki-67 level in the presence of metformin is not a predictor factor of pCR.

Conclusion: This is the first study conducted in a Latin American population showing that metformin with systemic therapy increases pCR regardless of the intrinsic molecular subtype or Ki-67 levels. These findings encourage prospective studies to evaluate the role of neoadjuvant metformin in this population.

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Keywords: Breast cancer, Latin America, metformin, neoadjuvant therapy

Introduction

According to the World Health Organization, breast cancer (BC) is one of the leading causes of morbidity and mortality worldwide with approximately 14 million new cases in 2012.¹ In 2015, Costa Rica reported a BC incidence rate of 42.25 per 100000 women.²

BC is considered not a single disease, but a group of different entities with diverse pathological features, clinical implications, and outcomes.³ Estrogen and progesterone receptors as well as HER2 expression (using immunohistochemistry), in conjunction with clinicopathological characteristics such as tumor grade, tumor size, and nodal involvement, are useful for establishing the management and prognosis of these patients.³ Gene expression microarrays in BC have led to identifying of at least five molecular subtypes, labeled as luminal A, luminal B, HER2+, basal-like, and...
normal breast-like. These BC subtypes have been associated with significant differences in overall survival as well as response to systemic therapy.

A recent meta-analysis showed that patients with BC who attain a pathological complete response (pCR) in the breast and axilla after neoadjuvant systemic treatment might have a better survival rate, mainly in aggressive tumors such as HER2 or triple-negative (TN) subtypes. Following this finding, the use of presurgical chemotherapy became very common, and biomarkers such as HER2, and hormone receptor (HR) status have been frequently used to indicate the implementation of this therapeutic approach. Furthermore, other markers have been explored to predict pCR, including Ki-67, which is a non-histone nuclear cortex protein expressed in the cell nucleus during the G1, S, G2, and M phases of the cell cycle. High Ki-67 levels have been associated with an increased chance of achieving pCR and therefore improved survival.

It has been proposed that hyperinsulinemia, insulin resistance as well as obesity influence breast cancer prognosis. Various mechanisms have been hypothesized, including increased circulating plasma levels of estrogen, insulin, insulin-like growth factor, and other hormonal factors that act to promote tumor growth and increase the risk of metastatic disease.

Metformin is an oral biguanide used for treatment of type 2 diabetes that inhibits hepatic gluconeogenesis and increases insulin sensitivity at peripheral level, thereby improving the glycemic control. Taking into account these pharmacological properties and the increasing evidence for the antitumor effect of metformin, we decided to conduct a cross-sectional study to evaluate the impact of metformin on achieving pCR in breast cancer patients who received neoadjuvant chemotherapy in a Central American population. To our knowledge, this is the first study conducted in this region.

Methods

Study population

After receiving approval from the institutional ethics committee, we conducted a cross-sectional study from January 2007 to December 2015 in Mexico Hospital, San Jose, Costa Rica, to evaluate the pCR rate in patients with invasive breast cancer receiving neoadjuvant systemic therapy with or without metformin. We included all women with early-stage or locally advanced, biopsy-proven invasive breast cancer who received systemic treatment neoadjuvantly. The following exclusion criteria were used: metformin use in a different proposed dosage (< 1 g/day), pregnancy, unknown HR or HER2 status, ECOG > 2, intolerance or hypersensitivity to metformin, not giving informed consent, having secondary or concurrent malignancies except for controlled non-melanoma skin cancer or cervical carcinoma in situ.

Treatment and pathology assessments

Patients included were previously evaluated in the multidisciplinary tumor board. Overall, all patients received preoperative systemic therapy based on anthracyclines (epirubicin) and cyclophosphamide with or without 5-fluorouracil, followed by taxanes (paclitaxel). In case of HER2 overexpression—determined using IHC or fluorescence in situ hybridization (FISH) trastuzumab was added to taxanes. Neoadjuvant hormonal treatment was allowed without concomitant chemotherapy. After systemic therapy, patients underwent surgery (radical mastectomy, simple mastectomy, or conserving surgery) according to initial stage of the disease and response to systemic treatment. Also, the axilla was managed with sentinel node biopsy or axillary lymph node dissection. Adjuvant radiotherapy was given according to standard practice. In HR-positive tumors, adjuvant endocrine therapy was used, and HER2-positive tumors had to complete one year of adjuvant trastuzumab. When oral metformin was incorporated, the permitted dosage was 500 mg twice a day in conjunction with systemic therapy.

Two experienced BC pathologists reviewed all the biopsies. The initial diagnosis was made using a breast or axillary node core needle biopsy. The report had to include a complete IHC (ER, PR, HER2, and Ki-67 percentage), tumor grade, and histologic subtype. In case of equivocal HER2 status by IHC, FISH would be performed. A pCR was defined as the absence of invasive carcinoma in breast or the axillary nodes in the surgical specimen (ypT0, ypN0).

Statistics

Demographic data are summarized and showed using descriptive statistics, and categorical variables are presented as percentages. Continuous variables are given as medians and ranges. The Fisher exact test was used to determine the association between metformin, immunophenotype, and pCR. A Multivariate logistic regression analysis was implemented to find potential predictors of pCR. We used the receiver operating characteristic (ROC) curve to establish the best cut-off point of Ki-67 for prediction of pCR. A P value less than 0.05 was considered statistically significant. Data were analyzed using SPSS (V. 20.0, Chicago, IL).

Results

We identified 81 women with invasive breast cancer who received neoadjuvant treatment. Fifty-three women with early-stage or locally advanced breast cancer fulfilled the inclusion criteria and were enrolled. Of them, 14 patients received metformin (500 mg bid PO) during chemotherapy, and 39 were...
treated with chemotherapy alone. (Figure 1)

Patient demographics and clinical characteristics of the groups are presented in Table 1. The mean age of the patients was 52.48 (range: 22–83) years. Overall, 56.6% of the patients did not have any comorbidities, 28.3% had hypertension, and 15.1% had type 2 diabetes mellitus. Two patients reported dyslipidemia, 1 patient had colon carcinoma more than five years before accrual, 1 patient had hypothyroidism, and 1 patient suffered from anxiety syndrome.

Infiltrating ductal carcinoma was the most common histology (n = 49, 92.45%), followed by infiltrating lobular carcinoma (n = 2, 3.77%), medullar carcinoma (n = 1, 1.89%), and poorly differentiated carcinoma (n = 1, 1.89%). We found that 21 patients (40%) had luminal subtype, 20 (38%) had TN tumors, 7 (13%) coexpressed estrogen receptor and HER2, and 10 cases (19%) were HER2+/HR-.

Twenty-eight patients (53%) had grade3 tumors, 22 (43%) exhibited grade 2 invasive breast cancer, and only 3 patients (4%) had grade 1 differentiation. In total, 35 women (67%) had lymphovascular invasion.

After neoadjuvant therapy (Table 2), 30 women (57%) had breast-conserving surgery and 23 (43%) had radical mastectomy. Eighteen patients (34%) achieved a pCR. We analyzed the pCR rate according to tumor subtype determined by IHC (Table 3).

HER2-overexpressing tumors tended to predict pCR (OR: 3.88, P = 0.071), in contrast to luminal tumors, which did not achieve a pCR (OR 0.09; P = 0.01). When metformin was added to systemic therapy, a pCR rate of 64.3% was achieved, while the pCR rate in the systemic therapy-alone arm was 23.1% (OR: 6.0, 95% CI: 1.60–22.53, P = 0.008). After adjustment for potential confounders (e.g., intrinsic breast cancer subtype assessed using immunohisto-

Table 1. Patient demographics and clinical characteristics by treatment group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Systemic therapy + metformin (N=14)</th>
<th>Systemic therapy alone (N=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50.3 (38–66)</td>
<td>53.1 (22–83)</td>
</tr>
<tr>
<td>&lt;50 years</td>
<td>6 (42.8%)</td>
<td>14 (35.9%)</td>
</tr>
<tr>
<td>≥50 years</td>
<td>8 (57.2%)</td>
<td>25 (64.1%)</td>
</tr>
<tr>
<td>Clinical stagea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>II</td>
<td>7 (50.0%)</td>
<td>17 (43.6%)</td>
</tr>
<tr>
<td>III</td>
<td>7 (50.0%)</td>
<td>22 (56.4%)</td>
</tr>
<tr>
<td>Tumor IHC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2+</td>
<td>5 (35.7%)</td>
<td>12 (30.8%)</td>
</tr>
<tr>
<td>HER2-</td>
<td>9 (64.3%)</td>
<td>27 (69.2%)</td>
</tr>
<tr>
<td>RE+</td>
<td>3 (21.4%)</td>
<td>19 (48.7%)</td>
</tr>
<tr>
<td>RE-</td>
<td>11 (78.6%)</td>
<td>20 (51.3%)</td>
</tr>
</tbody>
</table>

*TNM Stage according to AJCC Cancer Staging Manual 7th Ed.*
chemistry), metformin use was independently associated with a higher likelihood of pCR (OR: 5.56, 95% CI: 1.27–24.3, P = 0.02) (Table 4). We also noted a trend for achieving a higher pCR rate in TN tumors when metformin was incorporated (OR: 10, 95% CI: 1.03–67.1, P = 0.053).

We decided to incorporate Ki-67 as a predictor marker for achieving pCR, with an 86% sensibility and 57% specificity when the cut-off was set at 55% (corresponding to the highest area under the ROC curve = 0.755). We found a higher chance of obtaining a pCR when Ki-67 percentage was greater than 55% (Table 5); however, when metformin was added to this model, we did not find a statistically significant increase in the rate of pCR in patients with Ki-67 > 55% (OR: 4.0, 95% CI: 0.36–44.1, P = 0.26).

We did not register any serious adverse events with metformin. Mild diarrhea was observed in 3 patients, which was controlled with short-term antidiarrheal medications. No other side effects were found.

**Discussion**

Preoperative systemic therapy (neoadjuvant) is becoming popular nowadays for early-stage or locally advanced breast cancer. When this modality of treatment is given, the main purpose is to downstage the tumor for a less aggressive surgery with less postoperative complications and better cosmetic outcomes. Neoadjuvant treatment also allows prompt tumor response evaluation, and the residual disease can be a prognostic factor of tumor recurrence and overall survival, mainly in high-risk histologies such as TN tumors or HER2+ invasive breast carcinomas. For this reason, there is a particular interest in exploring new therapies to increase the pCR rate. We decided to look at the effect of metformin plus standard therapy on the rate pCR. All the included patients were discussed in our institutional tumor board. As a result, there was a clear trend toward including more HER2+, TN, or HR-positive invasive breast cancer with high-risk features such as advanced disease or high histologic grade. This modality of treatment was also preferred in young patients, which explains the median age of 52.48 (±13.41) years. Most of the patients in our study were healthy (56.6%). This fact is very

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**Table 2. Neoadjuvant systemic treatment used**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>N</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEC* 75 followed by weekly paclitaxel.</td>
<td>6</td>
<td>11.32%</td>
</tr>
<tr>
<td>FEC/EC 100 followed by weekly paclitaxel</td>
<td>21</td>
<td>39.62%</td>
</tr>
<tr>
<td>Epirubicin followed by weekly paclitaxel + trastuzumab</td>
<td>14</td>
<td>26.42%</td>
</tr>
<tr>
<td>Weekly paclitaxel + trastuzumab</td>
<td>1</td>
<td>1.89%</td>
</tr>
<tr>
<td>Weekly paclitaxel alone</td>
<td>1</td>
<td>1.89%</td>
</tr>
<tr>
<td>Dose-dense (epirubicin followed by paclitaxel)</td>
<td>6</td>
<td>11.32%</td>
</tr>
<tr>
<td>Platinum/epirubicin/paclitaxel</td>
<td>2</td>
<td>3.77%</td>
</tr>
<tr>
<td>Anastrozole</td>
<td>2</td>
<td>3.77%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>53</td>
<td>1%</td>
</tr>
</tbody>
</table>

*F= 5-Fluorouracil, E= Epirubicin, C= Cyclophosphamide

**Table 3. Association between IHC-determined tumor subtype and pathological complete response rate**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>With pCR, n (%)</th>
<th>Without pCR, n (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR+/HER2-</td>
<td>1 (6.7)</td>
<td>14 (93.3)</td>
<td>0.09 (0.01–0.74)</td>
</tr>
<tr>
<td>HR+/HER2+</td>
<td>2 (28.6)</td>
<td>5 (71.4)</td>
<td>0.75 (0.13–4.31)</td>
</tr>
<tr>
<td>HR-/HER2+</td>
<td>6 (60)</td>
<td>4 (40)</td>
<td>3.88 (0.93–16.19)</td>
</tr>
<tr>
<td>Triple-negative</td>
<td>9 (42.9)</td>
<td>12 (57.1)</td>
<td>1.92 (0.60–6.10)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>18</td>
<td>35</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 4. Multivariate analysis of potential predictors of pathological complete response**

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>5.56 (1.27–24.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>RE+</td>
<td>0.16 (0.01–2.39)</td>
<td>0.18</td>
</tr>
<tr>
<td>RE-/HER2+</td>
<td>3.23 (0.83–29.16)</td>
<td>0.28</td>
</tr>
<tr>
<td>Triple-negative</td>
<td>1.27 (0.17–9.31)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

**Table 5. Comparison of pCR by Ki-67 percentage**

<table>
<thead>
<tr>
<th>Ki-67 (number)</th>
<th>With pCR, n (%)</th>
<th>Without pCR, n (%)</th>
<th>OR (95% CI %), P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;55% (14)</td>
<td>2 (14.3%)</td>
<td>12 (85.7%)</td>
<td>8.0 (1.42–45.06), 0.013</td>
</tr>
<tr>
<td>&gt;55% (21)</td>
<td>12 (57.1%)</td>
<td>9 (42.9%)</td>
<td>-</td>
</tr>
</tbody>
</table>

relevance because other retrospective studies using metformin in this setting had evaluated diabetic patients.39

Overall, the pCR rate (ypT0/is ypN0) obtained in our research was 34%, which is higher than that reported in other studies (ranging from 14 to 19.8%).39 Nevertheless, we have to emphasize that our study population was selected deliberately, including very responsive tumor subtypes. We found that the pCR rate was significantly higher when metformin was added to standard systemic therapy, even after adjustment for the tumor subtype, meaning that adding metformin to systemic therapy increased the likelihood of achieving pCR 5.56 times.

Metformin has shown anticancer properties, including activation of the AMP activated protein kinase (AMPK) pathway,31 antioxidant activity, induction of apoptosis, and many others.22 Patients with type 2 diabetes are hyperinsulinemic, and there is evidence that this condition contributes to tumorigenesis. In this scenario, metformin reverses, at least partially, hyperinsulinemia and exhibits antiproliferative properties, which might increase the effect of chemotherapy in a synergy. Preclinical research has demonstrated that metformin can sensitize tumor cells to chemotherapy through inhibition of the expression of PI3K/AKT proteins31 and, consequently, controlling the activity of the mammalian target of rapamycin (mTOR). The combination of metformin with chemotherapy appears to inhibit mTOR activity, inducing cell cycle arrest.34 Our study, although limited by its cross-sectional design and sample size, provides clinical evidence that metformin in conjunction with chemotherapy increases the pCR rate in the context of non-diabetic Latin American patients, which is relevant to planning prospective studies.

Ki-67 is a non-histone nuclear cortex protein expressed in all cell cycle phases except in the quiescent phase G0, reaching the peak level during mitosis. Ki-67 can serve as an indicator of tumor proliferative activity.18,25,26 Higher level of Ki-67 has been hypothesized to be a predictor marker of pCR.25,26 A recent meta analysis involving 53 studies (10,848 patients) showed that tumors with high Ki-67 score had a better chance to respond to neoadjuvant treatment, although there was no clear cut-off.26 In our study, there was a higher chance of obtaining a pCR when Ki-67 index was greater than 55% using ROC curve; however, when metformin was added, we were unable to find a specific Ki-67 cut-off. This might mean that, regardless of Ki-67 levels, the most important factor to obtain the pCR was metformin.

This finding must be confirmed by future studies since this has not been evaluated in this specific approach, as far as we know, either in retrospective or prospective studies.

In conclusion, this is the first cross-sectional study conducted in a Latin American population showing that the use of metformin in combination with systemic therapy can increase the pCR rate in locally advanced or early breast cancer, irrespective of the histologic subtype, grade, or Ki-67 levels. These findings can encourage prospective studies using metformin in the neoadjuvant setting in our population.

Acknowledgment
Participant patients, and Caja Costarricense de Seguro Social.

Conflict of Interest
We have read and understood the journal’s policy on disclosing conflicts of interest and declare that we have none.

References
Seroma Formation After Mastectomy and Axillary Dissection; A Comparison Among Blunt Dissection With Hemostat, Sharp Dissection With Metzenbaum Scissors, and Dissection With Harmonic Scissors


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†Medical student, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

**Background:** Seroma formation is a common complication after surgery for breast cancer. It may originate from dissected lymphatic ducts in axillary area. Two important predictive factors are the surgical technique, and instruments used during surgery. This study was conducted to determine the impact of three axillary dissection techniques, namely, blunt dissection with hemostat, sharp dissection with Metzenbaum scissors, and dissection with harmonic scissors, on seroma formation.

**Methods:** Patients with a tissue diagnosis of breast cancer who did not have metastasis, and were candidates for either breast conservation surgery (BCS) and sentinel lymph node biopsy (SLNB) with or without axillary lymph node dissection (ALND), or modified radical mastectomy (MRM) were included in a prospective study. Patients were randomly allocated to one of the above mentioned three groups. The incidence of seroma formation was compared among the groups.

**Results:** Sixty patients (age: 50.25 ± 10.33 years) were enrolled for the study. At the end of the study, after four weeks of postoperative follow up, 19 patients developed seroma (31.6%), of whom 5 (26.3%) had dissection with harmonic scissors, 6 (31.5%) with Metzenbaum scissors, and 8 (42.1%) were dissected bluntly. There was no significant difference among groups regarding seroma formation ($P = 0.583$).

**Conclusion:** Application of harmonic scissors for axillary dissection has no significant impact on seroma formation. However, MRM leads to significantly more seroma formation compared with BCS.

Introduction

Breast cancer has remained the second cancer-related cause of death in women worldwide and accounts for a significant volume of cancer surgeries. Surgical procedures for breast cancer include breast conservation surgery (BCS), with or without sentinel lymph node biopsy (SLNB) or axillary lymph node dissection (ALND), and modified radical mastectomy (MRM), with the choice depending on the stage of the disease. Each technique has its own advantages and complications.

Seroma formation is among the most common complications of surgery for breast cancer, with an incidence of 3% to 85%. It may significantly delay adjuvant therapy and increase the risk of infection. On the other hand, surgery may be indicated for prolonged unresponsive cases. The mechanism of seroma formation is not well understood; however,
some risk factors or predictive factors are as follows: age, size of the breast, comorbidity, the number of involved axillary lymph nodes (LNs), history of previous breast or axillary surgery, and treatment with tamoxifen or heparin. Lower fibrinogen levels in seroma compared with those in postoperative plasma supports the theory that seroma most probably originates from lymph nodes. MRM carries a greater risk of seroma formation than BCS does, and an RCT found that seroma formation is much less after SLNB than after ALND.

In conventional techniques, such as blunt dissection with hemostat and sharp dissection with Metzenbaum scissors, hemostasis is generally attained with electrocautery or ligation. Argon diathermy, laser scalpel, and ultrasonic scalpel decrease intraoperative blood loss. However, they are time-consuming and increase postoperative seroma formation and the risk of tissue thermal damage.

This prospective study was carried out to compare the prevalence of seroma formation following surgery for breast cancer, when axillary surgery was performed using harmonic scissors, hemostat scissors, or Metzenbaum scissors.

Methods

This was a prospective study conducted in a tertiary referral university hospital during 2013 and 2014. The primary goal of the study was to measure seroma formation in breast cancer patients who underwent BCS and SLNB (with or without ALND), or MRM when axillary dissection was performed with harmonic scissors, hemostat, or Metzenbaum scissors.

Inclusion criteria were (1) having histopathologically diagnosed breast cancer, (2) having no distant metastasis, and (3) being candidate for BCS and SLNB (with or without ALND), or BCS and ALND, or MRM. Exclusion criteria were having (1) previous breast or axillary surgery, (2) history of radiation to breast, axilla, or upper limb, (3) history of systemic chemotherapy for any reason, (4) any pathologic process involving the skin of the breast, axilla, or upper limb, (5) a motor disorder of upper limb, and (6) no consent.

Sampling was done by excel quadratic block method. Patients were randomly allocated to one of the following groups:

1. Axillary dissection using blunt dissection with hemostat. Bleedings were either cauterized or ligated.
2. Axillary dissection using sharp dissection with Metzenbaum scissors. Bleedings were either cauterized or ligated.
3. Axillary dissection using harmonic scissors. Axillary dissection was performed entirely by harmonic scissors.

Allocation was done by simple randomization technique. Patients entering the operating room were allocated to group 1, then 2, and then 3, and this round was repeated.

The surgeon, preoperative preparation, and postoperative care (including wound care, ambulation, limb activity, and physical therapy) were the same for each patient. Diagnosis of seroma formation and its evaluation was performed by the same research team member, who was a 4th-year resident of surgery. Only the operating surgeon knew which patient belonged to which group, and the patients and the researcher (the 4th-year resident) were blinded.

The study variables were age, the type of breast surgery, the type of axillary dissection, the technique of axillary dissection, the number of involved lymph nodes (pN), tumor size (pT), tumor grade, microscopic diagnosis, estrogen receptor (ER) status, progesterone receptor (PR) status, human epidermal growth factor receptor 2 (HER2) status, Ki-67 status, and seroma formation.

Seroma formation was defined as any clinically detectable collection of serum requiring aspiration. Drainage in the first three postoperative days was recorded. Patients were then visited 1, 2, and 4 weeks after the operation and before the start of adjuvant therapy, and were examined for seroma formation. Data were recorded, finalized, and analyzed using IMB SPSS software, version 20.

Informed consent form was signed by every patient before entering the study. Data confidentiality was observed and the patients were free to leave the study at any time. No extra charge was imposed on the patients. The researchers were committed to the World Medical Association’s Declaration of Helsinki throughout the study.

Results

Sixty patients (age: 50.25 ± 10.33) were assigned to three groups of 20 each: blunt dissection with hemostat, sharp dissection with Metzenbaum scissors, and dissection with harmonic scissors (Table 1).

Of 20 patients who had blunt axillary dissection with hemostat, 16 (80%) underwent MRM and 4 (20%) BCS; of 20 patients who received axillary dissection with Metzenbaum scissors, 10 (50%) underwent MRM and 10 (50%) BCS; and of 20 patients in the group who had axillary dissection with harmonic scissors, 12 (60%) underwent MRM and 8 (40%) BCS.

In total, 38 (63.3%) patients underwent MRM. Axillary dissection was performed using harmonic scissors in 12 (31.5%), using Metzenbaum scissors in 10 (26.5%), and using hemostat in 16 (42%) patients. Twenty-two (27.7%) patients underwent BCS. Axillary dissection was performed with harmonic scissors in 8 (36.5%), with Metzenbaum scissors in 10 (45.5%), and with hemostat in 4 (18%) patients.

The most common tissue diagnosis was invasive
After preoperative chemotherapy, a breast-conservation surgery was ductal carcinoma, (n = 47, 78.3%), followed by invasive lobular carcinoma (n = 10, 16.7%) and infiltrating ductal carcinoma (n = 3, 5%). The mean tumor size was 3.4 ± 1.53 cm, and the mean number of involved axillary lymph nodes was 2.86 ± 3.27 (Table 1).

Ten (16.7%) patients were at stage Ia, 17 (28.3%) at stage Ib, 21 (35%) at stage II, and 12 (20%) at stage III of breast cancer, according to TNM classification (Table 1).

Also, 34 (56.7%) patients were ER+ and PR+, 12 (20%) were HER2-positive, and 4 (6.7%) were Ki-67-positive (Table 1).

No significant difference was found among the three groups regarding age (P = 0.4), tumor size (P = 0.944), the number of involved lymph nodes (P = 0.789), pathologic type of tumor (P = 0.668), and the stage of disease (P = 0.978). Neither was there any significant difference in receptor status (ER: P = 0.934, PR: P = 0.934, HER2: P = 0.892, and Ki-67: P = 0.329) (Table 1).

s possible in 22 patients (55%), and the rest of them underwent mastectomy. Pathological complete response (ypT0/is ypN0) was achieved in 13 patients (33%). Only 3 patients (15.8%) with AR-positive tumors achieved a pCR. The rate of pCR according to the expression or absence of AR, ER, PR, and HER2 is reported in Table 2. AR-positive tumors had 82% less chance of achieving a pCR compared with patients with AR-negative tumors (OR = 0.18; 95% CI, 0.04–0.75).

After four weeks of follow up, and at the end of the study, 19 (31.6%) patients developed seroma. The frequencies of seroma formation in patients in different groups were 5 (20%) (dissection using harmonic scissors), 6 (30%) (Metzenbaum scissors), and 8 (40%) (hemostat). Analysis using chi-square test showed no significant difference in seroma formation among the groups (P = 0.583) (Figure 1).

Table 2. Pathological complete response according to the Immunohistochemical expression of breast cancer receptors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Blunt dissection with hemostat</th>
<th>Dissection with Metz</th>
<th>Harmonic scalpel</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50.25 ± 9.27</td>
<td>48.65 ± 10.74</td>
<td>51.85 ± 11.16</td>
<td>0.400</td>
</tr>
<tr>
<td>Tumor size</td>
<td>34.15 ± 13.96</td>
<td>35 ± 15.55</td>
<td>33.85 ± 17.17</td>
<td>0.944</td>
</tr>
<tr>
<td>Number of involved lymph nodes</td>
<td>2.55 ± 2.45</td>
<td>2.95 ± 3.05</td>
<td>3.10 ± 4.20</td>
<td>0.789</td>
</tr>
<tr>
<td>Stage of the disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ia</td>
<td>2 (10%)</td>
<td></td>
<td>4 (20%)</td>
<td>0.978</td>
</tr>
<tr>
<td>Ib</td>
<td>2 (10%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1 (5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1 (5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histologic type of tumor</td>
<td></td>
<td></td>
<td></td>
<td>0.668</td>
</tr>
<tr>
<td>Invasive lobular</td>
<td>4 (20%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infiltrating ductal</td>
<td>5 (25%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive ductal</td>
<td>6 (30%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER expression</td>
<td>12 (60%)</td>
<td>9 (45%)</td>
<td>11 (55%)</td>
<td>0.934</td>
</tr>
<tr>
<td>PR expression</td>
<td>12 (60%)</td>
<td>9 (45%)</td>
<td>11 (55%)</td>
<td>0.934</td>
</tr>
<tr>
<td>HER2 expression</td>
<td>4 (20%)</td>
<td>7 (35%)</td>
<td>3 (15%)</td>
<td>0.892</td>
</tr>
<tr>
<td>Ki-67 expression</td>
<td>2 (10%)</td>
<td>5 (25%)</td>
<td>2 (10%)</td>
<td>0.329</td>
</tr>
<tr>
<td>Technique of surgery</td>
<td></td>
<td></td>
<td></td>
<td>0.134</td>
</tr>
<tr>
<td>MRM</td>
<td>16 (80%)</td>
<td>10 (50%)</td>
<td>12 (60%)</td>
<td></td>
</tr>
<tr>
<td>BCS</td>
<td>4 (20%)</td>
<td>10 (50%)</td>
<td>8 (40%)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Frequency of seroma in study groups

Figure 2. Frequency of seroma after MRM and BCS
Correlation of demographic and pathologic characteristics with seroma formation was also evaluated. No correlation was found between seroma formation and age \((P = 0.858)\), tumor size \((P = 0.920)\), disease stage \((P = 0.415)\), the type of cancer \((P = 0.271)\), ER and PR status \((P = 0.781)\), HER2 expression \((P = 0.775)\), or Ki-67 expression \((P = 0.778)\).

Of 38 patients who underwent MRM, 16 (42.1%) developed seroma: 5 were dissected using harmonic scissors, 5 using Metzenbaum scissors, and 6 using hemostat. Only 3 (9%) out of 22 patients who had BCS developed seroma: 1 was dissected using Metzenbaum scissors and 2 using hemostat. None of the patients who underwent BCS and were dissected with harmonic scissors developed seroma. The difference in the incidence of seroma formation between MRM and BCS patients was significant \((P = 0.022)\) (Figure 2).

**Discussion**

Although the mechanism of seroma formation is not fully understood, some of the risk factors or predictive factors are as follows: age, size of the breast, comorbidity, the number of involved axillary lymph nodes (LNs), history of previous breast or axillary surgery, and treatment with tamoxifen or heparin.\(^4\)\(^5\) One theory holds that seroma forms because of chronic inflammatory reaction inflicted by surgery.\(^7\) This reaction leads to increased serosal fluid accumulation in response to elevated fibrinolytic activity in seroma and lymphatic fluid.\(^8\) On the other hand, low fibrinogen level in seroma compared with that in postoperative plasma\(^6\) supports the hypothesis that seroma is most probably originated from lymph nodes. A combination of different techniques and instruments affects seroma formation\(^1\) and may be the reason for various amounts of seroma reported in different studies. Radical mastectomy leads to higher rates of seroma formation than simple mastectomy do.\(^9\)\(^10\) This difference is not significant between radical mastectomy and MRM.\(^11\) Immediate breast reconstruction after MRM decreases the risk of seroma formation compared with delayed reconstruction.\(^12\)

To obviate the dead space, Halstead recommended a short upper skin flap sutured to pectoral fascia at the level of lower margin of the 1st rib; the remaining wound would be covered with skin graft.\(^13\) Orr, in 1951, used tension sutures to tighten the skin flaps to the chest wall.\(^14\) Keyes and colleagues used multiple sutures to attach the skin flaps to the chest wall.\(^15\) Other techniques such as suturing the flaps to subcutaneous tissue and avoiding drainage in BCS were also implicated to reduce seroma formation.\(^16\)\(^17\)

No study, as yet, has shown the efficacy using laser scalpel or ultrasonic scissors in prevention of seroma formation.\(^18\) Other techniques such as avoiding drainage in BCS\(^19\) were also implicated to reduce seroma formation. Whitfield and Rainbury did not found any significant difference in seroma formation between suction drainage and closed siphon drainage.\(^20\) Pathologic specifications of tumors and patient-related factors do not seem to affect seroma formation, except for weight, which shows a direct relation with seroma formation.\(^2\)\(^2\)\(^1\) No particular relationship has been reported between seroma formation and hormone receptors, axillary lymph node involvement, the grade of the tumor, or the stage of disease.\(^15\)\(^2\)\(^1\) This lack of relationship also been observed for anemia, smoking, diabetes, and breast size as well as tumor size, tumor location, the size of the resected specimen, and the pathologic type of cancer.\(^14\)\(^2\)\(^4\)\(^2\)

Instruments based on piezoelectric mechanism induce coagulation by denaturing collagen and elastin in soft tissue, blood, and lymphatic vessels through high-frequency vibration \((50–60 \text{ MHz})\).\(^22\) This produces less heat and results in less thermal energy transfer to adjacent tissues compared with electrosurgery.\(^23\) Harmonic Focus,\(^2\) introduced more than a decade ago, cuts and coagulates lymphatic ducts safely and is utilized for hepatic, thyroid, and breast surgery among others.\(^24\)

The primary outcome of this study was postoperative seroma formation, which occurred in 19 patients. There was no significant difference in age, tumor size, number of involved lymph nodes, type of the tumor, the stage of disease, and the expression of ER, PR, HER2, and Ki-67 among the groups, so the groups were matched by these variables. The studied variables including demographic specifications, the pathologic variety of the tumor, or the methods of surgery were not associated with postoperative seroma formation.

The frequency of seroma formation was lower in the harmonic scissors group than the other groups, although the difference was not significant. No patients who underwent BCS with harmonic scissors developed seroma. Although it cannot be substantiated statistically, it can be taken as a suggesting clue for future study.

There was no correlation between seroma formation and age, tumor size, the stage of disease, the type of cancer, or the expression of ER/PR/HER2/Ki-67. Therefore, it can be concluded that these factors do not relate to seroma formation.

The only variable that showed a significant correlation with seroma formation was the principal technique of mastectomy, i.e., MRM vs. BCS. Regardless of the method used for axillary dissection, seroma formation rate was higher after MRM than after BCS.

Orr et al published the data of 72 patients who underwent MRM, partial mastectomy with axillary dissection, or simple mastectomy. The total incidence rate of seroma formation was 11%, the highest incidence (45.67%) being observed in patients who had simple mastectomy and were
discharged at postoperative day 1.\textsuperscript{10} Deo and Shukla\textsuperscript{11} reported the application of harmonic scissors in MRM for the first time. They showed a decrease in intraoperative bleeding and duration of drainage compared with conventional clamp and tie. Galatius and colleagues\textsuperscript{21} studied 59 patients who underwent surgery using harmonic scissors (n = 30) or conventional scissors (n = 29). On follow-up, 20 patients in each arm had developed seroma: a total of 40 (67\%). There was no significant difference in the frequency of seroma formation between harmonic scissors and conventional ones. Lumachi \textit{et al} conducted an RCT and showed that using harmonic scissors, compared with surgical scalpel and tying, led to significantly less seroma formation.\textsuperscript{12}

Although no study with a similar design has been published yet, studies have produced inconsistent results. Khan and colleagues studied 150 patients who were operated using either harmonic scissors or electrosurgery.\textsuperscript{22} In the harmonic scissors group, the incidence of seroma formation was 21.3\% vs. 33.3\% in the electrosurgical group, which was significantly less. While we had just about the same percentages in harmonic vs. Metzenbaum, the difference was not significant.

The relationship between MRM and seroma formation has been studied formerly. Gonzalez and colleagues reported seroma formation rate of 19.5\% for MRM and 9.2\% for BCS ($P = 0.001$).\textsuperscript{23} In a meta-analysis by Kuroi \textit{et al}, 51 clinical trials, 7 cohort studies, and 7 retrospective studies were included. They concluded that MRM had a significant association with seroma formation. The present study reached the same finding: MRM led to significantly higher seroma formation compared with BCS ($P = 0.022$).\textsuperscript{24}

The limitations of this study were the small number of patients in each group, the heterogeneity of groups considering techniques of axillary dissection, heterogeneity of groups considering the extent of mastectomy, and the use of simple randomization. Also, we should have ideally divided the BCS patients into those who had BCS + SLNB, BCS + SLNB + ALND, and BCS + ALND. Again, the small number of patients in each of these subgroups would impede meaningful statistical analysis.

These limitations may have hypothetically affected our results. Therefore, we believe that a large-scale study with a better randomization method comparing the use of harmonic vs conventional scissors in patients with the same extent of mastectomy may better clarify the issue.

The use of harmonic scissors for axillary dissection in MRM, BCS with SLNB (with or without ALND), and BCS with ALND does not decrease the incidence of postoperative seroma formation significantly. Also, there is no association between age, tumor specifications, or molecular markers status and seroma formation. The only effective factor is the extent of mastectomy, with MRM leading to significantly higher rates of seroma formation than BCS.

\textbf{Conflict of Interest}

The authors have none to declare.

\textbf{References}


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ABSTRACT

Background: Adenoid cystic carcinoma (AdCC) of the breast is a rare type of invasive breast cancer, accounting for less than 1% of all breast cancer cases. It is usually a low-grade tumor that rarely metastasizes to the axillary lymph nodes and distant organs. AdCC of the breast is mostly negative for estrogen receptor, progesterone receptor, and HER2-neu. However, despite being triple-negative, it has favorable outcome, and patients with AdCC of the breast generally have a good to excellent long-term survival.

Case presentation: Here we report a case of a 51-year-old female presenting with left breast mastalgia who was finally diagnosed with AdCC of the left breast. The patient underwent breast-conserving surgery and axillary staging followed by adjuvant chemoradiation.

Conclusion: Because of the rarity of AdCC of the breast, it has been difficult to conduct prospective trials to determine the best treatment option. Different types of therapy, including BCS and mastectomy with or without axillary staging with or without adjuvant chemoradiation have been reported, all with overall good to excellent long-term results. Prospective controlled trials will help in determining the best treatment.

Introduction

Adenoid cystic carcinoma (AdCC) of the breast is a rare type of invasive breast cancer, accounting for less than 1% of all breast cancer cases. It is mostly seen in the 5th and 6th decades of life and affects both genders, although females are affected more frequently.

Breast AdCC is a low-grade tumor that rarely metastasizes to the axillary lymph nodes and distant organs. Distant metastasis generally occurs in the absence of axillary lymph node involvement and lungs are the most commonly involved organs.

Breast AdCC is usually negative for estrogen receptor, progesterone receptor, and HER2/neu (triple-negative). However, unlike other triple-negative invasive breast cancers, breast AdCC has a favorable prognosis, and 5-, 10-, and 15-year survival rates have been reported to be 94–98.1%, 85–100%, and 91.4%, respectively.

Because of the rarity of breast AdCC, it has been difficult to conduct prospective trials to compare different types of local and systemic treatments; therefore, there is currently no standard treatment recommendations. Different types of surgery including breast-conserving surgery (BCS) and mastectomy, with or without axillary staging, with or without adjuvant chemoradiation or hormone therapy, have been used in different studies, with overall similar good results.
Here, we report a case of a 51-year-old female presenting with left breast mastalgia who was finally diagnosed with AdCC of the left breast.

**Case Presentation**

A 51-year-old woman referred to our breast clinic with bilateral breast pain, especially in her left breast. She had no history of previous medical or surgical diseases and was taking no medications. Her age at menarche was 13; she was 21 at her first live birth and had two children. She had an abortion and had reached menopause 3 years before. There was no family history of breast or ovarian cancers. On her physical examination, an area of tissue thickening, measuring 30 mm, was palpated in the upper central portion of her left breast. Examination of the right breast and bilateral axillary regions was unremarkable.

Bilateral digital mammography and breast ultrasound were requested. Mammography revealed an asymmetry with irregular borders in the left breast in medial and deep retroareolar area. This finding was reported to be highly suspicious for malignancy. Also, an asymmetry was detected in axillary tail of the right breast which was stable in comparison with the last year’s mammography. Ultrasonography showed an irregular mass (18 × 12 mm) at 10 o’clock position in the left breast, and a hypoechoic lesion (7 × 4 mm) without blood flow in the axillary tail of the right breast. Ultrasound-guided core-needle biopsies (CNB) of both lesions were performed. Pathologic examination reported the left breast lesion as invasive carcinoma. Immunohistochemistry (IHC) staining of the lesion was negative for estrogen receptor, progesterone receptor, HER2/neu, CK20, GCDFP-15, mammaglobin, synaptophysin, and chromogranin, and positive for Ki-67 (10–20% of tumor cells) and CK7. Pathologic examination of the right breast lesion showed no evidence of malignancy.

According to pathology report of the left breast mass, and considering the patient’s clinical stage, breast conserving surgery and sentinel node biopsy with the use of technetium 99 (99Tc) injection was planned. After excision of the breast specimen, surgical margins were evaluated using frozen-section analysis and were declared clear. However, despite the injection of 99Tc at the standard dosage in the periphery of the lesion before the surgery, sentinel lymph nodes were not identified. Therefore, limited axillary dissection was done for the axillary staging.

Gross examination showed an infiltrative tumor measuring 18mm. Microscopic examination showed solid and cribriform nests of basaloid cells consistent with adenoid cystic carcinoma. (Figure 1) Immunostaining confirmed the dual epithelial and modified myoepithelial differentiation of tumor cells. (patchy staining for CK5/6, CK7, EMA and P63) The solid component constituted more than 30% of tumor, consistent with grade 3. No tumor calcification, perineural and vascular invasion were reported; clear surgical margins was confirmed. There were six lymph nodes in the axillary specimen which all showed reactive changes. (Figure 2)

According to the pathology report, which confirmed high-grade breast AdCC, and after multidisciplinary review, metastatic workup was pursued; whole-body bone scan and thoracic CT scan were normal. Abdominal ultrasound showed an echogenic mass in the posterior segment of the right lobe of the liver, followed by abdominal CT scan showing a 27 mm low attenuation mass in the right hepatic lobe in favor of metastatic lesion. For further clarification of the lesion, abdominal MRI was done which showed a 38 mm round mass with low signal intensity on T1 and high signal intensity on T2 images at the 6th segment of the right hepatic lobe, which was suggestive of a metastatic lesion. Ultrasound-guided percutaneous needle biopsy of the lesion was decided, but the patient refused to undergo biopsy. Therefore, for deciding on the best management, we had an expert radiologist repeat the ultrasound and review the MRI images. He strongly suggested the possibility of a liver hemangioma, so 99mTc-RBC scanning of the liver was performed.

The scan demonstrated a hemangioma in the right

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**Figure 1.** A: Photomicrograph shows breast tissue (arrow) infiltrated with cribriform and solid nests of basaloid cells morphologically consistent with adenoid cystic carcinoma, x100, H&E. B: Higher magnification of tumor, x400, H&E.
Unlike AdCCs of other organs like head and neck, breast AdCCs are low-grade tumors with a good to excellent prognosis and long-term survival that rarely metastasizes to regional lymph nodes and distant sites. In our patient, axillary lymph nodes were not involved, although the tumor was of high grade. Distant metastasis usually occurs in the absence of lymph node involvement. The most common sites of distant involvement are lungs, although metastasis to brain, bone (vertebrae), liver, or kidneys has also been reported.

In a review by Miyai et al. of seven studies on patients with breast AdCC, lymph node involvement was reported to be 0–6.1%, distant metastasis (including bone, liver, lung, and kidney) 1–20%. Also, 5- and 10 year survival rates were reported to be 88–94% and 90–94.9%, respectively. In another study by Chen et al. on 86 breast AdCC patients, 88.4% of patients had grade 1 or 2 tumors; 100% had stage 1–2 cancer; and the rate of lymph node involvement was 2.3%. A retrospective study of 244 women with invasive breast AdCC found that 92.2% of the patients had localized disease, 4.9% had regional disease, and only 7 patients had distant metastasis. The 5-year and 10-year relative cumulative survival rates were 95.5% and 93.5%, respectively, and lymph node involvement ratio was 5.5%. In a retrospective study by Ghabach et al. on 338 women with breast AdCC, regional and distant disease was reported in less than 5% of case. The ratio of lymph node involvement was 2.5%, and the overall 5-, 10-, and 15-year relative survival rates were 98.1%, 94.9%, and 91.4%, respectively. Women aged <50 years and ≥50 years had an excellent 5-year relative survival of 94.4% and 99.0%, respectively. Ten-year relative survival was similar for both age groups (94.4%), although 15-year survival rate was slightly lower for older patients compared with the younger ones (88.9% vs

**Discussion**

Breast AdCC is a rare type of invasive breast cancer accounting for less than 1% of all breast cancers. It is mostly seen in the 5th–6th decades of life, although there are reports of patients with younger and older ages at the time of diagnosis. Although breast AdCC predominantly occurs in women, there have been reports of men with breast AdCC. Signs and symptoms of breast AdCC are usually similar to those of other types of breast cancer, including breast mass and breast pain. It mostly involves subareolar region, as in our patient; although, in a retrospective study on 338 women, the most involved quadrant was the upper, outer quadrant. Despite the preference of the malignancy for areolar region, nipple discharge is not a common symptom.

Unlike AdCCs of other organs like head and neck, breast AdCCs are low-grade tumors with a good to excellent prognosis and long-term survival that rarely metastasizes to regional lymph nodes and distant sites. In our patient, axillary lymph nodes were not involved, although the tumor was of high grade. Distant metastasis usually occurs in the absence of lymph node involvement. The most common sites of distant involvement are lungs, although metastasis to brain, bone (vertebrae), liver, or kidneys has also been reported.

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**Figure 2.** Immunostainings for CK5/6 (A), CK7 (B), EMA (C) and P63(D) confirm the presence of dual population of epithelial and modified myoepithelial cells in tumor.
Breast AdCCs are usually triple-negative,\(^1\,^3\,^4\,^7\,^8,\,^11\) as was the case for our patient, although hormone receptor-positive breast AdCCs have also been reported.\(^7\) Unlike other triple-negative invasive breast cancers, which have worse outcomes than their positive counterparts, all breast AdCC tumors, including triple-negative ones, have good prognosis overall.\(^1\,^3\) In Chen et al., the rate of triple-negative tumors was 77.9%, and Kim et al. reported that all of their 6 patients were triple-negative.\(^4\)

Different types of treatment including BCS and mastectomy with or without axillary staging, with or without adjuvant chemotherapy, radiation therapy or hormone therapy have been used for breast AdCC, mostly with no significant difference in outcomes.\(^2\,^3\) In our case, with the initial diagnosis of invasive carcinoma, the patient underwent BCS with sentinel lymph node biopsy followed by adjuvant radiation and chemotherapy, because of the high grade of the tumor. Because of the rarity of these tumors, it has been difficult to conduct prospective randomized trials to compare different treatment options and elucidate the best option. Existing data are mostly the results case series and retrospective studies; therefore, there is no universal guideline for treatment of breast AdCC.

The surgical approach for local breast disease control ranges from BCS to mastectomy. Regarding BCS, however, there is no consensus about the adequate surgical margin. Ro et al. suggested a grading system for breast AdCC based on the proportion of solid growth of the tumor: no solid element (grade 1), <30% solid element (grade 2), and >30% solid element (grade 3). Consequently, they proposed local excision, simple mastectomy, and mastectomy with axillary node dissection for grade 1, 2, and 3 tumors, respectively.\(^1\) Another issue is the role of adjuvant treatments, as there is no strong evidence for determining the type of adjuvant therapy (i.e., radiation vs chemotherapy) and identifying the patients who will benefit most from these therapeutic modalities.

According to some studies, mastectomy is the preferred local surgical treatment. In one study, recurrence rate following local excision was reported to be 6–37%.\(^9\) This lower rate of recurrence may explain why mastectomy is the preferred choice of clinicians, despite the fact that there is no randomized controlled trial comparing BCS to mastectomy. A meta-analysis found that recurrence-free 10-year survival rates following mastectomy and BCS were 85.1% and 45.7%, respectively (p < 0.05). Six of 10 local recurrences and 7 of 8 distant metastases had occurred 5 years or later after initial treatment. It concluded that patients with breast AdCC should be treated using mastectomy followed by extended follow-up.\(^14\)

Most reports favor BCS as the preferred local treatment, especially along with adjuvant radiation. In a case report by Ichikawa et al., a patient with breast AdCC underwent BCS with axillary lymph node dissection. After a 5-year follow-up, without adjuvant therapy, they reported that the patient had no clinical recurrence of the disease.\(^15\) In another study, women with breast AdCC were treated with lumpectomy (41 patients) or mastectomy (20 patients), and 40 of them received adjuvant radiotherapy (35 after lumpectomy, 5 after mastectomy). The median follow-up was 79 months, and 5-year overall survival (OS), disease-free survival (DFS), and 5-year locoregional control (LRC) rates were 94%, 82%, and 95%, respectively. Univariate analysis revealed that neither the type of surgery nor the use of postoperative radiotherapy affected survival. The 5-year LRC rate was not different between the mastectomy and BCS groups (100% vs 93%, \(P = 0.16\)). A significant correlation was observed between the use of radiotherapy and LRC for the BCS group (\(P = 0.03\)). The 5-year LRC rate was 95% for the radiotherapy arm compared with 83% (95% CI: 54%–100%) for the no-radiotherapy arm. In patients with positive margins, all of whom received postoperative radiotherapy, no local failure occurred. The authors concluded that BCS should be considered the preferred treatment for patients with breast AdCC and that postoperative radiotherapy should be proposed in the case of BCS.\(^12\)

In another study, patients with breast AdCC underwent simple or modified radical mastectomy (n = 22) or lumpectomy (n = 6). Of the 6 patients who had lumpectomy, 5 also received postoperative radiation therapy. No local recurrence occurred in either group. The 5-year DFS and OS rates were 100% and 85%, respectively. The authors concluded that breast AdCC has very favorable biologic characteristics and that both lumpectomy with radiation and simple mastectomy result in good local control.\(^16\)

In a retrospective study, Sun et al. followed up 478 patients with breast AdCC for a median of 59 months and reported 10-year cause-specific survival (CSS) and OS rates of 87.5% and 75.3%, respectively. The 5-year CSS rates for different treatment modalities were as follows: lumpectomy + adjuvant RT = 96.1%, lumpectomy alone = 91.8%, mastectomy alone = 90.2%, and mastectomy + adjuvant RT = 94.1% (\(P = 0.026\)). Multivariate Cox analyses revealed that lumpectomy + adjuvant RT could be an independent prognostic factor for CSS and OS. Patients who underwent lumpectomy + adjuvant RT had better survival rates than patients who had lumpectomy only (CSS: \(P = 0.018\); OS: \(P = 0.031\)) or mastectomy only (CSS: \(P = 0.010\); OS: \(P = 0.004\)). They concluded that breast AdCC had an excellent prognosis and suggested that BCS be used as the treatment of choice for patients with breast AdCC, along with adjuvant RT to improve survival rates.\(^17\)
In another study on 376 patients who underwent surgery with or without postoperative adjuvant RT, 10-year absolute OS and CSS benefits were 21% and 7%, respectively (P = 0.005 and 0.12, respectively). Multivariate analysis revealed RT to be a significant predictor of overall and cause-specific survival with hazard ratios of 0.44 (95% CI: 0.22–0.88) and 0.1 (95% CI: 0.01–0.88), respectively. They concluded that RT after local surgical therapy for breast AdCC resulted in better cause-specific and overall survival rates and that “use of RT in this rare tumor should be considered in patients otherwise eligible for RT.”

Boujelbene et al. contend that BCS with postoperative radiotherapy is comparable to mastectomy alone in terms of survival, although they also acknowledged that the value of adjuvant systemic therapies is not established. They suggested that patients with breast AdCC should be under long-term follow-ups as late relapses are possible.

Considering very low involvement of regional lymph nodes in breast AdCC and the inherent complications associated with axillary staging, another dilemma is whether to pursue axillary lymph node staging or dissection in patients with clinically negative axillary lymph nodes or not.

In a study on 20 patients with breast AdCC, preoperative axillary ultrasound was normal in 10 and suspicious in 3 of 13 women who had a subsequent negative lymph node fine-needle aspiration (FNA). Fifteen patients (75%) had sentinel lymph node surgery and were pathologically node-negative, while the remaining 5 had no axillary surgery. After a median follow-up of 3.6 years (range: 0.2–38.6 years), three patients experienced an in-breast recurrence. They observed no cases of nodal metastasis in 20 patients and concluded that preoperative axillary ultrasound with FNA of suspicious nodes could accurately predict pathologic nodal stage, and that surgery might be omitted safely in patients with pure breast AdCC and a clinically negative axilla. In the study of Khanfir et al., which was previously mentioned, 84% of the 61 node-negative women with breast AdCC had axillary lymph node dissection or sentinel node biopsy, concluding that axillary lymph node dissection or sentinel node biopsy might not be recommended. In Arpino et al., axillary lymph node dissection was performed in 23 patients with breast AdCC, only one of whom (4%) had histologic positive lymph nodes (2 of 10), and no recurrence was detected for the patient. They concluded that axillary lymph node dissection is not helpful in clinical management of patients with breast AdCC.

Considering all these reports and reviews, mastectomy as well as BCS with adjuvant radiation appear to be reasonable surgical approaches for local treatment of breast AdCC, with equivalent oncologic outcomes. The need for axillary staging in clinically negative axillary lymph nodes, as well as the need for adjuvant chemotherapy, is still unclear. The importance of prospective randomized trials to elucidate the best treatment option cannot be overemphasized.

Breast AdCC is a rare type of invasive breast cancer with good prognosis and low rate of regional lymph node and distant organ involvement. Although there is currently no universal agreement on the best treatment, different types of therapy, including BCS and mastectomy— with or without axillary staging, with or without adjuvant chemoradiation have been used, all with overall good to excellent long-term results. Prospective controlled trials will help in determining the best treatment options.

The decision to report this case was completely explained to the patient and her informed consent was obtained.

Conflict of Interest
None to declare.

References


Since Angelina Jolie broke the news of her bilateral mastectomy and oophorectomy, most breast surgeons have regularly been facing the question of this type of surgery whatever the age of the patient. Familial breast cancers have received a lot of media coverage. They are often aggressive with bad prognosis, and the discovery of predisposing genes, such as mutated BRCA1 and BRCA2, has opened a window of hope. For the first time it was possible to prevent cancer by the identification of the gene and removal of the targeted organ.

Surgery and the identification of constitutional genomic alterations have been merged for the benefits of our patients. However, several difficulties appeared quickly in the management of the patients according to their presentation with cancer or before the cancer diagnosis. Bilateral mastectomy could be discussed when the patient is tested positive for BRCA mutation. However, when the patient had cancer in a high-risk family, until recently, the results of the tests took very long to obtain, and this prevented the medical team from offering the best choice to the patient. This is not the case anymore, and the results can now be obtained in a few weeks for a reasonable price. On the other hand, aside from the BRCA mutations, new predisposition genes have been discovered, including high-penetrance p53, and PALB2, and other genes called VUS (variants of unknown significance) in which the risk is unknown or very low. Therefore, delivering comprehensive information is increasingly becoming critical for clinicians.

To go back to the title of the paper, I will describe three situations in which legal aspects were involved, and allow to open a discussion. The first one is the story of a patient who tested positive for BRCA1 and was offered a bilateral prophylactic mastectomy with immediate reconstruction and bilateral oophorectomy. Several members of her family had died of breast cancer and she was initially very happy to undergo surgery. A couple of years later, she told me at the clinic, “Thank you, Doctor, for saving me from dying, but I am not a woman anymore.” Early menopause, loss of libido, difficult sexual relationship, depression, and a future divorce had made her life very difficult. The patient had had several sessions of counseling with the geneticist, cancer surgeon, plastic surgeon, her gynecologist, and a psychologist before surgery. Despite all the medical advice and the fact that all the medical management was uneventful, she remains actually severely depressed and she will probably lose her job soon.

The second one describes a patient who was initially treated for breast cancer with breast-conserving therapy followed by chemotherapy, radiation therapy, but not hormonal therapy, because she had triple-negative breast cancer. She was a heavy smoker. Her younger sister developed breast cancer some years later. They both were tested for the mutation and were positive for BRCA1. She was advised to undergo prophylactic surgery, but she delayed the decision for 8 years for personal reasons. Finally, she decided to be operated on and asked for a bilateral deep inferior epigastric perforators (DIEP). After the surgery, she had a bilateral DIEP necrosis, with several months of healing with vacuum assisted closure (VAC) therapy and secondary healing. Finally, her breasts were reconstructed with several lipofillings over a 3-year period. Like the first patient, she was severely depressed and lost her job.

The third story is the story of a 35-year-old woman who was diagnosed with triple-negative...
breast cancer. She received neoadjuvant chemotherapy, followed by a lumpectomy with an oncoplasty and a contralateral symmetrization during the same operative time. There was a complete pathologic response. During the neoadjuvant treatment, the medical oncologist had asked for the presence of the mutation. The result, however, was delivered to the surgeon a month after the surgery and was positive for BRCA1.

Any surgeon involved in breast cancer management and reconstruction has similar stories to tell. How can we protect ourselves from legal issues arising from the cases similar to the first two cases?

The legal aspects of this new field of prophylactic surgery, i.e., removing an organ to prevent the occurrence of cancer, varies according to the countries. In France, for instance, surgery constitutes “voluntary assault and battery,” but, since it is performed to prevent life-threatening conditions, it is not subject to legal action. Moreover, in the United States, when a patient decides to be operated on, if he or she signs the informed consent form, normally no lawsuit is filed against the surgeon. The legal aspects vary from one country to another.

As doctors, we must remain aware that our patients are in a permanent state of stress, first of all, because they have cancer. In addition, they have a predisposing mutation, and everything is again increased when a postoperative complication occurs. Complications after the surgery are always disastrous in breast cancer patients, but it is even worse when they occur after the hope given by the idea of prevention.

This is our responsibility as surgeons to avoid and limit the side effects of our treatments by providing the most comprehensive information possible. However, when a patient in the USA requires, due to the presence of an identified mutation, a bilateral prophylactic mastectomy for a T1N0 breast cancer, we can be surprised. Actually, about 50% of American women ask for this type of surgery. The data referring to a 30%–40% complication rate seems unable to prevent the patients from this obviously unnecessary surgery. However, American scholarly journals, as well as media, sponsored by famous surgeons, keep presenting data in favor of this type of surgery, supported by the female advocacy organizations.

If we want to avoid needless surgery in treatment of our patients and limit the consequent lawsuits, bilateral surgery must be exclusively reserved to the carriers of the mutated genes (except patients with severely dystrophic breasts).

The multidisciplinary approach (involving a geneticist, cancer surgeon, plastic surgeon, gynecologist, and a psychologist or psychiatrist) is mandatory to give the patient a more objective and comprehensive preoperative information.

The constant postoperative depression is increased by bilateral surgery, and the patients must be correctly informed about it before and after surgery. Although a good cosmetic result would limit these side effects, it is not sufficient to prevent the personal problems induced by the surgery, which will be most certainly complicated over the postoperative course. In addition, it is important to protect ourselves from patients’ or their families' aggressiveness when deciding this complex surgery. Documentation of all the given information, orally and written, is necessary and can help demonstrate the objectivity and honesty of the medical team facing a patient who had put a great hope in the preventive surgery to remove the fear of cancer death but actually has to deal with the usual postoperative problems. There are few chances are that artificial intelligence will be able to solve that kind of questions in the near future.

References
**Background:** Women with atypical hyperplasia are about 4 times more likely to develop breast cancer compared with the general population. Atypical hyperplasia has been recommended to be used as a criterion for the inclusion of women in chemoprevention programs. Chemoprevention offers promise as a strategy for reducing the incidence of breast cancer in high-risk population.

**Methods:** A literature search was conducted in PubMed and Scopus databases using the search terms “breast atypia,” “chemoprevention,” and “risk-reducing therapy” for papers published from 1966 to Aug 2017. The search was limited to English-language papers and human studies. It yielded 114 search items. Article selection for possible inclusion was performed using the title and abstract. Finally, 12 studies were identified as eligible for inclusion in the review.

**Results:**

The rates of atypical ductal hyperplasia (ADH) ranged from a low of 2 per 10000 mammograms in 1995 to a high of 6 per 10000 mammograms in 2011. Lobular neoplasia was an incidental finding in 0.5%–3.5% of core biopsies. True incidence of lobular neoplasia is unknown. Women with atypical breast lesions have a 5%–11% risk of developing breast cancer within 5 years and a 17%–26% risk of developing breast cancer within 10 years. The reported risk of breast cancer with atypical hyperplasia (ADH and ALH are often grouped together) is approximately 19% within 15 years. It is believed that the initiation of chemoprevention would be appropriate; if the 10-year breast cancer risk is 4% to 8%. Breast cancer risk reduction by chemoprevention is reported to be 32% to 55% in breast atypia.

**Conclusion:** According to our findings, patients with a diagnosis of ADH, ALH, or severe ADH should be considered for chemoprevention if they are at least 35 years of age and have no contraindications to treatment. Only 4%–20% of high-risk women decide to take chemoprevention, on average.
women with atypical hyperplasia [atypical ductal hyperplasia (ADH) and lobular hyperplasia (ALH) are often grouped together] is approximately 19% within 15 years. The propensity score demonstrated that 54.7% of cancers in women could have been prevented with the use of chemoprevention at a mean follow-up of 68 months.

The standard of care for ADH found on core biopsy remains excision. Following excision, patients with ADH should be referred for breast cancer risk assessment and risk reduction counseling. Routine excision following a core biopsy diagnosis of lobular neoplasia is not indicated in cases with radiographic-pathologic concordance. Patients with a core biopsy diagnosis of lobular neoplasia (which includes ALH and classic LCIS), deemed concordant with imaging, should be counseled regarding the increased future breast cancer risk conferred by these lesions and the need for continued surveillance.

Age and estrogen exposure (early menarche, late menopause, nulliparity, use of exogenous hormones) are risk factors for breast cancer. High risk populations for breast cancer are considered to be women with ductal or lobular carcinoma in situ, women with a history of thoracic radiotherapy before the age of 30 years, women who have a 5-year breast cancer risk of ≥ 1.7%, and women with atypical hyperplasia. According to the modified Wellings-Jensen model of breast cancer evolution, benign breast lesions such as ADH, a result of hyperplastic enlarged lobular unit formation, may evolve into ductal carcinoma in situ and, eventually, develop into invasive breast malignancy. Currently, women with atypical hyperplasia are not included in the guidelines for high-risk women, and it has been recommended that the updated versions include them. Multiple models have been developed to help with prediction of breast cancer risk, including the Gail model, the Tyrer-Cuzick model, the Claus model, and several others.

Chemoprevention uses pharmacologic or natural agents to prevent cancer development. Women aged 35 years and older who are at high risk of breast cancer (with a 5-year predicted risk for breast cancer of at least 1.66%) are recommended to take breast cancer risk-reducing agents such as tamoxifen. A key driver of breast carcinogenesis is estrogen receptor α (ESR1) signaling. Consequently, agents that modulate ESR1 activity or deplete its ligand decrease breast cancer incidence. Tamoxifen has previously been shown to reduce circulating IGFBP-1, a surrogate endpoint biomarker for phase II chemoprevention trials. Tamoxifen significantly downregulates known estrogen response genes, such as ESR1 and SERPINA3, but, notably, the Ets oncogene family transcription factor ETV4 was also significantly downregulated. Tamoxifen exerts a significant antiproliferative effect in some, but not all women, regardless of menopausal status.

Tamoxifen has been shown to reduce the incidence of estrogen receptor-positive, but not estrogen receptor-negative, breast cancer. NSABP P-1 trial recommended the use of atypical hyperplasia as a measure for the inclusion of women in chemoprevention programs.

The purpose of this study was to review the literature on the risk of breast cancer based on ductal or lobular atypia to determine the effectiveness of chemoprevention in decreasing this risk. Information provided in this review will benefit health care providers who care for women at high risk for breast cancer by providing necessary information on development and delivery of breast cancer prevention. It will help readers of the chemoprevention literature to understand its strengths and weaknesses.

Methods
PubMed and Scopus databases were searched in August 2017 using the search terms “breast atypia,” “chemoprevention,” and “risk-reducing therapy.” The search returned 114 results. After initial screening of titles and abstracts, 62 papers were identified for potential inclusion in the review. Reference lists of these papers were also reviewed for relevant articles. We identified 28 studies that addressed breast cancer chemoprevention in breast lesions with atypia. We searched for papers whose main goal was evaluation of breast cancer risk reduction in atypical breast lesions (ADH and ALH) by chemoprevention. Our search was limited to English-language papers and human studies. A total of 12 studies met all inclusion criteria.

Results
Incidence of Atypical breast lesions
The rates of ADH diagnosis ranged from a low of 2 per 10 000 mammograms in 1995 to a high of 6 per 10 000 mammograms in 2011. Lobular neoplasia was an incidental finding in 0.5%–3.5% of core biopsies. True incidence of lobular neoplasia was unknown.

Atypia as a cancer precursor
Women with atypical breast lesions have 5%–11% risk of developing breast cancer within 5 years and a 17%–26% risk of developing breast cancer within 10 years. McEvoy and colleagues retrospectively evaluated the outcomes of the lesions in women under age 35 diagnosed with ADH, ALH, lobular carcinoma in situ (LCIS), and severe ADH from 1987 to 2010. They concluded that young women with atypical breast lesions were significantly more likely to develop breast cancer and recommended that they be followed closely. A meta-analysis by Dyrstad et al found the summery risk estimate for developing breast cancer following
a biopsy showing atypical hyperplasia not otherwise specified was 3.93 (95% CI: 3.24–4.76). A large cohort study at the Mayo Clinic also found a similar increased risk of breast cancer in women with atypical hyperplasia. They found that 30% of the women had developed breast cancer (in situ or invasive) 25 years after the atypical hyperplasia was confirmed by biopsy and that the number of foci of the hyperplasia was correlated with the risk of breast cancer.  

Khan et al. suggested that the use of antiestrogen agents such as tamoxifen might be an effective strategy for preventing breast cancer in women with benign breast disease (BBD) as patients with BBD who had estrogen receptor-positive epithelium were more likely to develop breast cancer later in life.  

**Table 1. Risk estimate of breast cancer after diagnosis of atypical lesion**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Atypical lesion</th>
<th>OR</th>
<th>Cancer development</th>
<th>Mean (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyrstad et al.24</td>
<td>Atypical hyperplasia</td>
<td>3.24-4.76</td>
<td>(0.95 CI)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADH</td>
<td>2.54-4.23</td>
<td>(0.95 CI)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ALH</td>
<td>2.81-5.47</td>
<td>(0.95 CI)</td>
<td></td>
</tr>
<tr>
<td>Zhou et al.27</td>
<td>ADH</td>
<td>2.93</td>
<td>(0.95 CI)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ALH</td>
<td>5.14</td>
<td>(0.95 CI)</td>
<td></td>
</tr>
<tr>
<td>Coopey et al.5</td>
<td>ADH</td>
<td>17.3%</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ALH</td>
<td>20.7%</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LCIS</td>
<td>26.7%</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe ADH</td>
<td>26%</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atypical lesion</td>
<td>184 (11.1%)</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>(1658 patients without treatment)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McEvoy et al.25</td>
<td>Atypical lesion (58)</td>
<td>7 (12%)</td>
<td>68</td>
<td></td>
</tr>
</tbody>
</table>

Tamoxifen treatment has also been shown to reduce the risk of BBD by 28% (RR = 0.72, 95% CI: 0.65–0.79) and the risk of biopsy by 29% (RR = 0.71, 95% CI: 0.66–0.77).  

Coopey and colleagues evaluated women’s risk of breast cancer based on atypia type to determine the effectiveness of chemoprevention in decreasing the breast cancer risk. They analyzed 2938 women diagnosed with atypical breast lesions and 2459 patients with atypia in 1999 and beyond. They reported that chemoprevention had significantly reduced breast cancer risk for all atypia types (P = 0.001). In terms of age at atypia diagnosis, women aged 50 years and older (age range: 50–93) had a statistically significant reduction in breast cancer risk with chemoprevention (P = 0.001, HR = 0.34). The hazard ratio for invasive cancer with chemoprevention compared with no chemoprevention was 0.48, suggesting a 52% reduction in risk with chemoprevention (P = 0.029). Similarly, the hazard ratio for ductal carcinoma in situ with chemoprevention compared with no chemoprevention was 0.45, suggesting a 55% risk reduction with chemoprevention (P = 0.024). They calculated that if all women diagnosed with atypia in 1999 and beyond took chemoprevention, they could have prevented 69 out of the observed 126 (54.7%) cancers that occurred during that time period. They concluded that the breast cancer risk in atypical breast lesions could be reduced by an estimated 50% at 5 years (from 8.3% to 4.1%) and 65% at 10 years (21.3% to 7.5%) using chemoprevention.  

Four large trials with long-term follow-up have been conducted to evaluate the efficacy of tamoxifen treatment in preventing breast cancer in high-risk women: the Royal Marsden tamoxifen prevention trial, National Surgical Adjuvant Breast and Bowel Project Breast Cancer Prevention Trial (NSABP-P1), an Italian study, and the International Breast Cancer Intervention Study I (IBIS-I).  

The NSABP-P1 (1992–1997) included a total of 13954 patients, of whom 13388 (95.9%) were randomly assigned to receive, in a double-masked manner, 20 mg/day of either tamoxifen or placebo for 5 years. The incidence of breast cancer, both invasive and noninvasive, was reduced significantly as a result of tamoxifen administration. The number of cancer events in the placebo group was almost twice as many as that in the tamoxifen group. The number of invasive breast cancer cases was 175 in the placebo group vs 89 in the tamoxifen group (P < 0.00001), corresponding to a 49% reduction in the overall risk. Women who were ≥ 60 years old at randomization experienced a greater tamoxifen-associated reduction in breast cancer incidence compared with those who were < 50 at randomization (55% vs 44%). Subgroup analyses revealed that chemoprevention had reduced the risk of breast cancer by 56% among women with a history of LCIS and by 86% in women with a history of atypical hyperplasia.  

The Royal Marsden trial randomized 2471 eligible high-risk women to take either placebo or tamoxifen (20 mg daily) for eight years.
follow-up evaluation after a median of 18.4 years from randomization (years after the 8 years of tamoxifen was complete), the researchers noticed a 32% reduction in ER/PR-positive primary breast cancer incidence, a demonstration of the consistent “carryover protective effect” of long-term estrogen deprivation therapies used to treat or prevent breast cancer.

In a meta-analysis by Ropka and associates, patients’ decision about breast cancer chemoprevention was evaluated. They reviewed 9 studies that had reported the hypothetical uptake, i.e., the rate of participants’ showing interest in getting chemoprevention medication, and 5 reporting real uptake, namely, acceptance of chemoprevention medication (mean uptake rates: 24.7% and 14.8%, respectively). Using logistic regression, they found significant correlation with type of decision (hypothetical versus real, OR: 1.65; 95% CI: 1.26–2.16), educational or decision support intervention (provided vs not, OR: 0.21; 95% CI: 0.17–0.27), and cohort risk for breast cancer (high-risk vs general population, OR: 0.65; 95% CI: 0.56–0.75). Increased uptake was consistently correlated with perceived vulnerability, and reduced uptake was correlated with concern for adverse effects.

Discussion

In this study, we reviewed published studies evaluating the association of biopsy-proven atypical lesions of the breast with the risk of developing breast cancer. Proliferative BBD with atypia confers a relative risk for future breast cancer of 4.1 24. The mean duration from initial diagnosis of BBD to diagnosis of breast cancer was 9.4 years.25 As the screening methods improve, the diagnosis of atypical lesions at an early stage increases, and this relative risk is shown to be even higher in more recent studies. There are reports indicating that women with atypical breast lesions have a 5%–11% risk of developing breast cancer within 5 years and a 17%–26% risk of developing breast cancer within 10 years. For LCIS, prior studies have found a 4% risk of breast cancer at 5 years, a 7%–13% risk at 10 years, and a 17%–28% risk at 15 years.2 In the Mayo Clinic study also found a similar increased risk of breast cancer in women with atypical hyperplasia. They found that 30% of the women who had developed breast cancer (in situ or invasive) 25 years after the atypical hyperplasia was confirmed by biopsy and that the number of foci of the hyperplasia was correlated with the risk of breast cancer.

A recently published consensus statement on the use of preventive therapy for breast cancer recommends that the initiation of chemoprevention would be appropriate if the 10-year breast cancer risk is 4%–8%.27 It is estimated that up to 50% of breast cancers could be prevented among women at least 35 years old at elevated risk using currently available chemoprevention strategies.2 The American Society of Clinical Oncology recommends discussion of chemoprevention with women who have an increased 5-year risk for breast cancer and endorses consistent use of benign breast biopsy morphology for risk classification.28 According to our findings, patients with a diagnosis of ADH, ALH, LCIS, or severe ADH meets this criterion and should be considered for chemoprevention if they are at least 35 years old and have no contraindications to treatment. Contraindications to treatment with tamoxifen include the following: history of deep venous thrombosis, thrombotic stroke, pulmonary embolus, known inherited clotting trait, transient ischemic attack, and pregnancy.29 Furthermore, the risk to benefit ratio of chemoprevention needs to be individualized for each patient based on menopausal status, presence of a uterus, and other comorbidities.

Most women elect not to take chemoprevention agents despite the overall risk reduction established. Only 4%–20% of high-risk women decide to take chemoprevention, on average.3 Contributing factors to this decision include fear of potential side effects, physicians’ not recommending it, and patients’ perception that their breast cancer risk is not that high.3

### Table 2. Chemoprevention effect on breast cancer risk reduction in breast atypia

<table>
<thead>
<tr>
<th>Study</th>
<th>No.</th>
<th>Person-Years</th>
<th>Observed events</th>
<th>Expected events</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayo-cohort 1</td>
<td>331</td>
<td>4543</td>
<td>66</td>
<td>17</td>
<td>0.388</td>
<td>3.00–4.94</td>
</tr>
<tr>
<td>NSABP-P1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women assigned to tamoxifen</td>
<td>6681</td>
<td>26154</td>
<td>89</td>
<td>0.51</td>
<td>0.39–0.66</td>
<td></td>
</tr>
<tr>
<td>Women assigned to placebo</td>
<td>6707</td>
<td>26247</td>
<td>175</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boston 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atypia group Without chemoprevention</td>
<td>1658</td>
<td></td>
<td>184 (11.1%)</td>
<td>21.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atypia group assigned to chemoprevention</td>
<td>615</td>
<td></td>
<td>36 (5.8%)</td>
<td>7.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RMPT 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atypia group assigned to chemoprevention</td>
<td>2471</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*OR* = odds ratio, *HR* = hazard ratio.
Atypical hyperplasia is associated with a significant increase in the risk of developing breast cancer. This necessitates the development of management strategies such as risk-reducing therapies for women with benign breast lesions with atypia. There is still much room for improvement. Physicians should counsel patients with ADH and ALH, especially those with a life expectancy of ≥10 years, about the effectiveness of chemoprevention in decreasing their breast cancer risk. These women are considered to be at increased risk for breast cancer and must receive tailored counseling on how they might decrease their risk of breast cancer. Furthermore, the risk to benefit ratio of chemoprevention needs to be individualized for each patient.

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ABSTRACT

**Background:** For many years, the acceptable margins of the resections for ductal carcinoma in situ (DCIS) has been 2 mm, although, in some reports and the recent updates of some guidelines, the closer margins are also declared as acceptable in some circumstances. Despite these new recommendations, the safe margin in DCIS remains a matter of controversy in many institutional and national guidelines.

**Case Presentation:** A woman with invasive breast cancer with associated DCIS presented to our clinic. She underwent breast-conserving surgery, and pathology report showed one focus of DCIS at a distance of < 1 mm from inked margin. This case was presented in the weekly breast multidisciplinary team session of the Department of Breast Surgery, Tehran University of Medical Sciences.

**Question:** The question was whether the patient should be operated again to obtain more extensive margins for DCIS or the radiation therapy would be enough as the next step in her treatment.

**Conclusion:** According to the latest published guidelines, the members of panel decided to accept the margin and informed the patient about the risk of recurrence and the need for adjuvant radiotherapy and follow-up modalities.

**Introduction**

Ductal carcinoma in situ (DCIS) is the growth of noninvasive malignant cells in the lumen of the mammary ducts. Screening mammography helps to detect breast lesions in an early stage. Therefore, the diagnosis rate of DCIS has increased in the past two decades because of early detection.¹

Breast-conserving surgery (BCS) with adequate margins and radiotherapy reduces the risk of invasive recurrence in low-risk DCIS group, although radiotherapy is more likely to be planned for patients with DCIS who have large (> 15 mm), intermediate- or high-grade tumors, or present with comedo-type central necrosis.²

The adequate margin in BCS for DCIS is a challenging subject. In one study, the recommended minimum adequate clear margins in low- or intermediate-grade DCIS, measuring less than 2.5 cm, was ≥ 3 mm.³ A consensus statement by the Society of Surgical Oncology (SSO) and the American Society for Radiation Oncology...
(ASTRO) considered 2 mm as standard margin that should be complemented by whole-breast irradiation. These therapeutic options are associated with lower rates of ipsilateral breast tumor recurrence (IBTR) and reexcision. Moreover, the consensus marked “no ink on tumor” as the adequate margin for invasive tumors. The acceptable free margin is 1 mm for both in situ and invasive cancers according to the Association of Breast Surgery (ABS). However, a new meta-analysis found no significant difference between 2-mm and more than 2-mm surgical margins in resection of DCIS. The study recommended 2 mm as the minimum acceptable safe margin in patients undergoing BCS and radiotherapy.

The present article aims to explore this challenge and make the best decision for a patient whose DCIS was removed with the resection margins of <1 mm.

Case Presentation
A 60-year-old woman with no family history of breast cancer referred to our breast clinic with the chief complaint of the right breast mass. The mass (30 × 20 mm) was palpated in the upper outer quadrant of her right breast (near zone) without palpable axillary lymph node.

In mammography, there was an ill-defined mass containing suspicious microcalcifications in the upper outer quadrant of the right breast with a BI-RADS score of 5 (Figure 1). Ultrasonography reported a 16 × 14 mm irregular, hypoechoic mass in upper outer part of the right breast, and the axillary lymph nodes were reported as reactive. The results of core needle biopsy of the breast mass was indicative of invasive ductal carcinoma with concomitant in situ component with comedo necrosis.

After preparation of patient for surgery, she underwent breast conserving surgery along with oncoplastic repair (Round Block technique). Specimen mammography during surgery was performed to be certain of complete excision of the microcalcifications. Frozen study for sentinel lymph node was performed reported negative for malignancy and was confirmed in permanent evaluation. Lumpectomy specimen revealed a 35 mm firm ill-defined mass with histologic diagnosis of invasive and in situ ductal carcinoma. Ductal carcinoma in situ constituted about 20% of the tumor and lymph vascular invasion was also noted. All surgical margins were defined free except for the inferior margin which involved by DCIS with distance of 2 mm. Immunohistochemistry assessment was positive for estrogen receptor, but negative for progesterone receptor and HER2 neu with proliferative activity (K167) of about 20%.

The patient was scheduled for further imaging studies to assess systemic distribution of the tumor as well as reoperation to excise the involved margin (figure 2, 3, 4). In the second operation, the inferior margin re-excision was performed with acceptable distance from the tumor grossly. Complete excision of residual microcalcifications (tow groups that were localized with wires before second surgery) certained by specimen mammography (figure 5).

In pathologic assessment of re-excised inferior margin, multiple foci of low grade DCIS with minimum distance of 1 mm to inked surgical margin was reported. The cosmetic outcome after the second operation was good again, and there were no complications associated with either surgery. All the systemic radiologic assessments were negative for metastasis.

Figure 1. Bilateral mammography showed microcalcifications in upper outer quadrant of right breast.
Figure 2. Mammography of right breast. In CC and MLO views microcalcifications and surgical clips can be seen.

Figure 3. Mammography of right breast. Wire localization of microcalcifications are shown in CC and MLO views.

Figure 4. Focal compression magnification view (CC)

Figure 5. Specimen mammography which shows microcalcifications have been excised.

Question
The case was presented in the weekly breast multidisciplinary team session of the Department of Breast Surgery, Tehran University of Medical Sciences. The question was whether the patient should be operated again to obtain more extensive margins for DCIS or radiation therapy would be enough as the next step in the treatment of the patient.

Discussion
Breast-conserving therapy is the mainstay of treatment in most breast cancer patients for both invasive and in situ carcinomas. The therapy includes BCS, i.e., excision of the tumor with a margin of normal breast tissue, followed by whole-breast radiation therapy (WBRT). For many years, the acceptable resection margin for DCIS has been 2 mm, although, in some reports and the new updates of some guidelines, the closer margins are also marked as acceptable in some circumstances. Despite these new recommendations, the safe margin in DCIS remains a matter of controversy in many institutional and national guidelines.

Changes in guidelines in the past years
According to the Society of Surgical Oncology (SSO) and the American Society for Radiation Oncology (ASTRO) guidelines in 2014, the rule of “no ink on tumor” is now accepted and efficient for invasive breast tumors. Nevertheless, in the case of DCIS, the recommendations in different guidelines have changed during the past few years. While the rule of “2-mm margins” was recommended by SSO-
ASTRO-ASCO (American Society of Clinical Oncology)\textsuperscript{4,9} and the National Institute for Health and Clinical Excellence (NICE),\textsuperscript{9} the Association of Breast Surgery (ABS) of the United Kingdom emphasized in 2015 that just 1-mm margin would be sufficient in resection of DCIS.\textsuperscript{10} Recently, the National Comprehensive Cancer Network (NCCN) reiterated that “no ink on tumor” rule would be enough in management of DCIS as well as invasive ductal carcinoma. This recommendation was based on the National Surgical Adjuvant Breast and Bowel Project (NSABP) trials in 2017\textsuperscript{9,11} and the American Society of Breast Surgeons (ASBS) recommendations.\textsuperscript{9} This discrepancy has led to variations in management practice in breast surgery centers. For example, a study of the variation in margin policy for BCT in 79 sites in the UK and Ireland in 2016, found that 53.2% of units accepted 1-mm and 38% accepted 2-mm margins for DCIS.\textsuperscript{8}

Which margin width is supported by evidence?

A meta-analysis of 20 studies including a total number of 7883 DCIS patients with known margin status treated with BCT assessed the impact of margin width on ipsilateral breast tumor recurrence (IBTR). The status of ink on tumor (i.e. positive margin) was associated with higher rates of IBTR, whereas negative margins—especially those of at least 2 mm wide—had lower rates of IBTR. The effectiveness of margins when they were more than 2 mm is not supported by evidence.\textsuperscript{4,7,12,13} It should be taken into consideration that “negative margins” does not mean that there are not any residual DCIS in the breast.\textsuperscript{4,14} Even in unicentric DCIS, tumor can be multifocal with the skip involvement of a segment of the breast.\textsuperscript{7} This may happen in about two-third of well-differentiated DCIS cases. It is worth mentioning that the skipped lesions are mostly in 0 to 5 mm distance from the main lesion.\textsuperscript{11}

Evidence shows significant decrease in IBTR for 2-mm margin in comparison with no ink on tumor (\textgreater{} 0 or 1 mm).\textsuperscript{4} The other important factors in IBTR risk are biology of the tumor, younger age, symptomatic presentation, presence of necrosis,\textsuperscript{4,14} side of anterior (skin) or posterior (pectoral fascia) margins, remnant microcalcification on mammography after excision, volume of DCIS tumor near the margin, cosmetic status after reexcision, life expectancy of the patient,\textsuperscript{4} and the adjuvant chemotherapy for invasive component or endocrine therapy.\textsuperscript{14}

A study by Edinburgh Breast Unit on 466 patients with pure DCIS treated with BCS between 2000 and 2010, showed no increase in the rate of IBTR with margins of 1–2 mm in comparison with margins \textgreater{} 2 mm. The researchers concluded that margin width of 1 mm is sufficient in BCS of DCIS.\textsuperscript{12}

In a study by Memorial Sloan Kettering Cancer Center on 2996 cases of DCIS who underwent BCS from 1978–2010, in cases not receiving WBRT, wider margins were significantly associated with a lower rate of IBTR and may not be necessary in cases treated with WBRT.\textsuperscript{11}

In a study by MD Anderson Cancer team on 1216 patients with DCIS undergoing BCS, at 10 years of follow-up, the researchers concluded that there was no significant difference in IBTR for patients with < 2-mm margins (no DCIS at the inked margin) who received adjuvant WBRT compared with patients with \textgreater{} 2-mm margins, although local recurrence was significant in cases with < 2-mm margins not receiving WBRT.\textsuperscript{9}

What is appropriate in the guidelines?

Pure DCIS: Most guidelines emphasized on 2-mm margin for pure DCIS but a margin of < 2 mm by itself is not an indication for mastectomy.\textsuperscript{11}

DCIS with microinvasion: (size of invasive focus: \textless{} 1 mm) rates of IBTR in microinvasive carcinomas are more similar to DCIS\textsuperscript{14} and they should be managed as DCIS for optimal margin.\textsuperscript{11}

Invasive cancer with associated DCIS: When the \textit{in situ} component is more than 25% of the tumor bulk [called extensive intraductal component (EIC)], or the patient has lesser scattered foci of \textit{in situ} carcinoma, management is more similar to invasive cancer than pure DCIS, even when the closer margins contain DCIS.\textsuperscript{4} It is worth emphasizing that IBTR rates in patients with EIC-positive tumors with negative margins at BCS are equal to cases without EIC.\textsuperscript{14} Based on NSABP trials, which defined negative margin as “no ink on tumor,” clinical experience of the physician is required for the decision of reexcision in patients with margin width of < 2 mm.\textsuperscript{11}

Multidisciplinary team (MDT) recommendation

For this patient with foci of DCIS at a distance of < 1 mm from inked margin, members of breast MDT in Breast Division, Imam Khomeini Hospital, Tehran University of Medical Sciences recommended that neither reexcision of the margins for the inferior side of resection nor mastectomy was needed. Factors that promoted MDT to accept width margin were the presence of an invasive component in 80% of the specimen, the age of the patient, patient’s request for breast conservation, negative EIC, plan of adjuvant breast radiotherapy, plan of adjuvant chemotherapy and endocrine therapy. Thus, MDT members decided to accept the margin and informed the patient about the risk of recurrence and the need for adjuvant radiotherapy and follow-up imaging. The patient was referred to adjuvant chemotherapy and then adjuvant radiotherapy followed by endocrine therapy and follow-up imaging was planned for her.

References

1. Burstein HJ, Polyak K, Wong JS, Lester SC,
A Study on Factors Predisposing to Breast Ptosis

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\textsuperscript{b} Mallinckrodt Institute of Radiology, Department of Radiology, Washington University School of Medicine, Saint Louis, Missouri, USA
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\textsuperscript{d} Sina Trauma and Surgery Research Center, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

**Background:** Despite being a frequent plastic surgery complaint, the causes and predisposing factors for breast ptosis have not been studied profoundly. Studying ptosis causative factors will improve prevention, patient select and education, surgical outcome, and patient education. The present study aims to demonstrate the potential predisposing factors for breast ptosis.

**Methods:** In a 6-month study was conducted at the research department of Kaviani Breast Diseases Institute, Tehran, Iran, all female patients referring to the breast clinic were assessed. Patients with a background of severe comorbidities, history of breast surgery, and breast cancer were excluded. Data on demographic characteristics, current and past medical history, physical examination, and ptosis presence grade were collected.

**Results:** A total number of 141 patients, with the mean age of 35.8 years, were included. About 72% of the patients had varying grades of breast ptosis. Patients with ptosis tended to be of older age, weight, BMI, and brassiere size, were more likely to be menopausal, and had begun wearing brassiere at younger ages. The ordinal model revealed an association between ptosis and age, age at wearing brassiere, current breast size, and smaller cup size in patients.

**Conclusion:** We suggest age and breast size as the predisposing factors for breast ptosis. In our study, there was no relation between breast ptosis and history of lactation or the number of pregnancies. The effects of hormonal and menstrual status, as well as drinking and smoking habits, need to be investigated further.
the female body and physiology. To the best of our knowledge, very few studies have assessed the etiologic factors of breast ptosis or have addressed the present controversies regarding the probable causes, and most were focused on lactation from a maternity rather than plastic surgery aspect. The importance and need for such studies will be prominent when one considers that knowing the risk factors not only can improve the efficacy of prevention, patient selection for surgical corrections, and the clinical outcome of such interventions, but also helps to educate patients and clarify some cultural notions. For instance, the low rates of breastfeeding in both developed and developing countries have not changed after several years of education for fear of adverse effects on breast shape and cosmetics as one of the main mentioned causes. Surprisingly, this wrong notion does not seem to be restricted to specific cultural or geographical distribution. One can assume that understanding the actual predisposing factors will address the aforementioned advantages in plastic surgery. Considering these facts, we designed the present study to demonstrate and determine the probable risk factors for breast ptosis.

Methods

Study Design and Participants

Female patients referring to Kaviani Breast Diseases Institute in Tehran, Iran, between January and June 2013 were assessed for the presence of ptosis and its grade. We used the classification introduced by Regnault in 1976 for grading of ptosis. This classification is based on the position of the nipple: Grade I ptosis is described as the nipple being located at or up to 1 cm below the inframammary fold (IMF); in grade II, the nipple is located at a level 1 to 3 cm below IMF; and grade III ptosis is defined as the nipple being located more than 3 cm below IMF or at the inferior pole of the breast.

Patients with a history of breast surgery, breast cancer, or severe underlying medical conditions causing weight change in a short period (at least a month), including Cushing’s syndrome, Addison’s disease, severe thyroid diseases, and metabolic syndrome, and all patients requesting cosmetic breast surgeries were excluded from the study. All the questionnaires were anonymous, and patients’ data were kept confidential during the study. The study protocol was approved by the Tehran University of Medical Sciences Ethics Committee, and the informed consent was taken from patients after explaining the study and emphasizing on the confidentiality of the collected data.

Definition of Variables

The data regarding patients’ demographics and history, such as age, age at menarche, menopause age, age at beginning to wear brassiere, weight, BMI, education level, number of pregnancies, total number of lactating months, brassieres size before pregnancy and its changes, weight gain after each pregnancy were gathered through a structured interview (all the data were self-reported except for weight and height, which were measured by the same devices). In regard to smoking, patients were categorized into two groups of smokers/ex-smokers and nonsmokers. The same stratification was applied to alcohol consumption. Current brassiere and cup sizes were determined by a surgeon, using the underbust girth for the brassiere size according to the EN 13402 dress-size standard, and the alphabetic method for cup size, both of which are the current measuring systems in Iran as well.

Statistical Analysis

SPSS v. 19 (IBM Inc.) was used for data analysis. Data are presented as number (percent) or mean ± standard deviation. The means were compared using the student t test. Analysis of variance (ANOVA) was employed to compare the different grades of ptosis with interested risk factors. Factors showing significant association with ptosis in univariate analyses were put in a multivariate analysis to identify the independent associations between the potential risk factors of breast ptosis. A P value of 0.05 was considered the cut point for significant statistical difference.

Results

A total of 141 patients were included in the study. The patients’ mean age was 35.92 ± 9.42 years (ranging from 17 to 62 years). The mean age of menarche was 13.45 ± 1.58 years. The mean duration of lactation was 32.10 ± 18.70 months. Table 1 presents the detailed demographic specifications of the study population. A total number of 102 cases (72.3%) were diagnosed with varying grades of breast ptosis, while 37 (26.2%) did not have ptosis and 2 (1.5%) had pseudo-ptosis. The patients with pseudo-ptosis in the examination were assigned to the no-ptosis group. Moreover, we classified breast ptosis into two groups: minor ptosis (grade 1) and moderate to major ptosis (grades 2 and 3).

Patients with higher grades of breast ptosis tended to be of older age, higher weight, and BMI and were more likely to be menopausal. No significant differences were observed between the groups in the other variables (Table 2). Variables showing significant difference among the three groups in univariate analyses were put in an ordinal model to investigate the independent role of each one. As shown in Table 3, older patients were more commonly diagnosed with breast ptosis (95% CI: 0.020 to 0.109, P = 0.005). The younger age at wearing brassiere was associated with higher grades of breast ptosis (95% CI: -0.533 to -0.084, P = 0.007). Current brassiere and cup sizes were also
**Table 1.** Demographic characteristics of the study population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Categories</th>
<th>Mean ± SD or N (%)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>35.83 ± 9.42</td>
<td>65.68 ± 11.07</td>
<td>162.12 ± 5.85</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>24.48 ± 4.54</td>
<td>13.46 ± 1.57</td>
<td>45.91 ± 5.59</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>3.27 ± 1.36</td>
<td>31.86 ± 18.54</td>
<td>7.86 ± 4.53</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.227</td>
<td>0.529</td>
<td>0.529</td>
<td></td>
</tr>
<tr>
<td>Age at menarche</td>
<td>2.64 ± 1.36</td>
<td>1.73 ± 0.65</td>
<td>1.52 ± 0.65</td>
<td></td>
</tr>
<tr>
<td>Age at beginning to wear brassiere</td>
<td>14.97 ± 2.46</td>
<td>24.80 ± 4.56</td>
<td>58.71 ± 7.76</td>
<td></td>
</tr>
<tr>
<td>Age at first pregnancy</td>
<td>7.86 ± 4.53</td>
<td>27.54 ± 8.12</td>
<td>41.28 ± 5.18</td>
<td></td>
</tr>
<tr>
<td>Current or ex-smoker</td>
<td>0.001</td>
<td>0.078</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>Current or past alcohol consumption</td>
<td>0.001</td>
<td>0.078</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>Menopause age (N = 12)</td>
<td>0.001</td>
<td>0.078</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>0.001</td>
<td>0.078</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>Lactation duration (month)</td>
<td>0.001</td>
<td>0.078</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>Weight gain after each pregnancy (kg)</td>
<td>0.001</td>
<td>0.078</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>Current brassier size</td>
<td>0.001</td>
<td>0.078</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>Current cup size</td>
<td>0.001</td>
<td>0.078</td>
<td>0.078</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Comparison of the variables between ptotic and non-ptotic patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Categories</th>
<th>Mean ± SD or N (%)</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>30.05 ± 7.64</td>
<td>38.11 ± 9.77</td>
<td>38.24 ± 8.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at menarche</td>
<td>13.74 ± 1.73</td>
<td>13.48 ± 1.52</td>
<td>13.18 ± 1.52</td>
<td>0.227</td>
</tr>
<tr>
<td>Age at beginning to wear brassiere</td>
<td>14.97 ± 2.46</td>
<td>24.80 ± 4.56</td>
<td>58.71 ± 7.76</td>
<td></td>
</tr>
<tr>
<td>Age at first pregnancy</td>
<td>58.71 ± 7.76</td>
<td>27.54 ± 8.12</td>
<td>41.28 ± 5.18</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>22.13 ± 3.04</td>
<td>24.54 ± 4.25</td>
<td>28.88 ± 4.32</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>2.00 ± 1.26</td>
<td>2.20 ± 1.39</td>
<td>2.42 ± 1.36</td>
<td>0.610</td>
</tr>
<tr>
<td>Lactation duration (month)</td>
<td>36.11 ± 13.10</td>
<td>28.58 ± 15.65</td>
<td>35.09 ± 22.45</td>
<td>0.258</td>
</tr>
<tr>
<td>Number of breast-feedings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1 (10%)</td>
<td>1 (2.4%)</td>
<td>1 (2.8%)</td>
<td>0.437</td>
</tr>
<tr>
<td>1</td>
<td>2 (20%)</td>
<td>17 (40.5%)</td>
<td>14 (38.9%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6 (60%)</td>
<td>20 (47.6%)</td>
<td>13 (36.1%)</td>
<td></td>
</tr>
<tr>
<td>≥ 3</td>
<td>1 (10%)</td>
<td>4 (9.5%)</td>
<td>8 (22.2%)</td>
<td></td>
</tr>
<tr>
<td>Weight gain after each pregnancy (kg)</td>
<td>7.40 ± 3.16</td>
<td>37 (94.9%)</td>
<td>45 (97.8%)</td>
<td>0.764</td>
</tr>
<tr>
<td>Current or ex-smoker</td>
<td>No</td>
<td>37 (94.9%)</td>
<td>45 (97.8%)</td>
<td>0.764</td>
</tr>
<tr>
<td>Current or past alcohol consumption</td>
<td>Yes</td>
<td>2 (5.1%)</td>
<td>1 (2.2%)</td>
<td></td>
</tr>
<tr>
<td>Current brassier size</td>
<td>No</td>
<td>35 (89.7%)</td>
<td>44 (95.7%)</td>
<td>0.342</td>
</tr>
<tr>
<td>Current cup size</td>
<td>A</td>
<td>18 (46.2%)</td>
<td>3 (6.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>12 (30.8%)</td>
<td>13 (28.9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>9 (23.1%)</td>
<td>23 (51.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>0 (0%)</td>
<td>6 (13.3%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.** Multivariate analysis of factors with association with breast ptosis

<table>
<thead>
<tr>
<th>Variables</th>
<th>P value</th>
<th>95% CI</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.005</td>
<td>0.020</td>
<td>0.109</td>
<td></td>
</tr>
<tr>
<td>Age at beginning to wear brassiere</td>
<td>0.006</td>
<td>-0.533</td>
<td>-0.084</td>
<td></td>
</tr>
<tr>
<td>Current brassier size</td>
<td>&lt;0.001</td>
<td>0.117</td>
<td>0.268</td>
<td></td>
</tr>
<tr>
<td>Current cup size</td>
<td>&lt;0.001</td>
<td>0.489</td>
<td>1.461</td>
<td></td>
</tr>
</tbody>
</table>

*Nagelkerke = 58.8%*

Breast ptosis and predisposing factors

Despite being one of the most common cosmetic problems for women, only a few studies have evaluated the impact of possible risk factors on breast ptosis, and most of the studies about breast ptosis have referred to surgical techniques for solving the problem. As it can be assumed, breast ptosis is independently associated with ptosis (95% CI: 0.117 to 0.268, P < 0.001 and 95% CI: 0.489 to 1.461, P < 0.001).

The relationships between ptosis grade and different factors were investigated. The mean weight of patients was observed to be different for different grades of ptosis based on the ANOVA test (P < 0.001).
ptosis etiology has a multi-factorial nature and can be affected by dependent and independent factors such as race, culture, body composition, and age as well as hormonal changes. Regression of the glandular tissue due to hormonal changes after menopause or pregnancy, weight loss, skin pathologies, and previous surgery have been mentioned as potential causes of breast ptosis.\(^2^,\(^3\) All of the 12 menopausal patients in the present study had some degrees of ptosis, providing evidence for the aforementioned effect of menopause. None of the patients in this study had a history of breast surgery or dermatochalasis.

We observed an association between the cup size and ptosis grade. Patients with no ptosis were mostly size A, while patients with minor ptosis were size B, and more than half of the patients with moderate to major ptosis had a C cup size. In this study, we inquired the weight gain after the pregnancy to evaluate its effect. The mean weight gain after the pregnancy did not show statistically significant difference between the ptosis and no-ptosis groups. Unfortunately, we did not inquire the history of weight loss and were not able to evaluate its effect on breast ptosis.

The weight of the breast itself and the laxity degree of the breast-supporting ligaments are other proposed causative factors for breast ptosis. In the present study, we considered the breast cup size as a measure of the breast volume and mass. Although the cup size is not a perfect indicator of the breast weight due to variation in breast density and the several factors affecting it, the current cup size of the patients at the study time was higher in the ptosis group compared with the no-ptosis group. Meanwhile, as the mean weight of the ptotic patients was greater, the breast cup size and breast weight might have increased as a consequence of weight and body fat distribution. However, both factors lost their significant association with ptosis in multivariate analysis. Accordingly, the causative effect of the breast volume and weight need to be assessed in a more detailed study to check whether breast volume is an independent breast ptosis risk factor, or it is merely a manifestation of higher weight and BMI as main risk factors.

Laxity of the Cooper’s ligaments as another potential cause for ptosis, which is suggested mostly based on the individual observations, needs to be proved through scientific, experimental methods. As suggested by previous studies\(^1^8^,\(^1^9\), smoking is a cause for abnormal elastic characteristics of the skin, was studied in our patients along with drinking history. There were some limitations in our study to assess the effect of cigarette smoking or alcohol consumption on the development of breast ptosis. Considering a negative attitude towards female smokers in the society, we can assume that this fact might have influenced the overall prevalence of the smoking cases in our study through patients’ disclosure of their smoking status to the study team. Religious considerations can be applied to alcohol consumption besides the overall lower consumption of alcohol in the Iranian population, especially in females (relative frequency in total population = 2.31%, females = 0.56%).\(^2^0\)

None of the patients in the non-ptosis group were in the menopause phase of the reproductive physiology, while 11% (12 patients) of the ptotic patients were in this phase. This may be indicative of a protective effect of the female reproductive hormones, or it can just be a manifestation of the age factor. Our study not only confirms the previous finding that lactation has no effect on the development of breast ptosis, but adds to the literature by providing evidence for the lack of association between the number of pregnancies and breast ptosis.

Considering the available data, we suggest age and breast size as risk factors for breast ptosis. In addition, the age at beginning to wear brassiere was associated with having ptosis in our study, although it was not clear whether the patients began to wear brassiere at younger ages because of earlier onset of puberty or other considerations such as cultural issues. Obviously, further studies are needed to clarify the highly probable effects of female hormones as well as menstrual status on development of breast ptosis. The effects of smoking and alcohol consumption also need to be investigated in studies with greater sample sizes. In our study, there was no relationship between breast ptosis and breast-feeding, number of pregnancies, duration of lactation, or weight gain after pregnancy, a finding that can relieve the future mothers of the stress of developing breast ptosis following childbirth or lactation. Moreover, we suggest studies to demonstrate patients before pregnancy, during pregnancy, during lactation, and after weaning and compare with a control group. Also, another limitation of our study was generalizability of results as the included subjects were those referring to a private clinic..

**Conflict of Interest**
All the authors declare that they have nothing to disclose.

**References**
19. Boyd AS, Stasko T, King LE, Jr., Cameron GS, Pearse AD, Gaskell SA. Cigarette smoking-
Epstein-Barr Virus is Associated With Aggressive Subtypes of Invasive Ductal Carcinoma of Breast (Her2+/ER- and Triple Negative) and With Nuclear Expression of NFκB p50

Ashley James Ballard

ABSTRACT

Background: A growing body of evidence suggests a possible role for Epstein-Barr virus (EBV) in the pathogenesis of a subset of breast cancers, with many of these studies highlighting an increased association between EBV and aggressive forms of breast carcinoma. This study aimed to further investigate this issue by assessing the possible association between EBV and the Her2+/ER- and Triple negative subtypes of invasive ductal carcinoma (IDC).

Methods: An immunohistochemical marker for EBV (Epstein-Barr virus nuclear antigen 1 (EBNA1) clone E1-2.5) was applied to tissue micro array sections. The tissue microarray contained 58 cases of Her2+/ER- IDC, 57 cases of triple negative IDC and 67 cases of luminal like IDC. Each case was scored as positive or negative for nuclear expression of EBNA1 in tumour cells using standard light microscopy. Clinical and pathological details were noted for each case, as was the nuclear expression of NFκB p50.

Results: EBV infection was apparent in 43.2% of all cases. By subtype EBV was evident in 31 (57.4%) Her2+/ER- cases, 28 (49.1%) triple negative cases, and 14 (24.1%) luminal like cases; with a significant association being noted between the Her2+/ER- and triple negative cases and EBV infection (P 0.001). This association was primarily linked with ER negativity, Her2 status showed no significant association with EBV infection. There were no significant associations with other clinical and pathological characteristics. Of the 53 cases demonstrating NFκB p50 nuclear staining, 37 (69.8%) were also infected by EBV (P <0.001).

Conclusion: This study provides evidence that EBV is linked with aggressive subtypes of IDC (Her2+/ER- and triple negative) as well as providing evidence for a link between EBV and NFκB p50 nuclear expression, although the nature of these associations remains unclear.
EBV and high histological grade, nodal involvement, and young age of onset, while a meta-analysis published in 2012 appeared to demonstrate an association between EBV infection and elevated breast cancer risk. There is also some evidence to suggest a link between EBV and ER status. Using immunohistochemistry (IHC) to detect Epstein-Barr virus nuclear antigen 1 (EBNA1) Murray et al found a strong association between the 2B4-1 EBNA1 clone and ER negative tumours, although this was in the absence of PCR detectable EBV genetic material. Following on from this, two further studies using polymerase chain reaction (PCR) based methods demonstrated a clear relationship between EBV and ER negative breast tumours; although some investigators have failed to demonstrate significant associations between EBV and ER negative tumours this was largely due to small sample sizes, overall the data appears to be in favour of an association. However, as yet no clear molecular mechanism has been demonstrated to explain this. The relationship between EBV and Her2 status is unclear at present; there is evidence of Her2 overexpression in in-vitro models using EBV infected breast cancer cell lines. However, of the three studies to investigate this issue in human breast tissue, the first failed to demonstrate any association between EBV and Her2, while the second demonstrated a weak association between EBV and Her2 gene amplification. In the third study by Glenn et al. in 2012 all the Her2 expressing tumours were also found to be infected by EBV, although, due to the small sample size this was not considered significant.

From the evidence set out above it appears that breast tumours infected by EBV have a more aggressive nature, although this is not yet conclusive, and the manner of the relationship remains uncertain. Following on from the studies outlined above, the primary aim of this investigation was to further examine the relationships between EBV infection and the aggressive triple negative and Her2+/ER- subtypes of invasive ductal carcinoma (defined by receptor status). It was hypothesised that both of these subtypes would demonstrate significantly higher levels of EBV infection compared to the luminal like group (ER+/Her2- and ER+/Her2+/PR+). However, considering the evidence outlined above it was expected that ER status would have a greater impact than Her2 status. In order to test this IHC was used to examine expression levels of EBNA1 in 182 cases of breast cancer in tissue micro arrays (TMA’s), the use of IHC would allow EBV infection to be localised to tumour cells. The clinical and pathological characteristics of the tumours were also assessed to determine if there was a relationship with EBV infection; these included age, histological grade, tumour size, nodal involvement and Ki67 status. In addition to this NFkB nuclear expression was also assessed, as this transcription factor is known to be associated with EBV in gastric and nasopharyngeal carcinoma.

**Method**

Tissue specimens

The study used TMA’s (BR1503b, BR1504, BR1505, US BIOMAX) consisting of 410 cores of invasive ductal carcinoma in total, there were 2 cores from each case (total of 205 cases of IDC). The cases were grouped according to subtype of IDC (as defined by ER/PR/Her2 receptor expression). Cases that did not match the receptor expression patterns were discounted from the study. ER/PR and Her2 had been scored previously (information provided by US BIOMAX), ER/PR were considered positive if more than 1% of tumour cells were positive with an intensity of 1 (week) or stronger (>2 using the Allred scoring method). A Her2 score of 3+ was considered positive, a score of 0/1+ was considered negative. For the purposes of this study a score of 2+ was considered equivocal and the case was discounted (no FISH data available).

There were 58 cases of Her2+/ER- IDC (ER-, PR+/-, Her2+), 57 cases of triple negative IDC (ER-, PR-, Her2-) and 67 cases of luminal-like IDC (ER+, PR+/-, Her2- and ER+, PR+, Her2+). The proliferation level of each case had also been determined by US BIOMAX prior to the study using Ki67. The proliferation level was considered low if ≤20% of tumour cells expressed Ki67 or high if >20% of tumour cells expressed Ki67. Information about the clinical and pathological characteristics of the cases (Age, Grade, Size, and Nodal involvement) was provided by US BIOMAX, this data can be seen in Table 1. NFkB p50 nuclear staining was assessed by IHC within the testing laboratory using the method outlined below. TMA’s were stained using an EBNA1 monoclonal antibody, and were visualised according to the method outlined below. If the two cores from a single case did not display the same staining characteristics the result was considered equivocal and the case was discounted from the study.

**Antibodies and control material**

Commercially available control material was used that was known to stain strongly for EBV (245S, CELLMARQUE), and NFkB (326S, CELLMARQUE). To assess NFkB nuclear staining the NFkB p105/p50 antibody was used (clone 5D10, mouse monoclonal IgG1, ABCAM), diluted 1:200. To assess EBV infection the EBNA1 antibody was used (clone E1-2.5, mouse monoclonal IgG1, ABCAM), diluted 1:2000. Both antibodies were diluted using common antibody diluent (HK156-5KE, BIOGENIX).
**Immunohistochemistry (IHC)**

The tissue micro arrays were dewaxed in xylene taken through graded alcohols and rehydrated. TMA’s were then immersed in 10% hydrogen peroxide for 10 minutes to block endogenous peroxidase activity and retrieved according to manufacturer’s guidelines (heat mediated using the DAKO Pascal chamber and pH6.0 buffer [RE7113, NOVACASTRA]). After rinsing in wash buffer (HK583-SKE, BIOGENIX), the TMA’s were stained using a BIOGENIX i6000 with appropriate antibodies. Commercial control tissue was included with each staining run, the negative control was stained using a mouse IgG1 negative control (X0931 mouse monoclonal IgG1, DAKO). The antibodies were visualised using the SuperSensitive Polymer HRP (diaminobenzidine) kit (QD430-XAKE, BIOGENIX). Sections were then counterstained using Mayer’s haemalum, differentiated in 0.25% acid alcohol, blued using ammonium water and were dehydrated in alcohol, cleared in xylene and coverslipped. Sections were viewed and graded (positive/negative) using standard light microscopy, only nuclear staining within breast tumour cells was considered positive.

**Statistical analysis**

Statistical analysis was carried out using a statistical package for social sciences (SPSS) version 20 (PASW, IBM Corp. USA 2011). Associations between categorical data were assessed using the Chi-square test ($\chi^2$) or Fisher’s exact test where appropriate. The Mantel-Haenszel test was used when appropriate to assess the associations between categorical data where a third variable acted as a possible confounding factor to the two variables of primary interest. Using the Bonferroni correction, a $P$ value <0.005 was considered significant. Risk analysis was used to determine odds ratios (OR) and confidence intervals (CI).

**Results**

**EBV (EBNA-1 nuclear positivity) results**

182 cases of invasive ductal carcinoma were assessed using IHC for the presence of EBV (EBNA1) in the nuclei of tumour cells. 13 cases were either unreadable or displayed equivocal staining and were discounted from the study. 169 cases remained that were eligible consisting of 58 cases of luminal like IDC (34.3%), 54 cases of HER2+/ER- IDC (32.0%), and 57 cases of triple negative IDC (33.7%). EBNA1 staining patterns broken down according to the clinical and pathological characteristics of the cases can be seen in Table 1.

EBNA1 staining was evident in the tumour cells of 73 cases (43.2%), with a statistical power greater than 0.80; there was no evidence of EBNA1 staining in surrounding tissue or infiltrating lymphocytes. A significant association can be seen between EBV infection and the three IDC subtypes investigated [$\chi^2(2) = 13.85$, $P < 0.001$]; with EBNA1 positive breast tumour cells present in 31 (57.4%) of the HER2+/ER- cases and 28 (49.1%) of the triple negative cases, but only 14 (24.1%) of the luminal like cases. A three way analysis of the IDC subtypes association with EBV infection showed that the most significant difference was between the HER2+/ER- and luminal like subtypes [$\chi^2(1) = 12.88$, $P < 0.001$], with the odds ratio indicating that EBV infection was 4.24 times more likely in the HER2+/ER- subtype (95% CI, 1.89-9.50). The difference between the triple negative and luminal like sub types was also shown to be (borderline) significant [$\chi^2(1) = 7.74$, $P = 0.005$], with the odds ratio indicating that EBV infection was 3.03 times more likely in the triple negative subtype (95% CI, 1.37-6.71). There was no significant difference between the HER2+/ER- and triple negative sub types.

**Receptor, clinical and pathological staining patterns**

A significant association was shown between ER negativity and EBV infection [$\chi^2(1) = 13.07$, $P < 0.001$], with 53.2% of ER negative tumours shown to be EBNA1 positive, compared to only 24.1% of ER positive tumours. Based on the odds ratio, EBV infection is 3.57 times more likely in ER negative tumours (95% CI 1.76-7.24). It was noted that both the HER2+ and PR- groups overlapped significantly with the ER- group (54 HER2+ cases in the ER- group, 108 PR- cases in the ER- group), therefore the Mantel-Haenszel test was used to adjust for ER status when assessing PR status and HER2 status. Adjusting for PR status no significant association was found between PR status and EBV infection [$\chi^2(1) = 0.03$, $P = 0.863$], similarly no association was evident between HER2 status and EBV infection [$\chi^2(1) = 0.69$, $P = 0.405$].

Adjusting for tumour subtype a significant association was shown between NFkB p50 nuclear staining and EBV infection [$\chi^2(1) = 15.80$, $P < 0.001$], with 69.8% of tumour cells demonstrating NFkB p50 nuclear staining also shown to be EBNA1 positive. Based on the odds ratio EBV infection is 4.11 times more likely in those tumours demonstrating nuclear expression of NFkB p50 (95% CI 1.76-7.24). However, there were no significant relationships between EBNA1 positivity and age at diagnosis, histological grade, tumour size, nodal involvement or Ki67 status (see Table 1).

**Discussion**

Many of the investigations to date have utilised PCR or EBV encoded small RNAs (EBERs) in situ hybridisation as the primary detection methods. However, PCR has been criticised for detecting EBV DNA in infiltrating lymphocytes (laser capture micro dissection used in later studies should have
Table 1. EBV (EBNA1) staining according to the clinical and pathological characteristics (by case)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All</th>
<th>N (cases)</th>
<th>(EBNA1) EBV+ (%)</th>
<th>(EBNA1) EBV- (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>169</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>80</td>
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<td>30 (37.5)</td>
<td>50 (62.5)</td>
<td>0.10</td>
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<tr>
<td>≥50</td>
<td>89</td>
<td></td>
<td>43 (48.3)</td>
<td>46 (51.7)</td>
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</tr>
<tr>
<td>Histological grade</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>16</td>
<td></td>
<td>6 (37.5)</td>
<td>10 (62.5)</td>
<td>0.34</td>
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<tr>
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<td>57 (46.0)</td>
<td>67 (54.0)</td>
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<tr>
<td>Grade III</td>
<td>23</td>
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<td>7 (30.4)</td>
<td>16 (69.6)</td>
<td></td>
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<td>Tumour size</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 cm</td>
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<tr>
<td>≥2 cm</td>
<td>158</td>
<td></td>
<td>68 (43.0)</td>
<td>90 (57.0)</td>
<td></td>
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<td>Nodal involvement</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-</td>
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<td></td>
<td>58 (41.7)</td>
<td>81 (58.3)</td>
<td>0.27</td>
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<tr>
<td>N+</td>
<td>30</td>
<td></td>
<td>15 (50.0)</td>
<td>15 (50.0)</td>
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<td>Ki67 status</td>
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<tr>
<td>Low</td>
<td>91</td>
<td></td>
<td>38 (41.8)</td>
<td>53 (58.2)</td>
<td>0.40</td>
</tr>
<tr>
<td>High</td>
<td>78</td>
<td></td>
<td>35 (55.1)</td>
<td>43 (44.9)</td>
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<td>NFκB p50 status</td>
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<td></td>
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<tr>
<td>Negative</td>
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<td>36 (31.0)</td>
<td>80 (69.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>53</td>
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<td>37 (69.8)</td>
<td>16 (30.2)</td>
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<td>Estrogen receptor</td>
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<tr>
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<td></td>
<td>14 (24.1)</td>
<td>44 (75.9)</td>
<td>&lt;0.001</td>
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<td>Negative</td>
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<td>59 (53.2)</td>
<td>52 (46.8)</td>
<td></td>
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<tr>
<td>Progesterone receptor</td>
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<td></td>
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<tr>
<td>Positive</td>
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<td>12 (25.5)</td>
<td>35 (74.5)</td>
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<tr>
<td>Negative</td>
<td>122</td>
<td></td>
<td>61 (50.0)</td>
<td>61 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Her2 status</td>
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<td></td>
</tr>
<tr>
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<td>60</td>
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<td>33 (55.0)</td>
<td>27 (45.0)</td>
<td>0.40</td>
</tr>
<tr>
<td>Negative</td>
<td>109</td>
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<td>40 (36.7)</td>
<td>69 (63.3)</td>
<td></td>
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<tr>
<td>Subtype</td>
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<td>Luminal like</td>
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<td></td>
<td>14 (24.1)</td>
<td>44 (75.9)</td>
<td>0.001</td>
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<td>Her2+/ER-</td>
<td>54</td>
<td></td>
<td>31 (57.4)</td>
<td>23 (42.6)</td>
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<tr>
<td>Triple negative</td>
<td>57</td>
<td></td>
<td>28 (49.1)</td>
<td>29 (50.9)</td>
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</tr>
</tbody>
</table>

EBV and aggressive breast cancer

was chosen as it has been shown to be more reliable than the 2B4 clone.7,20

Previous investigations have demonstrated EBV positivity ranging from 21-63% with a mean of 35.5% (SD=10.9) when considering all forms of investigation, when considering only IHC staining using EBNA1 the range is 25-55% and the mean is 35.4% (SD=10.3).3-13,15,21 Although the results of the present study are slightly above the mean (43.2%), they are well within the range and fall within 1SD. The higher level of positivity in the current study may be due to the nature of the sample. Previous investigations used samples representative of the population norm in terms of receptor expression; due to the nature of this investigation the sample contained more ER- and Her2+ cases than would normally be expected. However, due to the nature of the investigation (using TMA’s), it was not possible to accurately classify lymphocytic infiltration of the tumour tissue. This should be considered when viewing these results.

Gene expression studies have identified five primary subtypes of breast cancer (invasive ductal carcinoma, no special type), Luminal A, Luminal B, Normal like, Basal like and Her2 positive.22 However, from an oncologist’s point of view breast cancer patients fall in to one of three groups, ER positive, Her2 positive and triple negative,23 as these groups represent distinct prognostic outcomes.24 A
strong association was demonstrated between EBV infection and tumour subtype (P=0.001), with EBV infection shown to be more prevalent in the Her2+/ER- and triple negative subtypes of invasive ductal carcinoma. This points towards a possible role for EBV in the pathogenesis of these breast cancer subtypes, both of which are typically high grade and aggressive. This study directly investigates the association between EBV infection and the high risk IDC subtypes (Her2+/ER- and Triple negative) in an organised manner, and backs up the work of previous authors associating EBV infection with aggressive (ER-) breast tumours.

To gain a greater understanding of the nature of this relationship receptor (ER, PR, Her2) receptor expression profiles were examined (although data on ER, PR, and Her2 clones used for this was not available), with Mantel-Haenszel analysis being used to adjust for ER status. As can be seen from the results ER negativity appears to be the dominant factor in the association between EBV and the aggressive breast cancer sub types, with the odds ratio suggesting that EBV infection is 3.57 times more likely in ER negative tumours (P <0.001). However, the nature of the relationship remains unclear, as no molecular mechanism has been outlined as yet to explain the association. It may be that the higher proportion of ER negative cells in the basal layer makes these cells more susceptible to the transfer of EBV infection from infiltrating lymphocytes, although the recent report by Khan et al. failed to find a correlation between EBV infected infiltrating lymphocytes and ER negative breast tumours. Further studies are needed to examine this issue, as the small size of the cores in the TMA’s limited the ability of the investigator to fully screen for infiltrating lymphocytes in the present study. No association was evident between the EBV infected tumour cells and PR receptor expression, confirming the findings of previous authors.

As outlined in the introduction the evidence for an association between EBV infection and Her2 expression is mixed, it was reported by Lin et al. that infection of breast cancer cell lines by EBV leads to activation of the Her2/Her3 signalling axis and elevated Her2 expression through the action of the BARF0 gene product. EBV has also been shown to induce EGFR expression in cervical carcinoma cell cultures through the action of NFkB p50. Recent evidence suggests that the ERBB2 gene is amplified through the action of NFκB p50, and recent studies have observed Ki67 status. In breast cancer it has been found to be associated primarily with grade 3 tumours greater than 50 mm with more than 3 lymph nodes positive for metastases. However, this study failed to demonstrate the presence of the EBV genome using PCR, and the reliability of the 2B4-1 clone must also be questioned as outlined earlier in the discussion. A further study in 2008 showed support for the association between EBV infection and high grade node positive tumours, although no significant association was evident between EBV infection and tumour size. Another study in 2011 demonstrated an association between EBV positivity and high tumour grade, however, no other clinical or pathological factors were significant (excluding receptor expression status). A final study by Glenn et al. indicated that EBV was associated with a younger age of diagnosis. In the present study no associations were evident between EBV infection of breast tumours and age, histological grade, tumour size or nodal involvement; this is in accordance with the majority of previous investigations, including a large multi centric study by Fina et al. in 2001. The scores used to grade the IDC sub-types in the TMA’s used were not available, so it was not possible to assess if EBV infection was associated with different scores for nuclear pleomorphism, or tubule formation. However, the relationship between EBV positivity and Ki67 status was assessed; Ki67 measures the proliferation activity of tumours and is becoming increasingly important in breast cancer prognostic tests such as IHC4+C. However, no significant association was evident. None of the previous studies investigating EBV and breast cancer have observed Ki67 status.

NFkB is a rapid acting primary transcription factor found in most cell types that acts as a master regulator of cellular responses to stress; it serves as a primary means of relaying signals from the extracellular environment to the nucleus in order to initiate a genetic program. Aberrant expression of NFkB has been associated with a number of cancers. In breast cancer it has been found to be correlated with ER/PR negativity, and is associated with the Her2 positive and basal like subtypes. NFkB p50 and p65 are both elevated in these cancer types, however, levels of NFkB p50 have been shown to be significantly higher than p65. NFkB has also been strongly linked to radiotherapy and anti estrogen therapy resistant forms of breast cancer.
Increased NFκB expression is normally the result of physiological changes leading to its activation. In the case of breast cancer, Her2 positive and basal like tumours have a solid growth pattern that leads to increased hypoxia towards the centre of the tumour mass. NFκB is one of the transcription factors governing cellular responses to hypoxia. EBV has been linked to NFκB in both gastric and nasopharyngeal carcinoma. In addition, a series of investigations have demonstrated EGFR receptor up regulation due to the activation of EGFR promoters by NFκB p50/p50/Bcl-3 complexes, indicating a distinct role for NFκB in tumour genesis. The results from the present study indicate that there is an unexpectedly strong association between EBV infection and nuclear expression of NFκB p50 in cases of invasive ductal carcinoma (P <0.001); the Mantel-Haenszel common odds ratio indicated that NFκB p50 nuclear expression was 4.11 times more likely in tumours infected with EBV (after adjusting for tumour sub-type). However, the nature of this association remains unclear; from the evidence outlined earlier in the discussion it is unlikely that EBV LMP1 is interacting with NFκB, it is possible that LMP2A interacts with NFκB in EBV linked breast cancer as is the case in EBV linked gastric carcinoma. It seems clear from the results of this investigation as well as from previously published data that EBV is not associated with Her2 in breast cancer; therefore, it is possible that LMP2A interacts with the NFκB pathway to confer resistance to apoptotic stimulus in cases of EBV linked breast cancer, as is the case in EBV linked gastric carcinoma. Further studies should be carried out to confirm these initial findings, and investigate the association between EBV and NFκB in breast cancer.

The primary aim of this study was to investigate a possible association between EBV infection and aggressive sub types of invasive ductal carcinoma. The data clearly indicates an association, with EBV infection significantly more prevalent in the triple negative (49.1%) and Her2+/ER- (57.4%) sub types compared to the luminal like sub type (24.1%). It was further demonstrated that a significant association is present between EBV infection and ER negativity, however, no significant associations exist between EBV infection and either PR status or Her2 status. The nature of the relationship between ER negativity and EBV infection remains unclear, with additional investigations needed to determine if ER negativity is involved in the aetiology of a possible sub set of EBV linked aggressive breast tumours, due to the limitations inherent with commercial TMA’s further work may be required to confirm these results. A surprisingly strong association was also noted between EBV infection and nuclear expression of NFκB p50 (independent of tumour sub-type), although the nature of this relationship remains unclear, and further studies are necessary to confirm these results and to determine how EBV interacts with NFκB. This study failed to find evidence of associations between EBV infection and other clinical and pathological characteristics (age, histological grade, nodal involvement, tumour size and Ki67 status).

Conflict of Interest
The author has no conflict of interest in relation to this article.

References


Methods: This case-control study (with 222 persons in each group) was conducted in Arash Women’s Hospital from 2014 to 2017. Women with laparoscopically proven endometriosis were considered as cases. Controls were selected from women who had previous laparoscopic surgery due to any reason, and the absence of endometriosis was confirmed in them.

Background: Endometriosis is a common chronic inflammatory, estrogen-dependent disease with characteristics similar to cancer. Epidemiological studies of the association between endometriosis and breast cancer have yielded inconsistent results. The present study aimed to investigate the association between endometriosis and breast cancer risk factors.

Conclusion: Women with endometriosis have some of the breast cancer risk factors in their history, and these risk factors (gravidity, age at first pregnancy, history of breast-feeding, and OCP or hormone use) can change the risk of endometriosis as they increase or decrease the risk of breast cancer.

Results: Multivariate logistic regression analysis by considering the risk factors (age, body mass index, gravidity, age at first pregnancy, age at menarche, history of breast-feeding, history of oral contraceptive and hormone use, history of miscarriage and induced abortion, breast cancer in first-degree relatives, and physical activity) revealed that endometriosis was positively association with age at first pregnancy (OR = 1.16, 95% CI: 1.08-1.25; P < 0.001), history of oral contraceptive use (OR = 3.91, 95% CI: 1.92-7.95; P <0.001), and history of hormone use (OR = 5.82, 95% CI: 2.70-12.56; P <0.001), and negatively associated with gravidity (OR = 0.61, 95% CI: 0.42-0.91; P = 0.01), and history of breast-feeding (OR = 0.37, 95% CI: 0.16-0.86; P = 0.02).

Endometriosis is a common chronic inflammatory, estrogen-dependent disease with characteristics similar to cancer. Estrogens causes hormonal abnormalities that will accompany her during reproductive life cycle and is considered an established risk factor for breast cancer.

Endometriosis is a common chronic inflammatory, estrogen-dependent disease which affects approximately 10%-15% of women of reproductive age. However, its prevalence may be as high as 25%-30% in infertile women. Many women are asymptomatic, while others experience varying...
degrees of chronic pelvic pain, dysmenorrhea, or dyspareunia. Endometriosis has characteristics similar to cancer, including cell invasion, uncontrolled growth, development of new blood vessels, and a decrease in the number of cells undergoing apoptosis. Furthermore, endometriosis, like cancer, is characterized by metastasis and recurrence, and the 5-year recurrence rate of endometriosis after laparoscopy has been reported to be 40%-50%.

However, endometriosis is not categorized as a malignant disorder, and malignant transformation of endometriosis occurs only in 1% of cases. Several studies have shown a possible association between ovarian cancer and endometriosis. A 1997 study in Sweden, with a large sample size and long-term (11.4 years) follow-up, reported that ovarian cancer rate in patients with endometriosis was about two times higher than the general population. They also found these women to have a 30% increased risk of breast cancer. However, subsequent epidemiological studies examining the association between endometriosis and breast cancer provided inconsistent results.

Therefore, the aim of this study was to evaluate the association between breast cancer risk factors and endometriosis.

Methods
Study Population
This case-control study was approved by the Ethics Committee of Tehran University of Medical Sciences. Oral informed consent was obtained from all participants. The study was performed in Arash Women’s Hospital during the period 2014-2017. Women with endometriosis were considered as cases, providing us with a case group of 222 women. The diagnosis was confirmed using laparoscopic surgery. An equal number of controls were selected from women who had previously undergone laparoscopic surgery due to any reason (pelvic pain, dysmenorrhea, unknown infertility, etc.), and the absence of endometriosis was confirmed in them.

Women with a history of any type of cancer, history of head and neck radiotherapy, and positive genetic test for breast cancer (BRCA1, BRCA2) were excluded. Demographic information and breast cancer risk factors [age, body mass index (BMI), gravidity, age at first pregnancy, age at menarche, history of breastfeeding, history of oral contraceptive and hormone use, history of abortion, history of breast cancer in first-degree relatives, and weekly physical activity (hours)] were obtained by a trained midwife through face-to-face interview. BMI is defined as weight divided by height squared (kg/m²). Weekly physical activity was the total hours per week that a woman exercised.

Statistical Analysis
Categorical and continuous variables are presented as N (%) and mean ± standard deviation, respectively. Pearson’s chi-square test and independent t test were used to assess differences between baseline demographic and clinical characteristics of the study groups. Multivariate logistic regression with backward selection mode was used to examine the association between endometriosis and the possible risk factors selected based on the prior knowledge. In this model, a P value of 0.2 was used as the criteria for entering a variable in the model, whereas a P value of 0.1 was considered the threshold for a variable to stay in the model. Results are presented as odds ratio (OR) with 95% confidence intervals (CI). Statistical analysis was performed using SPSS software (version 18, Chicago, IL, USA).

Results
Demographic and clinical characteristics of the subjects are shown in Table 1. The mean age of the subjects was 33.11 ± 5.88 (range: 19-45) years. Only three women were in postmenopausal status (one in the case and 2 in the control group). Independent t test showed no significant difference in age at menarche and physical activity between the case and the control groups (P > 0.05). However, other variables were significantly different between the two groups (P < 0.05) (Table 1). After univariate logistic regression analysis by considering the P value of < 0.2, the variables age, BMI, gravidity, age at first pregnancy, history of breastfeeding, history of miscarriage and induced abortion, and history of oral contraceptive and hormone use were selected for further analysis (data not shown in the table). Multivariate logistic regression results are presented in Table 2. The results revealed that endometriosis was positively associated with age at first pregnancy (OR = 1.16, 95% CI: 1.08-1.25; P < 0.001), history of oral contraceptive use (OR = 3.91, 95% CI: 1.92-7.95; P < 0.001), and history of hormone use (OR = 5.82, 95% CI: 2.70-12.56; P < 0.001), and negatively associated with gravidity (OR = 0.61, 95% CI: 0.42-0.91; P = 0.01) and history of breastfeeding (OR = 0.37, 95% CI: 0.16-0.86; P = 0.02).

Discussion
Breast cancer is the most common cancer in women. Therefore, many researchers are interested in early detection of breast cancer risk factors in high risk population. The risk of cancer development in patients with endometriosis has been investigated. We evaluated the breast cancer risk factors in endometriotic cases compared with a control group. The results of the present study showed that some of the risk factors for breast cancer, such as gravidity, age at first pregnancy, history of breastfeeding, and OCP or hormone use have the same effect on endometriosis as they have on breast cancer. It means the association of these risk factors with
Table 1. Comparison of demographic characteristics of the case and the control

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases (n = 222)</th>
<th>Controls (n = 222)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>32.03 ± 5.22</td>
<td>34.17 ± 6.25</td>
<td>&lt;0.001</td>
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<td>BMI (kg/m2)</td>
<td>24.03 ± 3.75</td>
<td>26.25 ± 4.49</td>
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<td>Duration of education (y)</td>
<td>15.13 ± 3.02</td>
<td>12.56 ± 4.00</td>
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<td>Gravidity (n)</td>
<td>0.53 ± 8.60</td>
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<td>0.002</td>
</tr>
<tr>
<td>Age at first pregnancy (y)</td>
<td>26.94 ± 4.98</td>
<td>22.81 ± 4.70</td>
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<td>Age at menarche (y)</td>
<td>13.08 ± 1.51</td>
<td>13.10 ± 1.57</td>
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<tr>
<td>Physical activity (h/wk)</td>
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<td>3.22 ± 7.18</td>
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<td>History of induced abortion</td>
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<td>History of breast-feeding</td>
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<td>147 (66.2)</td>
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<td>History of OCP usage</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>History of hormone use</td>
<td>88 (39.6)</td>
<td>36 (16.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Breast cancer in first-degree relatives

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>31 (13.9)</td>
<td>191 (86.1)</td>
</tr>
<tr>
<td>Controls</td>
<td>29 (13.1)</td>
<td>193 (86.9)</td>
</tr>
</tbody>
</table>

* Statistically significant at 0.05 level. Model includes age, body mass index (BMI), gravidity, age at first pregnancy, history of breast-feeding, history of OCP use, history of hormone use, history of miscarriage, and history of induced abortion. OCP: oral contraceptive pills; CI: confidence interval.

Table 2. Adjusted odds ratio for endometriosis according to known risk factors in multivariate analysis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Adjusted OR</th>
<th>Lower 95% CI</th>
<th>Higher 95% CI</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravidity</td>
<td>0.61</td>
<td>0.42</td>
<td>0.91</td>
<td>0.01</td>
</tr>
<tr>
<td>Age at first pregnancy</td>
<td>1.16</td>
<td>1.08</td>
<td>1.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of breast-feeding</td>
<td>0.37</td>
<td>0.16</td>
<td>0.86</td>
<td>0.02</td>
</tr>
<tr>
<td>History of OCP use</td>
<td>3.91</td>
<td>1.92</td>
<td>7.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of hormone use</td>
<td>5.82</td>
<td>2.70</td>
<td>12.56</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Statistically significant at 0.05 level. Model includes age, body mass index (BMI), gravidity, age at first pregnancy, history of breast-feeding, history of OCP use, history of hormone use, history of miscarriage, and history of induced abortion. OCP: oral contraceptive pills; CI: confidence interval.

Endometriosis and breast cancer risk factors

Endometriosis and breast cancer is similar. Therefore, such results indicate the possibility of an increased risk of breast cancer in these patients. The predominant risk factors were the use of oral contraceptives and other hormones and age at first pregnancy. However, gravidity and history of breast-feeding were associated with protective effects.

Consistent with our findings, two cohort studies, both based on hospital records of endometriotic patients, have reported overall increased risk of breast cancer after a diagnosis of endometriosis.\(^6\,7\) However, the findings of Schairer et al. were based on a limited number of breast cancer cases,\(^8\) and Brinton et al. didn’t find any apparent difference in risk according to the age of diagnosis.\(^9\)

A large case-cohort study by Bertelsen et al. reported the increased risk of breast cancer in women whose endometriosis were diagnosed at an older age.\(^10\) A recent publication by Kokcu et al. discussed about the possible mechanisms of increased risk of malignancy in patients with endometriosis and the contributions of genetic and epigenetic variations of endometriosis and universal carcinogens that may be potential risk factors of breast or other cancers in women with endometriosis.\(^11\)

However, the results of this study are not consistent with other studies.\(^12\,13\) The study by Brinton et al.,\(^4\) with longer follow-up, was not able to confirm the previous finding of the association between endometriosis and breast cancer.\(^11\) Furthermore, the positive association was not manifested in two case-control studies\(^12\,13\) and one cohort study.\(^14\) It is worth mentioning, however, that these investigations were conducted in postmenopausal breast cancer patients,\(^14\) and the selection of endometriosis cases was based on self-reported data,\(^12\,14\) whereas, endometriosis was confirmed by laparoscopic surgery in our study. Evidence has revealed that the etiology of endometriosis differs considering menopausal status of women, and women diagnosed with endometriosis before age 40 have reduced risk of developing breast cancer in the future. This may be attributable to cumulative exogenous estrogen levels throughout women’s life or the effect of antiestrogens in younger patients with endometriosis.\(^16\) Therefore, it seems that the differences in results could be due to the following factors: sample size, study design, diagnosis of patients (by the physician or self-reported), categorization of patients by the age of diagnosis or menopausal status, and different follow-up periods.

One of the most important risk factor for breast cancer is the family history of breast cancer. One study reported the proportion of women with a positive family history of breast cancer in first- and second-degree relatives was significantly higher in women with endometriosis compared with the controls.\(^15\) However, in the present study, the family history of breast cancer was similar in the case and the control group.

Cancer is a multifactorial disease influenced by strong genetic and lifestyle components, and
environmental factors and place of residency are important factors in the development of disease. Therefore, scientists should accept some differences between the results of causal studies due to the effect of environmental variables.

Consequently, it is necessary to establish a connection between world-wide cancer registration systems in order to draw a valid and reliable conclusion about the risks associated with endometriosis considering all risk factors.

This study had some advantages. Firstly, the study groups were selected according to laparoscopic results, and not based on self-reported data. Therefore, the method of selection (case and control) was more valid. Secondly, this was the first study on this subject in our population. Since the prevalence of cancer risk factors in Iran is high, and the trend is growing up, and breast cancer patients with advanced stages of disease are relatively younger (about 10 years) than their western counterparts, this type of study was needed to prevent the disease by setting up new screening programs in high-risk populations.

The limitation of this study was the study design. It would be better to conduct a retrospective cohort study in breast cancer patients and evaluate the history of endometriosis compared with the normal population, or carry out a prospective cohort study in endometriotic patients and follow them for a long time and evaluate them for the manifestation of any cancer in this population. The other limitation was the lack of knowledge about the stage of endometriosis and the origin of the disease.

In conclusion, we found some of the risk factors for breast cancer to have the same effect on endometriosis as they have on breast cancer. However, data on the association between endometriosis and breast cancer should be interpreted with caution because of the lack of consistency. Further study is needed to confirm the association between endometriosis and breast cancer.

For women with endometriosis, especially those with a history of OCP or hormone use and higher age at first pregnancy, counseling and screening for breast cancer are highly recommended.

Acknowledgment
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Conflict of Interest
No potential conflict of interest relevant to this article was reported.

References
16. Mousavi SM, Gouya MM, Ramazani R,


In Vitro Inhibition of MCF-7 Human Breast Cancer Cells by Essential Oils of *Rosmarinus officinalis*, *Thymus vulgaris* L., and *Lavender* x *intermedia*

Seyed Mahdi Tabatabaei, Fatemeh Kianinodeh, Mahdi Nasiri, Nasibeh Tightiz, Maryam Asadipour, Mahshid Gohari

**ABSTRACT**

**Background:** The essential oils of traditional medicinal plants, including *Rosmarinus officinalis*, *Thymus vulgaris* L., and *Lavender* x *intermedia* contain anticancer compounds such as lavandulyl acetate, rosmarinic acid and thymol. The aim of this study was to investigate the anticancer effects of the essential oils of *R. officinalis*, *T. vulgaris* L., and *L. x intermedia* on MCF-7 cells.

**Methods:** Essential oils were prepared from *R. officinalis*, *T. vulgaris* L., and *L. x intermedia* plants. Then, MCF-7 and Hu02 cells were treated with different concentrations of these essential oils for a given time. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay was used to determine the cellular viability and cytotoxicity in response to treatment with different extract concentrations. The morphological changes were studied by Hoechst and propidium iodide staining. The results were analyzed using the one-way ANOVA and Tukey test.

**Results:** All three essential oils inhibited the viability of the MCF-7 cell line in a dose-dependent manner. *T. vulgaris* L. was more potent against MCF-7 cells at 400 µg/ml concentration (IC₅₀ = 48.01 ± 0.94), while *R. officinalis* was moderate at 50800 µg/ml concentration (IC₅₀ = 47.39±0.91) and the concentration for *L. x intermedia* was 400 µg/ml (IC₅₀ = 47.39 ± 0.91).

**Conclusion:** *R. officinalis*, *T. vulgaris* L. and *L. x intermedia* show cytotoxic activity against breast cancer in vitro. *T. vulgaris* represents a potentially selective cytostatic factor and a safe target for future development of anticancer agents.

**Introduction**

Breast cancer is among the most common cancers of women all over the world and represents one of the major health threats that takes the lives of thousands of people every year. Factors affecting fertility, environment, lifestyle, and physical inactivity contribute to this process. Breast cancer ranks first among Iranian women with diagnosed cancers.

However, few studies have been conducted regarding clinical and pathologic features and age distribution of this disease in Iran. For over 3500 years, plants have been used to treat cancer, but their antiproliferative potential has only been evaluated since the late 1950s. Hartwell and colleagues used podophyllotoxin and its derivatives as the first anticancer agents for the first time in the late 1960s.

The recognition of medicinal plants and extensive research on their essential oils have led to their widespread use in medicine, such that the antimicrobial and antioxidant features of essential oils, especially their anticancer properties, have...
attracted a lot of attention. More than 60% of anticancer compounds are derived from plants, microorganisms, and marine sources. Today, medicinal plants are being considered safe due to their low side effects relative to chemical drugs. The anticancer plants contain a wide range of compounds, such as colchicine, vincristine, and podophyllotoxin, which are mitotic inhibitors binding to mitotic spindle tubular proteins and possessing alkaldoids such as vinca alkaloids (Vinca rosea) that are used in chemotherapy. Plants of the mint family (Lamiaceae) can be a rich source of phytochemicals such as phytoestrogens, flavonoids, carotenoids, and terpenoids that act as antioxidants, annihilating free radicals and stimulating the immune system. These compounds form DNA adducts inhibiting the activity of carcinogens and also blocking metabolic pathways of cancer.

_Thymus vulgaris_ is a medicinal plant with therapeutic properties such as antiinflammatory, antiseptic, antitussive, antispasmodic, and antimucus effects. The essential oil of this plant contains compounds such as eucalyptol (6.23%), camphor (15.14%), camphene (10.54%), α-pinene (8%), carvacrol (18.51%), and thymol (20.35%). Moreover, the plant contains inhibitor compounds for cyclooxygenase and oxidative stress, and the effect of these enzymes on the growth and segregation of cells (including cancer cells) has been confirmed.

*Lavandula x intermedia* belongs to Lamiaceae family and is a source of uric acid, oleanolic acid, and betulinic acid. The main components of the essential oils of these plants include linalool (32.8%), linalyl acetate (17.6%), lavandulyl acetate (15.9%), α-terpineol (6.7%), and geranyl acetate (5%).

*Rosmarinus officinalis* is also a good source of uric acid, oleanolic acid, and betulinic acid. Essential oil of rosemary contains carnosic acid (1%), carnosol (4.6%), rosmarinic acid (4%), uric acid (19.2%), and rosmanol (5%), among which rosmarinic acid is water soluble and other substances are soluble in organic solvents. Rosmarinic acid is a natural phenolic compound that has several biological attributes such as antidepressant, liver tissue protection, antiangiogenesis, antitumor, and anti HIV-1 infection. Several studies have shown anticancer effects of these three plants belonging to Lamiaceae family.

The aim of the present study was to investigate the cytotoxic effects of essential oils of _T. vulgaris, L. x intermedia, _and _R. officinalis_ on breast cancer cell lines.

**Methods**

The MCF-7 Cell line and Hu02 Cells

The MCF-7 human breast cancer cell line and Hu02 normal human fibroblast cells (control) were purchased from Iranian Biological Resource Center (IBRC), Tehran, Iran. Cell culture materials were obtained from Gibco Company (USA), but the flasks and microplates were acquired from Griner Company (Germany).

**Collection of Plant Samples**

The samples of _T. vulgaris, L. x intermedia, _and _R. officinalis_, which were harvested from Boroujerd city of Lorestan Province, were confirmed by (IBRC), Tehran, Iran. Cell culture materials were the Faculty of Natural Science and deposited at the Herbarium of Boroujerd Islamic Azad University. Next, the collected plant materials were dried in a dark place and were packed in paper bags in which they were stored until the experiments were performed.

**Essential Oil Extraction**

The essential oils were extracted using the Soxhlet method. First, 400 g of crushed dried samples from the leaves of each plant was placed in a tray and ethanol was added to it as solvent to make dough. Next, the dough was placed in the thimble inside the Soxhlet apparatus. Then, the ethanol was added slowly to the bottom flask, and the condenser was placed on the apparatus. An electric stove was used to boil solvent inside the flask. Consequently, the essential oil was collected and kept in sealed bottles at +4°C. The process lasted 5-8 hours.

**Cell Culture**

The cells were grown in RPMI-1640 (Gibco, UK) supplemented with 10% FBS (Gibco, UK), 2 mM/L glutamine, 100 unit/mL penicillin, and 100 mg/mL streptomycin (Sigma, US) in 96-well culture plates (Griner, Germany) at 37°C in a 5%-CO_2_ incubator (Heraeus, Hanau, Germany). All experiments were performed using cells from passage 12 or lower.

**MTT Assay**

The cell viability was determined using 3-(4,5-di-methylythiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric method. Briefly, MCF-7 cells were seeded at 10000 cells per well in 96-well culture plates and treated with different concentrations of the extracted essential oils for 24, 48, and 72 hours. Then, the cells were incubated with MTT solution (5 mg/ml in PBS) for 4 h, and the resulting purple formazan was solubilized in 100 μL of DMSO (Sigma). The absorption was measured at 570 nm and 620 nm as a reference using a 96-well plate reader (Bio-Rad, Hercules, CA, USA). Data were collected for four replicates each and were used to calculate the respective means. The percentage inhibition was calculated using the following formula. The IC₅₀ value was determined as the concentration of...
the complex that is required to reduce the absorbance to half of that of the control.

**Hoechst and Propidium Iodide Staining**

The effects of various concentrations of essential oils of *R. officinalis*, *T. vulgaris* L. and *L. x intermedia* on morphological changes were assessed by Hoechst staining. For this aim, we used a fluorescence microscope to examine cell morphology. Briefly, the cells were treated with different concentrations of essential oils of *R. officinalis*, *T. vulgaris* L., and *L. x intermedia* for 24, 48 and 72 hours. The medium was removed and the cells were washed with PBS (Gibson Company). Then, the samples were incubated with 100 µL of PBS + 100 µL of Hoechst solution for 6-8 minutes. Next, the cells were washed by PBS, and their nuclei were examined with a phase contrast microscope (Carl Zeiss, Jena, Germany) and finally photographed. In this stage, the cells appear blue in color. In the next stage, each well was washed with PBS and propidium stain was added. In this stage, this stain is attached to DNA grooves and makes dead cells’ nuclei look red.

**Statistical Analysis**

The data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey multiple comparison tests, on SPSS 20. The results are presented as mean ± SD. A P value of less than 0.05 was considered statistically significant.

**Results**

The results of the MTT assay are given in Tables 1-4 and Figures 1-4. Comparing the viability of MCF-7 breast cancer cells after 24, 48, and 72 hours showed significant differences among groups treated with various concentrations of *R. officinalis*, *T. vulgaris* L., *L. x intermedia*, and Taxol, which indicates that as the concentration is increased, the viability of MCF-7 cells is significantly decreased. As shown in Table 4, Taxol attenuated the cytotoxic effects of concentrations of essential oils in MCF-7 cells. In Hoechst and propidium iodide staining, live and dead cells respectively appear blue and red in color (figure 5). As the dose is increased, the number of red cells is also increased, which shows apoptosis resulting from the impact of essential oils.

**Effect of Essential Oils on the Viability of Cells**

In vitro cytotoxic effects of the essential oils on MCF-7 breast cancer cell line viability were evaluated by MTT assay and compared with the result of Taxol, a commercially available anticancer drug, the cytotoxicity of which is shown in Table 1. The comparison of cell viability at different essential oil concentrations (1000, 800, 400, 200, 100 µL/mL) and Taxol (10, 0.1, 0.01, 0.001µM) disclosed similar viability rates. As shown in Figure 1, the cytomorphological effects of essential oils on MCF-7 cells at different concentrations include activation of an intracellular suicide program characterized by morphological changes like cytoplasmic and cellular shrinkage, oxidative stress, coiling, and biochemical responses leading to apoptosis. It is quite obvious from the results that the apoptosis rate of MCF-7 cells is increased with increase in the concentration of essential oil. A dose-dependent increase in cell inhibition was seen after 24, 48, and 72 h incubation. The IC₅₀ for *T. vulgaris* was 400 µg/mL (48.01 ± 0.94). The complete inhibition (90%) of breast cancer cells was obtained at the maximum concentration of 1000 µg/mL. These results highlight the dose- and time-dependent increase in cytotoxicity. The IC₅₀ value suggests that the essential oil proves to be a promising drug for chemotherapeutic treatment. Figure 2 shows that the cytotoxic effect of *L. intermedia* essential oil is dose- and time-dependent at 1000 µg/mL (P < 0.05), but at lower concentrations (800, 400, and 200 µg/mL) the decrease in cell viability is only dose-dependent. Also, figure 3 demonstrates a dose- and time-dependent cytotoxic effect of *T. vulgaris* essential oil at 1000 and 800 µg/mL (P < 0.05), but only a dose-dependent effect at lower concentrations (400, 200, and 100 µg/mL).

The cytotoxic effect of *R. officinalis* essential oil is dose- and time-dependent at 1000 µg/mL (P < 0.05), but only dose-dependent at lower concentrations (800, 400, 200, and 100 µg/mL) (figure 4).

**Table 1.** 50% Inhibitory Concentrations (% v/v) for Taxol Against MCF-7 Cells (P < 0.05)

<table>
<thead>
<tr>
<th>Time</th>
<th>0.001 µg/mL</th>
<th>0.01 µg/mL</th>
<th>0.1 µg/mL</th>
<th>1 µg/mL</th>
<th>10 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>75.39 ± 0.16</td>
<td>67.45 ± 0.52</td>
<td>55.99 ± 0.86</td>
<td>51.73 ± 1.21</td>
<td>48.31 ± 1.02</td>
</tr>
<tr>
<td>48 h</td>
<td>63.98 ± 0.61</td>
<td>56.33 ± 0.69</td>
<td>47.55 ± 0.73</td>
<td>33.86 ± 0.97</td>
<td>26.91 ± 1.01</td>
</tr>
<tr>
<td>72 h</td>
<td>68.90 ± 0.22</td>
<td>49.94 ± 0.70</td>
<td>38.19 ± 0.82</td>
<td>27.62 ± 0.95</td>
<td>21.88 ± 1.31</td>
</tr>
</tbody>
</table>
Table 2. 50% inhibitory concentrations (% v/v) for *L. intermedia, T. vulgaris*, and *R. officinalis* Against MCF-7 Cells (P < 0.05)

<table>
<thead>
<tr>
<th>Time</th>
<th>100 µg/mL</th>
<th>200 µg/mL</th>
<th>400 µg/mL</th>
<th>800 µg/mL</th>
<th>1000 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L. intermedia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>83.61 ± 0.59</td>
<td>75.11 ± 0.72</td>
<td>66.09 ± 0.91</td>
<td>51.23 ± 1.03</td>
<td>44.52 ± 1.11</td>
</tr>
<tr>
<td>48 h</td>
<td>69.25 ± 0.51</td>
<td>57.89 ± 0.66</td>
<td>42.90 ± 0.89</td>
<td>29.74 ± 1.05</td>
<td>23.80 ± 1.14</td>
</tr>
<tr>
<td>72 h</td>
<td>61.28 ± 0.86</td>
<td>59.37 ± 0.75</td>
<td>44.93 ± 0.87</td>
<td>29.44 ± 0.91</td>
<td>20.89 ± 0.97</td>
</tr>
<tr>
<td></td>
<td>T. vulgaris</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>69.31 ± 0.70</td>
<td>59.81 ± 0.85</td>
<td>48.01 ± 0.94</td>
<td>40.22 ± 1.03</td>
<td>34.29 ± 1.06</td>
</tr>
<tr>
<td>48 h</td>
<td>47.25 ± 0.71</td>
<td>38.07 ± 0.79</td>
<td>30.86 ± 0.69</td>
<td>23.79 ± 0.82</td>
<td>18.95 ± 1.99</td>
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<tr>
<td>72 h</td>
<td>49.27 ± 0.65</td>
<td>38.94 ± 0.78</td>
<td>31.19 ± 0.60</td>
<td>19.94 ± 0.74</td>
<td>15.72 ± 0.88</td>
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<tr>
<td></td>
<td>R. officinalis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>75.41 ± 0.77</td>
<td>68.15 ± 0.89</td>
<td>55.62 ± 0.79</td>
<td>47.39 ± 0.91</td>
<td>40.21 ± 1.04</td>
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<tr>
<td>48 h</td>
<td>59.91 ± 0.66</td>
<td>46.73 ± 0.66</td>
<td>38.55 ± 0.80</td>
<td>25.33 ± 0.84</td>
<td>21.07 ± 1.02</td>
</tr>
<tr>
<td>72 h</td>
<td>57.34 ± 0.72</td>
<td>49.18 ± 0.83</td>
<td>38.99 ± 0.75</td>
<td>23.65 ± 0.80</td>
<td>18.48 ± 0.96</td>
</tr>
</tbody>
</table>

Table 3. 50% inhibitory concentrations (% v/v) for *L. intermedia, T. vulgaris*, and *R. officinalis* against Hu02 Cells (P < 0.05)

<table>
<thead>
<tr>
<th>Time</th>
<th>100 µg/mL</th>
<th>200 µg/mL</th>
<th>400 µg/mL</th>
<th>800 µg/mL</th>
<th>1000 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L. intermedia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>99.83 ± 0.14</td>
<td>90.63 ± 0.98</td>
<td>76.24 ± 0.69</td>
<td>61.44 ± 0.52</td>
<td>57.85 ± 0.48</td>
</tr>
<tr>
<td>48 h</td>
<td>82.63 ± 0.73</td>
<td>74.46 ± 0.84</td>
<td>67.32 ± 0.71</td>
<td>53.28 ± 0.62</td>
<td>48.41 ± 0.54</td>
</tr>
<tr>
<td>72 h</td>
<td>78.37 ± 0.64</td>
<td>73.92 ± 0.65</td>
<td>61.87 ± 0.76</td>
<td>46.63 ± 0.99</td>
<td>42.97 ± 0.79</td>
</tr>
<tr>
<td></td>
<td>T. vulgaris</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>98.54 ± 0.35</td>
<td>87.46 ± 0.68</td>
<td>68.38 ± 0.88</td>
<td>52.84 ± 0.77</td>
<td>49.76 ± 0.49</td>
</tr>
<tr>
<td>48 h</td>
<td>85.33 ± 0.47</td>
<td>77.59 ± 0.81</td>
<td>62.81 ± 0.63</td>
<td>46.93 ± 0.48</td>
<td>43.41 ± 0.78</td>
</tr>
<tr>
<td>72 h</td>
<td>76.79 ± 0.92</td>
<td>70.63 ± 0.75</td>
<td>56.12 ± 0.75</td>
<td>40.82 ± 0.56</td>
<td>35.88 ± 1.19</td>
</tr>
<tr>
<td></td>
<td>R. officinalis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>98.35 ± 0.83</td>
<td>89.32 ± 0.72</td>
<td>75.87 ± 0.63</td>
<td>60.98 ± 0.86</td>
<td>56.97 ± 0.85</td>
</tr>
<tr>
<td>48 h</td>
<td>90.26 ± 0.51</td>
<td>81.47 ± 0.94</td>
<td>63.91 ± 0.84</td>
<td>49.61 ± 0.58</td>
<td>46.35 ± 0.93</td>
</tr>
<tr>
<td>72 h</td>
<td>83.61 ± 0.69</td>
<td>75.84 ± 0.91</td>
<td>58.72 ± 0.93</td>
<td>42.19 ± 0.89</td>
<td>39.94 ± 0.97</td>
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</table>

Table 4. 50% inhibitory concentrations (% v/v) for *L. intermedia, T. vulgaris*, and *R. officinalis* essential oils + Taxol against MCF-7 cells (P < 0.05).

<table>
<thead>
<tr>
<th>Time</th>
<th>100 µg/mL + 0.001 µg/mL</th>
<th>200 µg/mL + 0.01 µg/mL</th>
<th>400 µg/mL + 0.1 µg/mL</th>
<th>800 µg/mL + 1 µg/mL</th>
<th>1000 µg/mL + 10 µg/mL</th>
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<tr>
<td></td>
<td>L. intermedia + Taxol</td>
<td></td>
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<tr>
<td>24 h</td>
<td>79.24 ± 0.67</td>
<td>68.57 ± 0.84</td>
<td>62.40 ± 0.88</td>
<td>46.84 ± 0.96</td>
<td>41.33 ± 1.04</td>
</tr>
<tr>
<td>48 h</td>
<td>62.53 ± 0.49</td>
<td>51.33 ± 0.59</td>
<td>36.67 ± 0.73</td>
<td>25.93 ± 0.85</td>
<td>21.58 ± 0.95</td>
</tr>
<tr>
<td>72 h</td>
<td>54.37 ± 0.36</td>
<td>52.48 ± 0.72</td>
<td>41.06 ± 0.69</td>
<td>24.57 ± 0.74</td>
<td>18.86 ± 0.73</td>
</tr>
<tr>
<td></td>
<td>T. vulgaris + Taxol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>48.05 ± 0.67</td>
<td>52.61 ± 0.77</td>
<td>40.54 ± 0.88</td>
<td>38.17 ± 0.92</td>
<td>31.04 ± 0.93</td>
</tr>
<tr>
<td>48 h</td>
<td>43.28 ± 0.54</td>
<td>35.81 ± 0.65</td>
<td>27.36 ± 0.58</td>
<td>20.66 ± 0.74</td>
<td>15.28 ± 0.82</td>
</tr>
<tr>
<td>72 h</td>
<td>37.95 ± 0.48</td>
<td>33.76 ± 0.53</td>
<td>28.29 ± 0.52</td>
<td>16.28 ± 0.63</td>
<td>13.87 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>R. officinalis + Taxol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>70.95 ± 0.82</td>
<td>64.55 ± 0.80</td>
<td>50.99 ± 0.95</td>
<td>42.38 ± 0.88</td>
<td>34.25 ± 1.27</td>
</tr>
<tr>
<td>48 h</td>
<td>52.08 ± 0.63</td>
<td>44.38 ± 0.72</td>
<td>34.58 ± 0.76</td>
<td>21.47 ± 0.79</td>
<td>20.64 ± 1.82</td>
</tr>
<tr>
<td>72 h</td>
<td>55.41 ± 0.14</td>
<td>45.67 ± 0.84</td>
<td>32.08 ± 0.81</td>
<td>20.98 ± 0.83</td>
<td>15.93 ± 0.94</td>
</tr>
</tbody>
</table>

Figure 1. The results of one of the triplicate MTT tests
Figure 2. The results of one of the triplicate MTT tests

Figure 3. The results of one of the triplicate MTT tests

Figure 4. The results of one of the triplicate MTT tests.
Figure 5. Morphologic changes in MCF-7 and Hu02 cells treated with essential oils at 100μg/mL
a: Untreated MCF-7 cells; b: Hu02 control cells; c-e: MCF-7 cells treated with T. vulgaris for 24, 48, and 72 hours; f-h: MCF-7 cells treated with R. officinalis for 24, 48, and 72 hours; i-k: MCF-7 cells treated with L. intermedia for 24, 48, and 72 hours; l-n: MCF-7 cells treated with 100 + 0.001 μg/mL essential oils of T. vulgaris + Taxol, R. officinalis + Taxol, and L. intermedia, respectively.
Discussion
Recently, many studies have been conducted on the anticancer and cytotoxic effects of herbal medicines as they are associated with low side effects. In this study, we evaluated the cytotoxic and inhibitory effects of *R. officinalis*, *T. vulgaris* L., and *L. x intermedia* essential oils against MCF-7 human breast cancer cells. Studies on the cytotoxic and inhibitory effects of rosemary essential oil on various cancer cell lines, including HL60, K562, MCF-7, MDA-MB-468, M14, A375, NCI-H82, DU-145, Hep-3B, and MDA-MB-231, has demonstrated that these effects vary depending on the cell lines. Research has also found that the flavonoids in rosemary inhibit the expression of Cox-2 by activating PPAR-\(\gamma\). Cox-2 is an enzyme that is upregulated in malignant and premalignant cancers such as breast, colon, pancreas, lung, stomach, head and neck, skin, and pharynx. Applying the essential oil of *R. officinalis* to the skin of mice prevents benzopyrene covalent binding to DNA and inhibits epidermal tumor formation. Moreover, the uric acid in *R. officinalis* prevents the binding of benzopyrene to epidermal cell DNA, and TPA to the membrane of these cells. Uric acid inhibits NF-\(\kappa\)B pathway in cancer cells possibly by suppressing the p65 component of NF-\(\kappa\)B and, thus, downregulating oncogenes such as Cox-2 and MMP-9, Cyclin D1, C-Jun, and C-Fas. Also, the carnosol in *R. officinalis* leaves functions in a similar manner and inhibits the activity of NF-\(\kappa\)B. In line with our findings, the anticancer properties of the compounds of the essential oils of *R. officinalis* and *L. x intermedia* have been demonstrated by other studies.

Regarding anticancer properties of *T. vulgaris*, Sertel and colleagues demonstrated in vitro inhibition of a head and neck squamous cell carcinoma cell line treated with the plant’s essential oil. The present study also showed the antitumor and antitoxic effects of essential oils of *T. vulgaris* on MCF-7 cells.

Assessment of morphological changes indicates that some changes, such as nuclear disintegration, formation of apoptotic bodies, and membrane blebbing, observed in the cells treated with Taxol and essential oils are indicative of apoptosis. However, based solely on these results, we cannot demonstrate the occurrence of apoptosis and this requires specific tests such as TUNEL assay or flow cytometry-based assays.

Eventually, it can be concluded that effective compounds in essential oils of *T. vulgaris*, *R. officinalis*, and *L. x intermedia* induce cell death in MCF-7 cancer cells. Literature review and the results of various studies, as well as our results, show that these three plants of Lamiaceae family have biological and pharmacological potential, and it seems that isolating effective components of these plants as well as determining their structure and exact mechanisms of action should be the topics for future research.

Acknowledgement
This study was financially supported by the vice-chancellor of research, Boroujerd Islamic Azad University, Lorestan, Iran.

Conflict of Interest
The authors declare that the research was conducted in the absence of any commercial relationships that could be construed as a potential conflict of interest.

References:


Apoptosis through both the mitochondrial apoptotic pathway and death receptor pathway in human colon adenocarcinoma COLO 205 cells. Food and Chemical Toxicology. 2011;49(2):485-93.
**Background:** Viral nanoparticles are biodegradable, biocompatible, self-assembling, and highly symmetric, and can be produced in large quantities. Several plant viral nanoparticles (VNPs) have been exploited in different areas of nanobiotechnology, especially drug delivery in cancer therapy. In this study, a flexuous plant virus called potato virus X (PVX) is presented with a unique nanoarchitecture which can increase tumor homing and penetration. Thus, this study aimed to investigate the potential of PVX for delivering Herceptin (HER) in different breast cancer cells and normal cells.

**Methods:** PVX was conjugated to HER by EDC/Sulfo-NHS in two steps. After confirming the conjugation, PVX-HER efficacy and drug activity were investigated in HER2-positive (SKBR3 and SKOV3), HER2-negative (MCF-7 and MDA-MB-231), and non-tumorigenic epithelia breast cancer (MCF-12A) cell lines after treatment with 10 and 20 µg of the drug. Then, PVX-HER was imaged in SKBR3 cells in to study the nuclear accumulation of the drug at different concentrations.

**Results:** An increased cytotoxic efficiency was observed for PVX-HER vs free-HER in SKBR3 and SKOV3 cell lines. However, the efficacy of PVX-HER failed to increase in MCF-7, MDA-MB-231, and MCF-12A cell lines compared with free-HER after 24 hours. In addition, compared with free-HER, Herceptin nuclear accumulation was increased in SKBR3 cells treated with PVX-HER. Further, the PVX-HER treatment resulted in reduced tumor growth in the HER2-positive cells lines. Finally, a direct relationship was observed between the imaging results and MTT assay in SKBR3.

**Conclusion:** PVX-HER displays a significantly greater cytotoxic activity compared with free-HER in HER2-positive cells.
VNPs from plants are preferable to those from animals due to their safety. One of the most popular rod-shaped VNPs is potato virus X (PVX), which is considered an important economic filamentous virus of \(515 \times 12\) nm in size belonging to the genus \(Potexvirus\) and the family \(Alphaflexiviridae\). The virus encompasses economically important pathogens worldwide, with variable symptoms. PVX has a single-stranded positive-sense RNA genome which is approximately 6400 nucleotides long with a cap and poly(A) tail. The genome contains five open reading frames (ORFs) which encode proteins of 166, 25, 12, 8, and around 26 kDa, respectively. Esfandiari et al. reported a new isolate of PVX (PVX-Iran, accession no. FJ461343) (figure 1). Over the last decade, virus research has been led toward the beneficial use of viruses independent of their potential pathogenesis.

PVX nanoparticles are used for imaging, drug delivery, and diagnosis. Further, PVX could increase tumor homing and penetration, compared to spherical cowpea mosaic virus (CPMV). Conjugation of PVX to doxorubicin (PVX-DOX) caused a reduction in tumor growth.

To prepare PVX-HER nanoparticles, we incubated PVX (5 µg µl\(^{-1}\)) with an EDC/Sulfo-NHS (N-hydroxysulfosuccinimide) cross-linker for 4 hours in reaction buffer (0.5 M NaCl, 0.1 M MES, pH 6). In order to remove the unconjugated free linker, the reaction was quenched by 2-mercaptoethanol and Biogel P-10 column (Bio-Rad). Accordingly, HER (50 µg µl\(^{-1}\)) was conjugated to PVX-EDC/Sulfo-NHS for 2 h in 0.1 M NaCl buffer (pH 7.5). Then, PVX-HER nanoparticles were purified by a Biogel P-10 column (Biorad). In the present study, the characterization was conducted using Zetasizer (ZEN 3600, Malvern) and also with Western blotting using a PVX-specific primary antibody (DSMZ, PV-0027) and a secondary goat polyclonal antibody against rabbit IgG-HRP (Abcam, ab6721).

**Methods**

Nicotiana benthamiana is a common experimental host in propagating PVX in large amounts. N. benthamiana leaves were inoculated with phosphate buffer (pH 7.2) for the purpose of purification, which caused an increase in virus accumulation after 2 to 3 weeks, and the leaves were harvested and purified as described previously.

To prepare PVX-HER nanoparticles, we incubated PVX (5 µg µl\(^{-1}\)) with an EDC/Sulfo-NHS (N-hydroxysulfosuccinimide) cross-linker for 4 hours in reaction buffer (0.5 M NaCl, 0.1 M MES, pH 6). In order to remove the unconjugated free linker, the reaction was quenched by 2-mercaptoethanol and Biogel P-10 column (Bio-Rad). Accordingly, HER (50 µg µl\(^{-1}\)) was conjugated to PVX-EDC/Sulfo-NHS for 2 h in 0.1 M NaCl buffer (pH 7.5). Then, PVX-HER nanoparticles were purified by a Biogel P-10 column (Biorad). In the present study, the characterization was conducted using Zetasizer (ZEN 3600, Malvern) and also with Western blotting using a PVX-specific primary antibody (DSMZ, PV-0027) and a secondary goat polyclonal antibody against rabbit IgG-HRP (Abcam, ab6721).

**Cell Culture**

SKBR3 and SKOV3, as HER2+ cells lines, and MDA-MB-231 and MCF-7 (as HER2- cells lines) were cultured in RPMI-1640 medium (RPMI, Gibco) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco) and 1% (v/v) penicillin-streptomycin (Pen-Strep, Gibco). Then, MCF-12A cells as a normal breast cancer was grown in Dulbecco’s modified Eagle’s medium (DMEM, Gibco) supplemented with 10% (v/v) FBS, 1% (v/v) pen-strep. Finally, all cells were seeded in 96-well plate (SPL, Life Sciences, Gyeonggi, Korea) and incubated at 37°C and 5% CO\(_2\) in a humidified environment.

**Cytotoxicity Evaluation**

Cells were washed with PBS after reaching a minimum 75% confluence. To this end, they were trypsinized with 0.25% (w/v) trypsin-EDTA (Gibco,
Tracking of PVX–HER in Cells

First, HER2/neu-positive breast cancer cells (SKBR3) were cultured. After reaching the cells confluency, they were seeded onto coverslips (SPL, 20012) in 24-well plates (5×10^4 cell/well) and incubated at 37°C, 5% CO2. In the next step, cells were washed and treated with free-HER (10 µg) and PVX-HER (10 or 20 µg). After 4 hours, the cells were washed, fixed in cold solution containing methanol/ethanol (1:1), and washed with PBS. Additionally, 4% BSA in 1X PBS was used for 30 min at room temperature. PVX antibody (DSMZ, PV-0027) was added and shaken for 90 minutes. Finally, the coverslips were washed with PBST (phosphate-buffered saline with Tween-20) and mounted by donkey anti-rabbit IgG-PE (Santa Cruz, sc-3745). The slides were imaged with a LABEX fluorescent inverted microscope.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA), along with LSD post hoc test, on SPSS 16.0. Results are reported as mean ± standard error of mean (SEM). Before determining the level of significance for the tests, the normality of data distribution was tested using Kolmogorov-Smirnov and Shapiro-Wilk tests. The difference in values was significant if the P value was less than 0.001. All experiments were performed in triplicate and repeated at least 3 times.

Results

After conducting the mechanical inoculation of N. benthamiana, mosaic symptoms were observed within 3 weeks. About 1 mg of pure PVX was extracted from each gram of infected plant. Western blotting with human antibody demonstrated a protein band of HER with a molecular weight of 55 kDa and PVX-HER conjugate with a molecular weight of 83 kDa. Also, the value obtained by Zetasizer for HER-PVX was -6.0 that was between PVX (-21.4) and HER2 (-1.48) charges (figure 2).

![Figure 2](image.png)

Figure 2. Scheme of potato virus X (PVX) conjugated to Herceptin (HER) with EDC/Sulfo-NHS in a two-step protocol western blot with PVX-specific antibody (DSMZ, PV-0027) and zeta potential was used to confirm conjugation.

In the present study, cytotoxic activity of HER-PVX against HER2-positive, HER2-negative, and normal cell lines was examined. SKBR3, SKOV3, MDA-MB-231, MCF-7, and MCF-12A cells were treated with 10 µg and 20 µg of PVX-HER and free-HER for 24 hours. The results of cell viability assay are presented in Table 1.
Table 1. The mean percentage ± SEM of cell viability for breast cancer cell lines by MTT

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>PVX-HER (10 µg)</th>
<th>Free-HER (10 µg)</th>
<th>PVX-HER (20 µg)</th>
<th>Free-HER (20 µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SKBR3</td>
<td>17.973 ± 0.005</td>
<td>47.697 ± 0.004</td>
<td>16.067 ± 0.004</td>
<td>42.510 ± 0.003</td>
</tr>
<tr>
<td>SKOV3</td>
<td>28.003 ± 0.005</td>
<td>37.641 ± 0.004</td>
<td>22.916 ± 0.006</td>
<td>33.518 ± 0.002</td>
</tr>
<tr>
<td>MCF-7</td>
<td>96.322 ± 0.010</td>
<td>98.478 ± 0.009</td>
<td>96.569 ± 0.0108</td>
<td>95.859 ± 0.0135</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>97.420 ± 0.009</td>
<td>97.856 ± 0.011</td>
<td>96.889 ± 0.005</td>
<td>96.661 ± 0.0092</td>
</tr>
<tr>
<td>MCF-12A</td>
<td>98.590 ± 0.010</td>
<td>98.561 ± 0.011</td>
<td>99.059 ± 0.006</td>
<td>98.559 ± 0.009</td>
</tr>
</tbody>
</table>

In addition, fluorescence microscopy was performed to monitor Herceptin accumulation in the cells in order to investigate the efficacy of PVX-HER compared with free-HER. SKBR3 cells were treated with free-HER (10 µg) and PVX-HER (10 µg and 20 µg) for 4 hours. Herceptin accumulated inside the nuclei of SKBR3 cells after 3 hours (figure 3). Localized delivery of Herceptin in the nucleus is more than that of cytoplasm. Fluorescence intensity is positively correlated with nuclear Herceptin accumulation. Thus, the greater fluorescence intensity in the cells treated with PVX-HER compared with free-HER indicates the greater efficacy of the PVX delivery system.

**Discussion**

The use of VNPs in medicine has expanded rapidly over the past 20 years to span a wide range of applications from imaging to targeted drug delivery. VNPs are naturally occurring bionanomaterials with unique symmetrical outer surface. They are biodegradable, nontoxic for mammals, and are produced at lower costs. Some studies have emphasized their use as contrast agents in MRI imaging and as facilitators in drug delivery.

PVX is the type member of the genus *Potexvirus* with flexible rod particles measuring 515 × 13 nm. PVX has proved more effective in tumor homing and penetration compared with spherical or isometric virus.

During recent years, the benefits of PVX have increased the use of this virus as a VNP, which have been emphasized in different cases such as the use of PVX for drug delivery system for Doxorubicin (DOX) and Herceptin for cancer therapy. The development of coadministered PVX-DOX in situ vaccination for treatment is a possibility.

Herceptin is the trade name for trastuzumab, approved by the FDA in 1998 in order to treat human epidermal growth factor receptor (HER) 2-positive breast cancer. Herceptin is an expensive monoclonal antibody which can cause serious side effects. The conjugation of drugs to nanoparticles helps to enhance therapeutic efficacy and reduce side effects.

In the previous study, PVX-HER nanoparticles significantly increased the death rate of HER2-positive cancer cells (SKBR3 and SKOV3). In the present study, the effect of this nanoparticle on other cell lines was investigated to complete the previous information. Thus, the effect of PVX-HER on cell viability was tested in SKBR3 (breast cancer) and SKOV3 (ovarian cancer) as HER2-positive cells, MDA-MB-231 (breast cancer) and MCF-7 (breast cancer) as HER2-negative cell lines, and MCF-12A as a nontumorigenic epithelial breast cell line. The results of ANOVA indicated a significantly increased cell death in SKBR3 (figure 4-A) and SKOV3 (figure 4-B) cell lines when treated with PVX-HER compared with free-HER. However, in MDA-MB-231 (figure 4-C), MCF-7 (figure 4-D), and MCF-12A (figure 4-E) cell lines, there was no significant difference in the mean absorption values between PVX-HER and free-HER. Therefore, the results provide a clear answer to how PVX-HER works on breast cancer cells. Further, SKBR3 cells were expected to address the question about the uptake of PVX-HER in the cells based on the results obtained by MTT assay. Fluorescence microscopy was used to monitor nuclear accumulation of Herceptin. As reported in previous studies, Herceptin accumulated within the nuclei of the SKBR3 cells after 3h. Then, a greater nuclear accumulation of Herceptin was observed in SKBR3 cells treated with 10 and 20 µg of PVX-HER compared with 10 µg of free-HER, indicating the greater efficacy of the PVX-based drug delivery systems.
PVX-HER displayed a significantly greater cytotoxic activity compared with free-HER in HER2-positive cells. Therefore, the relationship between the imaging results and the MTT assay following 24 hours of incubations with 10 and 20 µg of PVX-HER may be explained. However, PVX-HER failed to increase the cytotoxic efficacy in HER2-negative and normal epithelial breast cell lines compared with free-HER (figure 4-F).

In general, based on the drug delivery features of PVX, the results of the present study were consistent with the findings of the previous studies on VNPs. Doxorubicin was covalently conjugated to CMV, CPMV, PVX, and RCNMV in order to enhance the drug delivery.

The results of this study, as the first research on PVX-HER nanoparticles, together with that of the previous study, confirm the effect of PVX-HER nanoparticles on breast cancer cells.

As conclusion, the present study recommends the use of the new isolated PVX, as a plant viral nanoparticle, for delivering Herceptin. Based on the results, Herceptin loaded onto the surface of PVX increases the drug efficacy in HER2-positive cancer cells. The results can contribute to development of filamentous plant virus-based drug delivery systems in cancer treatment.

Acknowledgement
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Conflict of Interest
The author declares no conflict of interests.

References:
Delivering Bad News: Deal with Collusion for Love

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Bad news is “any news that adversely and seriously affects an individual’s view of his or her future,” and talking about one’s cancer diagnosis is one of them. Delivering bad news is a complex, necessary communication skill that should be effectively learned, especially by oncologists. Giving bad news has two key parts: transferring information to patients and responding to patients’ emotional reactions.  

Every patient has a unique cultural and family background; therefore, individualized care communication is critical.

On a typical day in my office, I was sitting behind my desk and waiting for the next patient to arrive. A man in his thirties poked his head around my office door and mentioned that the patient was his mother and desperately asked me not to reveal anything about her diagnosis to her.

“Doctor, my mother is 86 years old and hope is the only thing that has kept her alive. My father passed away last year, and I don’t want her to suffer more,” he said. Then he stared at me for a few seconds waiting for my approval.

He left the room and quickly returned while pushing his mother in a wheelchair. She was referred to me by her oncologist for palliative care consultation. The woman in the wheelchair had such a big smile on her face that I dared to crack a joke about how his son was pushing the wheelchair and if she was not happy about the ride, I would have his driver’s license revoked.

Noticing “terminal breast cancer” on her consultation sheet erased the smile from my face. Her oncologist had left the note that she was “NOT suitable for any invasive treatment.” The son was still standing behind the wheelchair signaling by constantly moving his hands to remind me to keep our big secret. It was so distracting that I decided to find an excuse to send him away. “Sir, please pick up a new patient folder from the front desk at the end of the corridor.”

As soon as he left, the mother leaned toward me and whispered, “I have advanced cancer and I’m going to die soon. I don’t want my son to know how bad this is. He has already had enough pain since his father passed away last year.” I instantly realized that the situation is more complicated than I expected, and I should act very tactfully. The son returned with the folder and I struggled to pretend nothing happened in his absence.

“What can I do for you, Mrs. A?” I asked. It was not my usual opening question. Before she had a chance to say anything, her son jumped in and said: “She has an old peptic ulcer that makes her uncomfortable, especially after eating; and also she needs some appetite pills to gain more weight.”

It was time for me to make a serious face and asking him to allow his mother to answer the questions. “Yes, yes, he’s right. My stomach is painful all the time, and I feel nauseous every time I try to …,” she stopped speaking. After an uncomfortable pause, she suddenly broke down in tears and asked her son to forgive her for not being able to stay with him long enough.

There was not much work for me to do. The “news” were broken. I was not certain how they would react to this new situation. Perhaps it would help to bring them closer to each other.

This is a common scenario in my clinic: “collusion for love!” Every day I see patients with terminal cancer who have no clue about their conditions, and I am warned by their relatives not to disclose the diagnosis or prognosis to them. In this particular case, it was more complicated: a tripartite collusion. Although collusion with relatives is not recommended, sometimes (especially in busy clinics) it is a more convenient option for physicians than disclosure.

While western medicine emphasizes honest
Disclosure of bad news to patients by physicians, depending on the cultural context, every patient needs an individualized response from the physician.

In today’s Iranian palliative care practice, considering the family dynamics is unavoidable for the physician to provide the best solution. This dynamic is referred to as “relational autonomy.” In eastern societies in which family unit is the main supporting source for the patients—economically, emotionally, socially, and spiritually—dealing with family’s involvement in patients’ medical decision making is deemed critical. Changing the paradigm from just giving bad news to “sharing news” is a more preferred policy. This strategy has its own challenges and barriers, but, by learning and following the available guidelines, physicians can achieve the best results.

Conflict of Interest
The authors have nothing to disclose.

References
Diagnostic Efficacy of Technetium-99m-Sestamibi Scintimammography in Comparison with Mammography to Detect Breast Lesions: A Systematic Review

Sahel Heydarheydari, Seyed Masoud Rezaiejo, Mohsen Cheki, Ehsan Khodamoradi, Karim Khoshgard

ABSTRACT

Background: To systematically review the performance of scintimammography compared with mammography in detecting breast lesions.

Methods: A literature search was performed in PubMed and ScienceDirect databases with “scintimammography AND breast lesions,” “mammography AND breast lesions,” “diagnostic value,” and “accuracy” as keywords to identify all related studies published in English from January 1, 2000, to August 1, 2017. Twenty-five studies, with a total of 4094 patients with clinically suspicious breast lesions, were included in the final analysis to assess the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of scintimammography vs. mammography in detecting breast lesions.

Results: The sensitivity and specificity of mammography were 75.82 ± 10.53 (95% confidence interval [CI], 50-84) and 59.58 ± 22.79 (95% CI, 20-91.4), respectively. The PPV and NPV of mammography were 75.60 ± 2.21 (95% CI, 42-93) and 61.62 ± 1.67 (95% CI, 39.1-86), respectively. The sensitivity of scintimammography was 86.64 ± 8.84 (95% CI, 58.3-100), and the specificity was 83.42 ± 10.74 (95% CI, 60-100). The PPV and NPV of scintimammography were 82.10 ± 11.65 (95% CI, 58-98.30) and 81.02 ± 17.00 (95% CI, 45-100), respectively.

Conclusions: Although mammography has a high sensitivity in the examination of older patients with fatty breast tissue, it is less reliable in detecting breast lesions in young and premenopausal patients with dense breasts. Diagnostic accuracy of scintimammography, as a functional imaging modality, is not affected by breast density, contrary to mammography. Therefore, scintimammography can improve the specificity of mammography.

Key words: Technetium-99m-sestamibi, scintimammography, mammography, breast lesions

Introduction

Breast cancer is the most frequent malignancy in women, affecting 1 in 13 women in their lifetime.1-4 Patients with breast cancer who have it detected at an early stage will have a better survival rate.1 The most widely used tool for detection of breast cancer, besides the physical examination, is mammography, which has a high accuracy in detecting breast lesions.1 The sensitivity of mammography in patients with dense breasts may be low;1,4 and, in patients with fibrocystic changes of the breast, mammographic detection of breast carcinoma may be difficult. Mammography has low specificity in distinguishing...
The aim of the present systematic review was to compare the performance of scintimammography with mammography in the detection of breast lesions.

Methods
Search strategy
A literature search was performed in PubMed and ScienceDirect databases using the following keywords: “scintimammography (mammoscintigraphy) AND breast lesions,” “mammography AND breast lesions,” “diagnostic value,” and “accuracy.” Articles that cited related studies were also searched to find any related publication (using PubMed, Europe PubMed Central, and Google Scholar citation tracking tools). An updated search strategy was developed in order to identify all related papers published in English from January 1, 2000, to August 1, 2017. The full search strategy is presented in Figure 1.

Selection of studies
Titles and abstracts obtained from the literature search were examined for eligibility. Information given in the titles and abstracts had to suggest that the study (1) included patients with suspected breast...

Figure 1. Flowchart summarizing search strategy and study selection

between malignant and benign processes. Therefore, it is impossible to detect breast lesions using mammography alone in some cases. The high rate of breast biopsies in patients with benign lesions has encouraged the use of non-invasive imaging modalities with greater accuracy such as magnetic resonance imaging (MRI), ultrasound (US), positron emission tomography (PET), and scintigraphy. Recently, it was demonstrated that technetium-99m-sestamibi (Tc-MIBI) accumulates in different types of tumors. For suspected breast lesions, some studies demonstrated that scintigraphy with Tc-MIBI differentiates benign from malignant lesions. Tc-MIBI scintigraphy is a noninvasive diagnostic modality in evaluating breast carcinoma in the field of nuclear medicine.

The aim of the present systematic review was to compare the performance of scintimammography with mammography in the detection of breast lesions.
lesions, (2) conducted scintimammography or mammography in those patients, and (3) evaluated diagnostic values of the tests (sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]). Full-text articles were retrieved for further assessment.

**Inclusion/exclusion criteria**

Studies were included if they assessed sensitivity, specificity, PPV, or NPV of scintimammography or mammography in the diagnosis of breast lesions. We included studies with or without comparator groups. Editorials, case reports, and review articles were excluded. All eligible papers were compared independently assessed by two authors for predefined inclusion criteria.

**Data extraction**

Two authors independently extracted the following data from each included study: the first author’s name, publication year, journal, country, details of study design, number of patients and their characteristics, index test or tests, reference standard, sensitivity, specificity, PPV, and NPV. The diagnostic performance of scintimammography and mammography in the detection of breast lesions was assessed by comparing index test results with the reference standards. Any discrepancies between the two researchers were resolved through consensus.

The patients were classified as true positive (TP) when both the index test (i.e., scintimammography or mammography) and the reference standard (pathological assessment) detected breast lesion, true negative (TN) when neither test detected breast lesion, false negative (FN) when the index test failed to detect a breast lesion identified by the reference standard, and false positive (FP) when the index test incorrectly suggested a breast lesion not detected by the reference standard. Sensitivity was defined as TP/(TP+FN) and specificity as TN/(TN+FP).

**Quality assessment**

We used the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) to evaluate the quality of the included studies. Assessment of the risk of bias and applicability concerns were performed in 4 domains including patient selection, index test, reference standard, and flow and timing. Applicability concern and risk of bias were judged as low, high, or unclear for the various QUADAS domains.

**Statistical analysis**

SPSS 16 was used for data analysis using descriptive statistics. Sensitivity, specificity, PPV, and NPV, along with 95% confidence intervals (CIs), were calculated for each study.

**Table 1. Main characteristics of the studies evaluating the diagnostic value of scintimammography**

<table>
<thead>
<tr>
<th>First author (Year)</th>
<th>Number of patients</th>
<th>Mean age (Year)</th>
<th>Index test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV* (%)</th>
<th>NPV** (%)</th>
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<td>96</td>
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<td>93.3</td>
<td>71.4</td>
<td>87.5</td>
<td>83.3</td>
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<td>172</td>
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<td>100</td>
<td>93.5</td>
<td>92.5</td>
<td>100</td>
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</tbody>
</table>

*PPV: Positive predictive value; **NPV: Negative predictive value
Results
Twenty-five studies assessing the sensitivity, specificity and/or PPV and NPV of scintimammography or mammography in the detection of breast lesions were included in the analysis. The studies included a total of 4094 patients (age: 51.68 ± 4.07 y) with clinically suspicious breast lesions. Table 1 shows the main characteristics of the 16 studies evaluating the diagnostic value of scintimammography, and Table 2 shows the main characteristics of the 3 studies assessing the diagnostic value of mammography. Six studies compared the diagnostic value of scintimammography with mammography (Table 3).

Sensitivity and specificity of mammography were 75.82 ± 10.53 (95% CI, 50-84) and 59.58 ± 22.79 (95% CI, 20-91.4), respectively. Also, mammography had a PPV of 75.60 ± 2.21 (95% CI, 42-93) and an NPV of 61.62 ± 1.67 (95% CI, 39.1-86).

The sensitivity of scintimammography was 86.64 ± 8.84 (95% CI, 58.3-100), and its specificity was 83.42 ± 10.74 (95% CI, 60-100). PPV and NPV of scintimammography were 82.10 ± 11.65 (95% CI, 58-98.30) and 81.02 ± 17.00 (95% CI, 45-100), respectively.

99mTc-MIBI scintigraphy plays an important role in localizing the breast tumors when ultrasound or mammography is not contributory. Diagnostic accuracy of scintimammography, as a functional imaging modality, is not affected by breast density, contrary to mammography, because of the advantages of labeling with 99mTc sestamibi radiopharmaceutical. Its uptake in the lesion involves several causes, including mitochondrial activity, angiogenesis, and presence of malformed vessels, but does not depend on the presence of architectural distortion and localized variation in breast density.

Discussion
Although mammography has high sensitivity in the examination of older patients with fatty breast tissue, it is less reliable in detecting breast lesions in patients with dense breasts, breast implants, and architectural distortion after radiation therapy or surgery.\(^7\) In a mammography unit with both a rhodium (Rh) anode and a molybdenum (Mo) anode, filtered with rhodium and molybdenum, respectively, the mammograms obtained by using the Mo/Mo combination were preferred. However, the mammograms obtained with the Rh/Rh combination were better than the Mo/Mo mammograms for young patients with dense breasts.\(^8\)

### Table 2. Main characteristics of the studies evaluating the diagnostic value of mammography

<table>
<thead>
<tr>
<th>First author (Year)</th>
<th>Number of patients</th>
<th>Mean age (Year)</th>
<th>Index test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV* (%)</th>
<th>NPV** (%)</th>
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<td>63</td>
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<td>Chen (2002)(^9)</td>
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<td>60</td>
<td>Mammography</td>
<td>84</td>
<td>80</td>
<td>93</td>
<td>63</td>
</tr>
<tr>
<td>Kotsianos-Hermle (2009)(^11)</td>
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<td>97</td>
<td>Mammography</td>
<td>76.5</td>
<td>91.4</td>
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</table>

*PPV: Positive predictive value; **NPV: Negative predictive value

### Table 3. Main characteristics of the studies evaluating the diagnostic value of scintimammography

<table>
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<th>First author (Year)</th>
<th>Number of patients</th>
<th>Mean age (Year)</th>
<th>Index test</th>
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<th>Specificity (%)</th>
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<td>28</td>
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<td></td>
<td>8</td>
<td>under 30</td>
<td>MG (young females)</td>
<td>50</td>
<td>20</td>
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<tr>
<td></td>
<td>24</td>
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<td>MG (previous surgery)</td>
<td>80</td>
<td>42</td>
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<td></td>
<td></td>
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<td>SM (dense breasts)</td>
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<td>90</td>
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<td></td>
<td></td>
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<td>SM (young females)</td>
<td>100</td>
<td>100</td>
<td>——</td>
<td>——</td>
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<tr>
<td></td>
<td></td>
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<td>SM (previous surgery)</td>
<td>80</td>
<td>100</td>
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<td>——</td>
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<tr>
<td>Sun (2000)(^9)</td>
<td>81</td>
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<td>SM (Planar)</td>
<td>83</td>
<td>47</td>
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<td>——</td>
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<td>87</td>
<td>47</td>
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<td>90.6</td>
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<td>72</td>
<td>81</td>
<td>57</td>
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<td>SM (Planar)</td>
<td>83</td>
<td>83</td>
<td>59</td>
<td>94</td>
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<td>46</td>
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<td>MG (Planar)</td>
<td>81</td>
<td>63</td>
<td>54</td>
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</table>

MG: Mammography; SM: Scintimammography; *PPV: Positive predictive value; **NPV: Negative predictive value
The intensity of \(^{99m}\text{Tc-MIBI}\) uptake varies from mild to high depending on factors such as the type, size, location, and hormonal factors. The size of the lesion affects sensitivity. The sensitivity for palpable lesions is significantly higher than that for nonpalpable ones. Another clinical application of \(^{99m}\text{Tc-MIBI}\) scintigraphy is the detection of patients with microcalcifications. Scintimammography seems to be helpful in differentiating malignant from benign calcifications and leads to a decrease in the frequency of breast biopsies. Some studies had separated the statistics for palpable and nonpalpable lesions because of the difference in management strategy. Palmedo reported that the total specificity and sensitivity of \(^{99m}\text{Tc-MIBI}\) scintimammography were 69% and 71%, respectively; for palpable lesions, however, the specificity and sensitivity of scintimammography increased to 91% and 83%, respectively. Palmedo reported similar results in another research and showed that the total sensitivity of scintimammography was 88%, increasing to 100% for palpable lesions. Another study reported that the sensitivity of scintimammography in detecting nonpalpable lesions was 78.3% compared with 89.1% for mammography; but, in palpable lesions, the sensitivity of scintimammography (91.3%) was higher than the sensitivity of mammography (78.2%). Based on the clinical studies, \(^{99m}\text{Tc-MIBI}\) scintimammography is more accurate than mammography in differentiating palpable breast lesions. Therefore, the utility of the technique has been emphasized to decrease the frequency of breast biopsies. As mentioned, the sensitivity of scintimammography can be affected by tumor size, and the specificity and sensitivity of \(^{99m}\text{Tc-MIBI}\) scintimammography increase for palpable breast lesions. Therefore, attempts have been made to enhance the sensitivity of scintimammography for the detection of cancer, especially for nonpalpable and ≤1-cm lesions. Myslivecek compared the specificity and sensitivity of scintimammography in detecting primary breast lesions with both single-photon emission computed tomography (SPECT) and planar images (Table 1). The results showed that SPECT scintimammography was slightly (10%) more sensitive than planar scintigraphy. Taillefer and Khalkhal reported PPV values of 97.7% and 76.9%, respectively, and NPV values were 81% and 97%, respectively. Palmedo reported that in 60% of false-negative (FN) mammograms, scintimammography was able to detect malignant lesions true-positive (TP). It is worth mentioning that scintimammography with \(^{99m}\text{Tc-MIBI}\) or \(^{99m}\text{Tc-sestamibi}\) scintimammography is a noninvasive imaging modality and highly sensitive test in detecting primary breast lesions.

The present systematic review demonstrates the high diagnostic value of scintimammography with \(^{99m}\text{Tc-MIBI}\) as a complementary method that improves the specificity of mammography and is potentially able to reduce the frequency of breast biopsies. Scintimammography is a noninvasive, low–radiation dose diagnostic method and an easy-to-perform procedure which may offer additional information above that provided by conventional radiology, especially in young, premenopausal patients with dense breasts and in patients who are on hormone replacement therapy, where the sensitivity of mammography is limited by the characteristics of the breast tissue. Technetium-99m-sestamibi scintimammography has the potential to determine the metabolic state of microcalcification, primary breast lesion, axillary lymph node detected by other imaging modalities. Scintimammography may discriminate between benign and malignant breast lesions in patients with a palpable mass or when the lesion size is more than 10 mm.

However, mammography has a high sensitivity in the examination of older patients with fatty breast and is associated with a small amount of radiation exposure. Mammography has a better spatial resolution compared with scintimammography. Nevertheless, the development and general availability of high-resolution cameras dedicated to breast imaging will probably allow scintimammography to become of routine use.

Conflict of Interest
The authors have no potential conflict of interest concerning the content of the present article.

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Management of Benign Phyllodes Tumor With Close Margins: A Case Presented in Multidisciplinary Session With Clinical Discussion and Decision Making

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ABSTRACT

**Background:** Phyllodes tumors (PTs) are a small group of fibroepithelial breast lesions, which are classified as benign, borderline, or malignant. Traditionally, a margin of 1 cm has been suggested as the standard of care for all groups of PTs. According to new studies and recent updates, the recurrence rate of benign PT is low and not associated with the surgical margin status. There is still a controversy for PT surgical margin.

**Case Presentation:** A woman with the primary diagnosis of fibroadenoma in core needle biopsy underwent surgery. The pathology report showed benign PT and margins about 1 mm in some areas. This case was presented in the weekly breast multidisciplinary team session of the Department of Breast Surgery, Tehran University of Medical Sciences.

**Question:** The question was whether re-excision was necessary to achieve the safe surgical margin.

**Conclusion:** In accordance with the latest published evidence, the members of the panel decided to accept the margin, informed the patient about the risk of recurrence, and recommended close follow-up.

Introduction

Phyllodes tumors (PT) are rare fibroepithelial tumors that can affect women of all ages, with the median age at presentation being 45 years. Since the description of “cystosarcoma phyllodes” by Johannes Mueller first in 1838, numerous synonyms and histologic classification systems have been introduced. The World Health Organization (WHO) prefers the use of “phyllodes tumors.” These tumors are classified as benign, borderline, or malignant based on histologic parameters such as mitotic activity, stromal cell atypia, stromal overgrowth, stromal heterologous component, stromal necrosis, and infiltration of normal breast tissue.

Core needle biopsy constitutes the pathological basis of the preoperative diagnosis of PT; however, it is not accurate enough to offer perfect guidance for surgical decisions. The high underestimation rate in these conditions makes the surgical planning complicated. When the tumor to breast ratio is too high to allow a satisfactory cosmetic outcome with segmental excision, total mastectomy either with or without reconstruction could be considered.

Axillary lymph node dissection is recommended to be performed only for clinically suspicious nodes, although it is unlikely that malignant cells would be found in them. There is no evidence for an effective role of adjuvant chemotherapy in the treatment of PT. Controversy exists as to the size of initial resection and need for revision surgery to achieve adequate margins. Guidelines have advocated wide excision with margins ≥ 1 cm for all kinds of PT. However, this cannot always be achieved, as the observation of margins closer than 1 cm in pathologic evaluation is common—a situation that...
often questions the necessity of a surgical revision of margins.

Case Presentation
A 46-year-old woman with a family history of breast cancer presented to our breast clinic with a chief complaint of the left breast mass. The mass, measuring 20 × 20 mm, was palpable in the upper outer quadrant of her left breast. It was soft, nontender, and mobile with a regular border. No palpable axillary lymph node was found. In mammography, there was an ill-defined mass in the upper outer quadrant of the left breast with a BIRADS score of 3. Ultrasonography reported an oval-shaped, well-circumscribed hypoechoic lesion measuring 19 × 15 mm with lobulated borders at the 2-o’clock position parallel to the skin. Internal vascularity was seen within the mass and ultrasonography did not show any suspicious axillary lymph nodes with scoring of 4a in BIRADS assessment categories. Pathology report of core needle biopsy of the mass was fibroadenoma. The patient underwent surgery out of concern due to the family history. Analysis of permanent pathology sections showed clefts of epithelial cells surrounded by hypercellular stroma. Stromal cells displayed mild pleomorphism with scanty mitotic figures (0-1 per 10 high-power fields [HPF]) with no evidence of necrosis. These findings suggested benign PT. All surgical margins were free, and the closest margins were located at the superior, and lateral area. They were both 1 mm.

Question
The case was presented at the weekly breast multidisciplinary team session at the Department of Breast Surgery, Tehran University of Medical Sciences. The question was whether the patient should be reoperated on to obtain more extensive margins.

Discussion
Each type of PT requires different management. Malignant PTs are characterized by marked stromal cellularity and atypia, infiltrative margins, high mitotic rate (more than 10 mitoses per 10 HPF), and the presence of stromal overgrowth. Surgery is the standard treatment for malignant PT. Surgical margins of ≥ 1 cm are accepted by most resources and guidelines.4,5,6,12-14 The surgery includes breast-conserving surgery or mastectomy depending on the size of the tumor.5,15-17 It has been accepted that lymph node dissection is not recommended.6,12-14 Adjuvant therapies (radiotherapy and chemotherapy) have no clearly defined role in the treatment of nonmetastatic malignant PT. Several studies suggest that adjuvant radiotherapy could reduce local recurrence when adequate surgical margins cannot be achieved.11,14,16,18-22 Hormone therapy is not effective against malignant PT.4,22,23 Borderline PTs have a greater degree of stromal cellularity and atypia, a mitotic rate of 4 to 9 mitoses per 10 HPF, microscopic infiltrative borders, and a lack of stromal overgrowth.24-26 The approaches to breast surgery, axillary management, and the surgical margins in the borderline PT are almost the same as malignant PT.2,5,9,12-14,27 Previous studies and guidelines suggested that margin-negative excision was not an adequate treatment for borderline PT and often recommended a margin width of at least 10 mm.7,8,24-26 Patients with borderline PTs are usually cured with surgery and should not be offered chemotherapy.14 The use of radiotherapy for borderline PT is controversial, but several studies recommend it when the sufficient margin cannot be achieved.15,16,19,21 Hormone therapy for borderline PT is ineffective.24-26

Benign tumors are characterized by an increased stromal cellularity with mild to moderate cellular atypia, circumscribed tumor margins, low mitotic rates (less than 4 mitoses per 10 HPF), and a lack of stromal overgrowth.23,28 Although surgical management of malignant and borderline PTs are similar, there is a controversy about management of benign PTs. The main discussion is about the surgical margins and the rate of recurrence. In earlier reports, benign, borderline, and malignant PTs had local recurrence rates of 8, 21, and 36 percent.27,28 A review article studied the relationship between surgical margins and the rate of recurrence in all kinds of PT (Table 1).25 For many years, the acceptable resection margin for PT has been 10 mm. The authors of some guidelines such as the National Comprehensive Cancer Network (NCCN) and MD Anderson believe that a margin of ≥ 1 cm should be applied to all types of PT.4,7 The editors of Diseases of the Breast agree with this approach.24,28 but the American Society of Breast Surgeons (ASBS) suggests that benign PT diagnosed after excision can be managed with close follow-up.13 According to Up-To-date, authors accept a narrower but clear margin for benign PT.14 The reason scientists have agreed with the wide excision is that surgical margin is one of the causes of recurrence.30,31 Wider excisions appear to reduce the risk of local recurrence, while close margins cause local recurrence in all types of PTs.30,36 Recent studies have shown that, regardless of surgical margin status, benign PT has a very low rate of local recurrence.1,3,8,37-39 Barth and Kim et al showed that no clinical, histologic, or surgical factor influences local recurrence of benign PT.24,37 Several researchers found that there was no statistical advantage of a 10-mm margin compared with a 1-mm one in benign PT.29,31,35,42-44 Furthermore, when benign PT is unexpectedly diagnosed at ultrasound-guided, vacuum-assisted excision, clinical follow-up may be preferred over further surgery.40,41 There are some data that support the negative margin for benign PT.30,42 Cowan et al found that patients with benign PT and positive margins on initial excision might be
managed conservatively. Recent studies suggest that the close follow-up approach could be sufficient in cases of benign PT diagnosed postoperatively, even if margins were < 1 cm. It seems that benign PTs are seen differently than borderline and malignant PTs. Onkendi and colleagues showed that the extent of surgical resection does not affect disease-free survival in patients with borderline and malignant PT. Sevigny recommended that revision surgery should not be performed for close or positive surgical margins for benign and borderline PT.

### Multidisciplinary team (MDT) recommendation

For this patient with benign PT and 2 close margins about 1 mm, members of breast MDT in Breast Surgery Department, Imam Khomeini Hospital, Tehran University of Medical Sciences, did not recommend re-excision. Factors that helped MDT to accept these margins were the very low rate of local recurrence of benign PT, good prognosis, and the patient’s concern. Thus, MDT members decided to accept the margin and informed the patient about the risk of recurrence and the need for follow-up imaging.

### Conflict of Interest

The authors have nothing to disclose.

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Effect of Acceptance and Commitment Group Therapy on Quality of Life and Resilience of Women With Breast Cancer

Fateme Sadeghi, Fariba Hassani, Suzan Emamipour, Hamidreza Mirzaei

Abstract

Background: Breast cancer diagnosis always causes a great deal of stress and results in significant changes in the patient’s routine life, which—apart from physical injuries—can lead to loss of social functioning and increased risk of mental disorders. Psychological resilience may be a protective factor in dealing with stressful clinical situations. The present study aimed at investigating the effect of acceptance and commitment group therapy on the quality of life and resilience of women with breast cancer.

Methods: This study had a quasi-experimental, pre-post intervention design with a control group. Twenty women with breast cancer (stages I or II) were randomly assigned to an intervention or a control group. The intervention consisted of eight weekly sessions of acceptance and commitment group therapy. Follow-up evaluations were carried out two months after the intervention. The Quality of Life Questionnaire-Core 30 (QLQ-C30), Quality of Life Questionnaire-Breast Cancer (QLQ-BR23), and the Connor-Davidson Resilience Scale (CD-RISC) were used in this study.

Results: Sixteen patients completed the study. Compared with the control group, significant improvements were observed in the total and subscale scores on QLQ-C30 (partial $\eta^2 = 0.40$), QLQ-BR23 (partial $\eta^2 = 0.73$), and CD-RISC scores (partial $\eta^2 = 0.94$) and ($P < 0.01$) in the intervention group.

Conclusion: Acceptance and commitment program appears to be an effective therapeutic intervention for improving quality of life and resilience of breast cancer patients. Therefore, we suggest that this intervention be provided along with medical treatments to improve quality of life and resilience of breast cancer patients.
to have a poor quality of life. Quality of life is an important consideration when providing care to cancer patients. It has been demonstrated that decreased health-related quality of life caused by chemotherapy side-effects may predispose patients to early discontinuation of treatment, while studies on breast cancer survivors have shown that a good quality of life may benefit breast cancer patients.

Quality of life is a broad, multidimensional concept reflecting patients’ perceptions of both positive and negative aspects of their life with regard to physical health, psychological state, level of independence, social relationships, personal beliefs and their relationships to salient features of their environment. The concept has attracted the attention of researchers, and there is a growing consensus that it should be one of the main components of medical practice and research.

In addition, the contribution of resilience to the compliance of patients with breast cancer is highlighted by a number of studies. Resilience is an individual’s ability to adaptively respond to hardship, stress, and adversity and has been defined as the capacity to “bounce back” from negative events without succumbing to despair. A study on survivors of breast, stomach, and lung cancers showed that resilience was associated with better quality of life and lower levels of depression. Another study examining fatigue in cancer patients undergoing radiation therapy showed that resilience was an important psychological predictor of quality of life and coping.

Acceptance and commitment therapy (ACT), which the American Psychological Association acknowledges as a modality of treatment for some psychiatric conditions, emphasizes acceptance and mindfulness and focuses on strategies to induce behavior change guided by the patient’s personal values to improve functioning and coping with persistent symptoms. The treatment approach in ACT is to encourage patients to acknowledge and accept mental experiences to increase their ability to work with problems that cannot be solved. ACT interventions involve addressing six core psychopathological processes including acceptance, cognitive defusion, self as a context, values, being present, and committed action. A recent review has identified studies using ACT in the contexts of lung, breast, blood, and bladder cancers. It is expected that ACT has the potential to become a particularly well-suited approach to treating people with breast cancer as it offers a model of healthy adaptation to difficult circumstances. This is the first study to use ACT to improve quality of life and resilience in people with breast cancer. If ACT proves effective in this trial, it could be recommended to be integrated into treatment plans for breast cancer patients.

However, despite providing valuable preliminary data, these studies either had a small sample or failed to report cancer stages. Therefore, this study was conducted to examine these hypotheses:

A: Acceptance and commitment group therapy affects the quality of life of women with breast cancer.

B: Acceptance and commitment group therapy affects the resilience of women with breast cancer.

Methods

A quasi-experimental study with a pre-post intervention design and a 2-month follow-up was carried out from March 2016 to December 2016. Breast cancer patients from the Oncology Department of the Cancer Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, were recruited for this study. Participation in the study was offered to all breast cancer patients who came for follow-up in the oncology wards in March and April 2016. The planned sample size was N = 20. In general, it is believed that the size of groups is set by the goals and priorities of groups, and it is recommended that the participants in each group be limited to 6 to 8 people because of paying attention to the needs of individual members. Then, 20 patients were randomly assigned to either the intervention or control group. All participants were Iranian women treated for breast cancer, and we used the following inclusion criteria: (1) having histologically confirmed primary breast cancer (stages I or II) (2) being aged 18 and above; (3) being literate and fluent in the Persian language; (4) having no history of mental disorder or psychiatric problem; (5) having no cognitive deficits; (6) being cooperative with treatments; and (7) having completed the initial surgical and oncological treatment at least 2 months before the inclusion. Participants were excluded from the study if (1) they had serious physical problems that would preclude them from following the intervention, (2) they missed more than two sessions of therapy, and (3) they participated in concurrent psychological treatment, studies or rehabilitation (i.e. relaxation, mindfulness, psycho-education, or ACT). Finally, 4 participants were excluded during the examination because they declined to participate or started to use psychiatric drugs.

The study received the approval of the local ethics committee. Each participant gave written informed consent for participation. The privacy of participants was protected, and also the confidentiality of records and personal accounts was maintained. It was also suggested to the control group that, after completing the research, they can attend another acceptance and commitment group therapy held by the researcher.

The Iranian versions of the Quality of Life Questionnaire-Core 30 (QLQ-C30) and Quality of Life Questionnaire-Breast Cancer (QLQ-BR23) were used to measure the quality of life in this study. The QLQ-C30 is the core module that examines...
the total quality of life in the course of cancer disease. The questionnaire contains 30 questions comprising a global health scale, 5 functional scales (physical, emotional, cognitive, social, and role-playing), 3 symptom scales (fatigue, nausea and vomiting, and pain), and a number of individual items (dyspnea, loss of appetite, insomnia, constipation, diarrhea, and financial difficulties). In the functional scales, higher scores represent a better level of functioning, while, in the case of symptom scales/items, higher scores mark a higher level of symptomatology or problems. The QLQ-BR23 is a 23-item breast cancer–specific questionnaire about the common side effects of therapy, body image, sexuality, and the outlook for the future, which cannot be used alone but has to be administered along with QLQ-C30. The QLQ-BR23 is scored in a similar fashion to the QLQ-C30. Both of the questionnaires have been translated into Persian and validated by Montazeri et al. Cronbach’s alpha coefficient for multi-item scales (to test the reliability of QLQ-C30) ranged from 0.48 to 0.95 at baseline and from 0.52 to 0.98 at follow-up administration of the questionnaire. The alpha coefficient of the Persian version of the QLQ-BR23 was 0.65 to 0.95 in the initial interview and 0.72 to 0.92 at the time of follow-up (two months).

The Connor-Davidson Resilience Scale (CD-RISC) is comprised of 25 items rated on a 5-point scale (0 to 4). The scale has a total score between 0 and 100, with higher scores corresponding to greater resilience. Reliability (0.87), validity (0.89), and factor analytic structure of the scale were evaluated, and reference scores for study samples were calculated. In general, the findings indicate that the Iranian version of CD-RISC provides a reliable and valid measure of resilience and can be used in clinical trials. Cronbach’s alpha coefficient is reported 0.89 and the validity of the scale is ranged from 0.41 to 0.64.

The process of the study was divided into four stages: (1) Recruiting subjects, carrying out pretests, and informing the participants of what the course would give them and what was required to attend the course. Also, the participants were offered the opportunity to discuss what the expected from the program. (2) Intervention: the intervention groups received eight 2-hour sessions (one session per week) of ACT, while the control group was placed on a waiting list. The description of therapeutic intervention is mentioned below:

Session 1: Providing an opportunity for participants to become acquainted with the purposes of the intervention; using creative frustration.

Session 2: Teaching the harmful role of suppressing feelings and thoughts; practicing mindfulness skills.

Session 3: Defining the concept of acceptance and identifying values; the practice of scanning the body according to mindfulness.

Session 4: Setting values based on goals; practicing how to solve barriers and problem; setting goals based on values; defusion exercise using the metaphor of bus passengers; classification of thoughts exercise.

Session 5: Practicing to consider the thought just as a thought; practicing defusion from verbal threats; mindfulness exercise. The assignment was designed with the aim of initiating work on the commitment and allowing barriers to be identified.

Session 6: Identifying traces in the value path and acceptance awareness training; identifying the goals along the path with values; examining useful solutions to barriers and learning daringness; examining the barriers to a valuable life.

Session 7: Learning the impact of thoughts; acknowledging that the disease (primary suffering) is unavoidable, but the meaning we give to the disease (secondary suffering) is a choice and also learning that pursuing commitment is not enough; commitment to actions and values even in the face of obstacles and exploring the barriers to satisfaction.

Session 8: Teaching primary suffering and secondary suffering by drawing a circular diagram; group satisfaction survey and teaching what one can achieve in his or her path of values.

(3) Carrying out the posttest. (4) Carrying out the follow-up test after two months and data collection. Since the study design is quasi-experimental with multivariate repeated measures, multivariate analysis of variance (MANOVA) with repeated measurements was used to evaluate the assumptions. After data collection, data normality was tested using the Kolmogorov-Smirnov test, and, based on their normal distribution, parametric tests were used. SPSS software version 16 was used for data analyses. The significance level was set at 0.05.

Results

According to Mauchly’s Test of Sphericity, the assumption of sphericity was violated for scores on QLQ-C30, $\chi^2(2) = 0.41$, $P < 0.05$. Therefore, the Greenhouse-Geisser correction was used in the present study. The scores on QLQ-BR23 met the sphericity assumption, $\chi^2(2) = 2.19$, $P > 0.05$; therefore, the Sphericity test was used in the present study.

### Table 1. Mean quality of life and resilience scores

<table>
<thead>
<tr>
<th>Measure</th>
<th>Groups</th>
<th>ACT</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>QLQ-C30</td>
<td>Pretest</td>
<td>39.28</td>
<td>47.00</td>
</tr>
<tr>
<td></td>
<td>Posttest</td>
<td>63.98</td>
<td>45.08</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>54.71</td>
<td>44.12</td>
</tr>
<tr>
<td>QLQ-BR23</td>
<td>Pretest</td>
<td>39.85</td>
<td>38.60</td>
</tr>
<tr>
<td></td>
<td>Posttest</td>
<td>71.13</td>
<td>38.22</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>61.15</td>
<td>39.76</td>
</tr>
<tr>
<td>Resilience</td>
<td>Pretest</td>
<td>14.25</td>
<td>16.44</td>
</tr>
<tr>
<td></td>
<td>Posttest</td>
<td>72.50</td>
<td>29.25</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>67.12</td>
<td>27.12</td>
</tr>
</tbody>
</table>
Statistical indicators

Table 2. Results of repeated Measures ANOVA to evaluate the effect of acceptance and commitment group therapy on quality of life

<table>
<thead>
<tr>
<th>Measure</th>
<th>Statistical indicators</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>QLQ-C30</td>
<td>Tests</td>
<td>1046.88</td>
<td>1.47</td>
<td>712.24</td>
<td>9.32</td>
<td>0.003</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Groups</td>
<td>1013.38</td>
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<td>1013.38</td>
<td>8.66</td>
<td>0.01</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Group*test</td>
<td>1727.08</td>
<td>1.47</td>
<td>1157.69</td>
<td>15.39</td>
<td>0.01</td>
<td>0.38</td>
</tr>
<tr>
<td>QLQ-BR23</td>
<td>Tests</td>
<td>1920.46</td>
<td>2</td>
<td>960.23</td>
<td>38.65</td>
<td>0.0001</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>Groups</td>
<td>3407.25</td>
<td>1</td>
<td>3407.25</td>
<td>93.13</td>
<td>0.0001</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>Group*test</td>
<td>2006.70</td>
<td>2</td>
<td>1003.35</td>
<td>40.39</td>
<td>0.0001</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Examination of hypotheses

A: Acceptance and commitment group therapy is effective in improving the quality of life of women with breast cancer

Quality of life (QLQ-C30): F = 9.32, partial η2 = 0.40 (P = 0.003). There was no difference in quality of life scores among the 3 time points in the control group (Table 1). The mean quality of life scores for the intervention group were: pretest = 39.28, posttest = 71.13, and 2-month follow-up = 61.15. The results indicate that quality of life score increased significantly during the trial. The difference between posttest and follow-up scores was not significant (P ≤ 0.01) so it could be concluded that intervention was effective, and the result was retained over time.

Quality of life (QLQ-BR23): F = 38.65, partial η2 = 0.73 (P ≤ 0.01). The mean quality of life scores for the intervention group were: pretest = 32.34, posttest = 67.70, and 2-month follow-up = 51.90. The mean scores on QLQ-BR23 functional scales for the intervention group were: pretest = 29.16, posttest = 81.38, and 2-month follow-up = 71.11; the mean scores on symptom scales were pretest = 75.34, posttest = 19.56, and 2-month follow-up = 26.60; and the mean scores on global health scale were pretest = 37.57, posttest = 67.70, and 2-month follow-up = 51.90. The obtained F values (155.71, 25.72, 7.34, 125.39, and 152.51) were significant for all scales (partial η2: 0.34-0.91, P < 0.01). Therefore, there were significant differences in the scores on scales of quality of life among the 3 time points and also between the intervention and control groups (P < 0.01). The mean scores on symptom scales of the QLQ-BR23; consequently, Huynh-Feldt correction was used in their analysis.

The results indicate that quality of life score increased significantly during the trial. The difference between posttest and follow-up scores was not significant (P ≤ 0.01). There was a significant difference between pretest and posttest quality of life scores; however, the difference between posttest and 2-month follow up values was not significant.

Table 3. Results of repeated measures ANOVA to evaluate the effect of acceptance and commitment group therapy on quality of life scales

<table>
<thead>
<tr>
<th>Subscale</th>
<th>Statistical indicators</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional scales (QLQ-C30)</td>
<td>Tests</td>
<td>7879.74</td>
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<td>3939.78</td>
<td>152.51</td>
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</tr>
<tr>
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<td>Groups</td>
<td>6422.07</td>
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<td>6422.07</td>
<td>119.44</td>
<td>0.0001</td>
<td>0.89</td>
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<tr>
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<td>Group*test</td>
<td>4902.00</td>
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<td>2451.00</td>
<td>94.88</td>
<td>0.0001</td>
<td>0.87</td>
</tr>
<tr>
<td>Symptom scales (QLQ-C30)</td>
<td>Tests</td>
<td>8070.22</td>
<td>1.39</td>
<td>5797.22</td>
<td>125.36</td>
<td>0.0001</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Groups</td>
<td>8516.04</td>
<td>1</td>
<td>8516.04</td>
<td>86.36</td>
<td>0.0001</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>Group*test</td>
<td>6728.76</td>
<td>1.39</td>
<td>4833.58</td>
<td>104.52</td>
<td>0.0001</td>
<td>0.88</td>
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<tr>
<td>Global health scales (QLQ-30)</td>
<td>Tests</td>
<td>2637.15</td>
<td>2</td>
<td>1318.57</td>
<td>7.34</td>
<td>0.03</td>
<td>0.34</td>
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<tr>
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<td>Groups</td>
<td>2134.48</td>
<td>1</td>
<td>2134.48</td>
<td>13.90</td>
<td>0.002</td>
<td>0.34</td>
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<tr>
<td></td>
<td>Group*test</td>
<td>1154.70</td>
<td>2</td>
<td>577.35</td>
<td>3.21</td>
<td>0.05</td>
<td>0.20</td>
</tr>
<tr>
<td>Functional scales (QLQ-BR23)</td>
<td>Tests</td>
<td>2645.69</td>
<td>2</td>
<td>1322.84</td>
<td>25.72</td>
<td>0.0001</td>
<td>0.64</td>
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<tr>
<td></td>
<td>Groups</td>
<td>1628.48</td>
<td>1</td>
<td>1628.47</td>
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<td>0.56</td>
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<tr>
<td></td>
<td>Group*test</td>
<td>2593.24</td>
<td>2</td>
<td>1286.62</td>
<td>25.21</td>
<td>0.0001</td>
<td>0.64</td>
</tr>
<tr>
<td>Symptom scales (QLQ-BR23)</td>
<td>Tests</td>
<td>1005.65</td>
<td>1.07</td>
<td>9325.65</td>
<td>155.71</td>
<td>0.0001</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Groups</td>
<td>1133.41</td>
<td>1</td>
<td>1333.41</td>
<td>3.62</td>
<td>0.001</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>Group*test</td>
<td>1715.87</td>
<td>1.07</td>
<td>1592.09</td>
<td>26.58</td>
<td>0.0001</td>
<td>0.65</td>
</tr>
</tbody>
</table>
We concluded that the intervention was effective, and the result was preserved over time. The scores for the control group were not significantly different among the 3 time points (Table 1).

**B: Acceptance and commitment group therapy is effective on the resilience of women with breast cancer**

According to Mauchly’s Test of Sphericity $\chi^2(2) = 16.89$, $P < 0.01$ variances of differences between all the combinations of the conditions related to the resilience of the studied groups were not equal; therefore, the Huynh-Feldt correction was used.

According to the obtained $F = 240.86$ and partial $\eta^2 = 0.94$, $P < 0.01$ (Table 4), there are significant differences among pretest, posttest, and follow-up resilience scores (Table 4). The mean resilience scores for the intervention group were: pretest = 14.25, posttest = 72.50, and 2-month follow up = 67.12. A reduction in posttest and follow-up scores can be seen in the control group (Table 1).

**Discussion**

The current study investigated the effectiveness of acceptance and commitment group therapy in improving the quality of life and resilience of women with breast cancer. The results indicated that ACT intervention was able to significantly enhance the scores on all scales of QLQ-C30, QLQ-BR23, and CD-RISC, and the effect almost remained stable over the follow-up period.

According to Yalom and Vinograd, most of the breast cancer patients are affected by pessimistic thoughts, hopelessness, despair, loneliness, and fear of death because of suppressing their feelings about their illness. Consequently, their participation in group psychotherapy sessions and dealing with their feelings can lead to a different perspective on the human’s life meaning. Also, all aspects of their lives are significantly influenced by the traumatic experience of “losing the breasts,” which are the attributes of femininity. Research has shown that reducing depression, anxiety, and other psychological complications caused by cancer and cognitive and emotional acceptance of the disease by the patient have higher effectiveness than standing against the disease. The findings of this research were in agreement with previous researches which all support the efficiency of acceptance and commitment therapy to improve patients’ quality of life. It could be concluded that ACT improves quality of life by directly focusing on effective life in valuable areas. ACT serves this purpose by exposing the patient to negative thoughts, emotions, and feelings in a controlled manner.

Our results also showed that acceptance and commitment group therapy promotes resilience among breast cancer patients, which is Consistent with previous studies. It could be that ACT helps people to play a more significant role in the challenges they face, regain hope, and step forward on the road to a meaningful and genuine life. Also, it can increase self-efficacy and the sense of empowerment, both of which contribute to increased resilience. A feeling of competence and capability is necessary for resiliency, and it is only in this case that an individual can step out of his passive position and start dealing with the challenges and managing his or her life.

A limitation of the present study was that it had only one intervention group, so the effect of ACT was not compared with other interventions. On the other hand, this is the first study in Iran that investigates the effect of acceptance and commitment group therapy on quality of life and resilience among women with breast cancer. There is a hope that the results of this research will encourage the practitioners to give more importance to psychological aspects of the disease, which can result in better and faster treatment of the disease and improvement of patients’ quality of life and resilience. Future studies should look into the effectiveness of this therapy in other types of cancers, with both genders.

**Conflict of Interest**

The authors have nothing to disclose.

**Acknowledgment**

Authors would like to express their gratitude to the professional staff and all the participants in this study. This study was supported and funded by Cancer Research Center, Shahid Beheshti University of Medical Sciences in Tehran. The study was conducted independently of funding sources.

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Association of FGFR2 and TOX3 Genetic Variants With the Risk of Breast Cancer in Iranian Women

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a Department of Science, Islamshahr Branch, Islamic Azad University, Islamshahr, Tehran, Iran
b National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran

ABSTRACT

Background: Breast cancer is the most common cause of cancer-related death in women worldwide. Novel genetic markers for breast cancer susceptibility have been identified in population-based studies. The aim of this study was to examine the association of two single-nucleotide polymorphisms (SNPs) of FGFR2 (rs1219648) and TOX3 (rs8051542) with the risk of breast cancer in Iranian women.

Methods: Breast cancer patients (n = 126) and healthy controls (n = 160) were genotyped for SNPs in FGFR2 (rs1219648) and TOX3 (rs8051542) using the tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). Also, immunohistochemical tests for human epidermal growth factor receptor-2, estrogen receptor, and progesterone receptor were carried out on breast tumor tissues.

Results: TOX3 (rs8051542) CC (OR = 1.24; 95% CI, 0.72-2.14; P < 0.001) and FGFR2 (rs1219648) GG (OR = 6.20; 95% CI, 23.63-162.66; \( \chi^2 = 132.775 \); P < 0.001) polymorphism was significantly associated with breast cancer. The association was also significant between breast cancer risk and TOX3 (rs8051542) TC and FGFR2 (rs1219648) AG variants.

Conclusion: Our findings suggested that genetic variants of FGFR2 (rs1219648 AG) and TOX3 (rs8051542 TC) can be potential candidate biomarkers for breast cancer risk.
(rs8051542 in TOX3 gene and rs1219648 in FGFR2 gene) and their association with BC risk. Also, the assessment of three markers including human epidermal growth factor receptor 2 (HER2), progesterone receptor (PR), and estrogen receptor (ER) was conducted in this study.

Methods

Study Design and Patients
In this study, 126 women with grade 4 breast tumors and 160 healthy controls were examined. All patients were provided with informed consent. Peripheral blood samples (3 mL) were collected from patients using K3EDTA as an anticoagulant.

DNA Extraction
DNA isolation from blood samples was performed using FlexiGene DNA extraction kit (Qiagen, Germany). The concentration of the isolated DNA was quantified using a NanoDrop spectrophotometer (Thermofisher, USA).

Genotyping
Tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method was used to identify the genotypes of DNA samples. The DNA sequences of the SNPs rs8051542 and rs1219648 were obtained from the NCBI SNP database and used for designing the primers. Sequences of the primers have been shown in Table 1.

Immunohistochemistry Study
Breast carcinoma tissues were obtained from the Pathology Department of Rare Diseases Medical Center and Hazrat-e-Rasoul Medical Complex, Tehran, Iran. Immunohistochemistry study was performed to investigate ER, PR, and HER2 status.

Statistical Analysis
We assessed the Hardy-Weinberg equilibrium using the chi-squared ($\chi^2$) test. The data were tested for association between the gene variations and breast cancer risk. The difference in the allele and genotype frequency between cancer patients and healthy controls was determined using standard $\chi^2$. The odds ratios (OR) along with their 95% confidence intervals (95% CI) were also calculated.

Table 1. Inner and outer primer sequences used in the tetra-primer ARMS-PCR method

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Cases, (N= 126)</th>
<th>Controls, (N= 160)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOX3</td>
<td>CC</td>
<td>57</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>50</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>FGFR2</td>
<td>AA</td>
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<td>9</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>10</td>
<td>146</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>84</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2. Genotype frequencies for cases and controls

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Cases, (N= 126)</th>
<th>Controls, (N= 160)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOX3</td>
<td>CC</td>
<td>57</td>
<td>36</td>
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</tr>
<tr>
<td></td>
<td>GG</td>
<td>84</td>
<td>5</td>
</tr>
</tbody>
</table>

Results

The present study evaluated the association between TOX3 (rs8051542) and FGFR2 (rs1219648) with breast cancer risk. We also examined the relationship between these genotypes and three hormonal markers and their association with breast cancer risk factors (Table 3, Table 4, and Figure 1).

In the current study, TOX3 (rs8051542) CC showed a significant association (OR = 1.24, 95% CI = 0.72-2.14; $\chi^2 = 16.608$; $P < 0.001$) and FGFR2 (rs1219648) GG; (OR = 62.0; 95% CI, 23.63-162.66; $\chi^2 = 132.775$; $P < 0.001$) with breast cancer.

However, there was a strong, inverse association between the low-risk allele and tumor markers (Table 3) and similar to TOX3 (rs8051542) TC (OR = 0.34; 95% CI, 0.21-0.54; $\chi^2 = 9.897$; $P = 0.002$) and FGFR2 (rs1219648) AG; (OR = 0.01, 95% CI, 0-0.02; $\chi^2 = 197.342$; $P < 0.001$)(Table 3,
and Figure 1). This may be attributable to significant polymorphisms of SNPs variants at low risk (Table 4).

\textit{FGFR2} (rs1219648) AG was associated with ER- and PR-positive tumor phenotypes. Also, the association of \textit{TOX3} (rs8051542) TC variant with PR- and ER-positive tumors was stronger than that of CC and TT variants.

**Discussion**

Recently, studies have indicated several SNPs as novel independent loci for diagnosis and prognosis of breast cancer. The aim of the present study was to explore the association of SNPs in \textit{FGFR2} and \textit{TOX3} genes with breast carcinoma subtypes. Previous reports focused on intronic SNPs in \textit{FGFR2} and proposed the gene to be a candidate locus for breast cancer. Also, it was reported in similar studies that rs8051542 was significantly correlated with breast cancer risk. Samson et al examined the \textit{FGFR2} SNPs in southern India and showed that there was no association between \textit{FGFR2} (C906T polymorphism) and breast cancer in this Asian population. Another study genotyped rs2981582, rs1219648, rs2981578, and rs7895676 polymorphisms in \textit{FGFR2} in breast cancer patients from northern India. The results showed that SNPs in intron 2 of \textit{FGFR2} may contribute to genetic susceptibility to breast cancer in northern Indian population. Zhang et al studied three SNPs in

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>OR</th>
<th>95% CI</th>
<th>( \chi^2 )</th>
<th>P value</th>
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<td>\textit{TOX3} (rs8051542)</td>
<td>CC</td>
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<td>0.72-2.14</td>
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<tr>
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<tr>
<td>\textit{FGFR2} (rs1219648)</td>
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<td>0.88-5.02</td>
<td>2.869</td>
<td>0.090</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>0.01</td>
<td>0-0.2</td>
<td>197.342</td>
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<tr>
<td></td>
<td>GG</td>
<td>6.2</td>
<td>2.33-16.26</td>
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**Table 4. Immunohistochemical analysis of HER2, ER, and PR status**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>ER+</th>
<th>ER−</th>
<th>PR+</th>
<th>PR−</th>
<th>HER2+</th>
<th>HER2−</th>
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<tr>
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<td>15</td>
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<td>15</td>
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<td>7</td>
<td>10</td>
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<tr>
<td></td>
<td>TC</td>
<td>22</td>
<td>2</td>
<td>22</td>
<td>2</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>11</td>
<td>2</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.64</td>
<td>0.11</td>
<td>0.11</td>
<td>0.81</td>
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<tr>
<td>\textit{FGFR2} (rs1219648)</td>
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<td>0</td>
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<td>0</td>
<td>3</td>
<td>4</td>
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<tr>
<td></td>
<td>AG</td>
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<td>9</td>
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<td>15</td>
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</tr>
<tr>
<td></td>
<td>GG</td>
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<td>0</td>
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<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.003</td>
<td>0.003</td>
<td>0.2</td>
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**Figure 1.** Frequencies of genotypes in cases (n = 126) and controls (n = 160)
The authors have nothing to disclose.

References
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ABSTRACT

Background: The present study aimed to investigate breast cancer trend, incidence, and mortality among Iranian women and was one of the first provincial and population-based studies to investigate breast cancer changes during 14 years in the largest province of Iran, Kerman.

Methods: This was a population-based longitudinal study. Information about women diagnosed with breast cancer from 2001 to 2014 was obtained from the Cancer Registry of Kerman University of Medical Sciences. Independent-samples t test, one-way analysis of variance, linear regression, time series graphs, and fitted line plots were performed using SPSS 22 and Minitab 17.

Results: A total of 2771 women were diagnosed with breast cancer in Kerman province from 2001 to 2014. The mean age of female patients was 49.52±12.88 years. The total incidence rate was 13.5 per 100,000 women and there was an increasing trend for incidence and age at diagnosis. Also, 254 women died from breast cancer during these 14 years and the mean mortality age was 54.16±14.33 years. There was also an increasing trend for mortality and age of death.

Conclusion: There is an increasing trend for incidence and mortality from breast cancer in Kerman province and this requires interventions such as appropriate screening programs. Also, enabling physicians and increasing patient awareness to identify breast cancer symptoms is necessary.

INTRODUCTION

Cancer, with 8.8 million deaths, was the second leading cause of mortality in 2015, as 1 out of 6 deaths in the world was caused by cancer.1 Approximately, 70% of cancer deaths occur in low- and middle-income countries,1 and cancer incidence and mortality is increasing in developing countries.2 Breast cancer is the second most common cancer worldwide,3 the most common cancer in women,4,5 and the leading cause of death from cancer in women.6 In 2015, 2.4 million new cases of breast cancer were diagnosed and 523 000 deaths from breast cancer were reported in the world.6 Although more than half of the global burden of breast cancer is currently from developed countries, its incidence is rapidly increasing in developing countries.7 Also, more than half of the deaths and about half of the new breast cancer cases occur in developing countries based on 2008 GLOBOCAN estimates.8
Breast cancer is also the most common cancer in Iranian women. It was the leading type of cancer in Iranian women, accounting for 24.6% of all cancers. The mean age of breast cancer diagnosis for Iranian women is 10 to 15 years lower than that for developed and Western countries. The mean age of diagnosis for American female breast cancer patients from 2008 to 2012 was 61 years. However, in Iran, most women diagnosed with breast cancer are younger than 50 years and most are diagnosed with invasive cancer at advanced stages. Studies done in Iranian women showed that low levels of knowledge and insufficient awareness about breast cancer symptoms were among the most important factors causing delays in breast cancer diagnosis. Further studies in Iran showed that empowering women through education can increase early detection of breast cancer.

In developed and Western countries, such as the US, most deaths from breast cancer occur among women older than 50 years. In Iran, not only are deaths from breast cancer on the rise, but a large number of deaths happen among women younger than 50 years.

The present study aimed to investigate breast cancer trend, incidence, and mortality among Iranian women and was one of the first provincial and population-based studies to investigate the trends of breast cancer during 14 years in the largest province of Iran, Kerman.

Methods
This was a longitudinal and population-based study. Information about women diagnosed with breast cancer was inquired from the Cancer Registry of Kerman Province from 2001 to 2014. This study was approved by the Deputy of Research (No: 93/460) and Ethics Committee of Kerman University of Medical Sciences. Other unknown information was collected by contacting the patient or her family.

In order to estimate the annual incidence rates, the number of new breast cancer cases for each year was divided by the total number of females in the same year. National census data for 1996, 2006, and 2011 and population growth rates were used to calculate the female population of the province in each year.

Descriptive statistics, independent-samples t-test, one-way analysis of variance, linear regression, time series graphs, and fitted line plots were performed with Excel 2016, SPSS 22, and Minitab 17.

Results
Totally, 2771 women were diagnosed with breast cancer in Kerman province from 2001 to 2014. The mean and median ages at diagnosis were 49.52 ± 12.88 and 48 years, respectively. Also, 254 patients died of breast cancer during these 14 years, and the mean and median ages at death were 54.16 ± 14.33 years and 54 years, respectively.

Patients from rural areas (P < 0.001) and those with grade 3 at the time of diagnosis (P = 0.035), had a significantly lower age at diagnosis. Although the mean age of patients with stage III at diagnosis was also lower than the other stages, the difference was not statistically significant (Table 1).

The trend in age at diagnosis for female breast cancer patients in Kerman province during the 14 years is shown in Figure 1. As shown in the figure, the mean age at diagnosis did not show a significant trend (P = 0.16).

Table 1. Female breast cancer patient's characteristics in Kerman province, Iran

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (%)</th>
<th>Age at diagnosis, Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>City</td>
<td>1677 (58.8)</td>
<td>49.92 ± 12.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rural</td>
<td>439 (15.4)</td>
<td>46.83 ± 12.37</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>735 (25.8)</td>
<td>50.25 ± 12.98</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2851 (100)</td>
<td>49.52 ± 12.87</td>
<td></td>
</tr>
<tr>
<td>Stage at diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14 (0.5)</td>
<td>50.21 ± 16.52</td>
<td>0.132</td>
</tr>
<tr>
<td>I</td>
<td>135 (4.7)</td>
<td>48.73 ± 10.78</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>567 (19.9)</td>
<td>48.64 ± 11.30</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>395 (13.9)</td>
<td>47.94 ± 11.86</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>89 (3.1)</td>
<td>51.52 ± 13.42</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>1651 (57.9)</td>
<td>50.17 ± 13.67</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2851 (100)</td>
<td>49.52 ± 12897</td>
<td></td>
</tr>
<tr>
<td>Grade at diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>290 (10.2)</td>
<td>50.05 ± 12.89</td>
<td>0.035</td>
</tr>
<tr>
<td>2</td>
<td>893 (31.3)</td>
<td>50.01 ± 12.759</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>402 (14.1)</td>
<td>48.09 ± 12.72</td>
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<tr>
<td>Unknown</td>
<td>1266 (44.4)</td>
<td>49.51 ± 12.98</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2851 (100)</td>
<td>49.52 ± 12.87</td>
<td></td>
</tr>
</tbody>
</table>
The trend in breast cancer incidence in Kerman province from 2001 to 2014 is shown in Figure 2a. As shown in the figure, breast cancer incidence has been increasing in Kerman province ($P < 0.001$).

The forecast of breast cancer incidence in Kerman province up to 2020 is shown in Figure 2b. The best fit for the data was a third-degree model. If the incidence of breast cancer increases as predicted in Figure 2b, about 27 cases of breast cancer will happen per 100000 females in Kerman province by 2020.
Figure 3 shows the mean age of death due to female breast cancer during the 7 years in urban and rural areas of Kerman province. The mean age of death among rural women was nonsignificantly lower than that of women living in cities (P = 0.074).

A large percentage of data was missing from 2001 to 2007 (91.9%), and because of this, data for these years were not included in Figures 4 and 5.

The trend in breast cancer mortality in Kerman province from 2008 to 2014 is shown in Figure 4. Breast cancer mortality did not show a significant trend during these 7 years (P = 0.063).

The trend in mortality age for female breast cancer patients in Kerman province from 2008 to 2014 is shown in Figure 5. The mean age of death due to breast cancer had an increasing trend from 2008 to 2014 (P = 0.046).
Discussion

The mean age at diagnosis for breast cancer was about 50 years among women in Kerman province. This age is lower in comparison with a developed country such as England, where the highest incidence of breast cancer happens after the age of 55.\(^2^\)

In the present study, the age-specific incidence rate was highest among women aged 40 to 55 years (46.22 per 100 000 females), and the median age of diagnosis was 48 years. However, according to the US National Cancer Institute, most women diagnosed with breast cancer in America were 55 to 64 years old and the median age of diagnosis of breast cancer was 62 years.\(^2^\) Also, Mousavi et al., in a population-based study of the data from Tehran Cancer Registry in Iran from 1998 to 2001, showed that the mean age at diagnosis was 51 years and that although 36% of patients were aged under 40 years, the highest incidence rate was reported among women aged more than 60 years.\(^2^\)

In the present study, the incidence rate for 2012 (17 per 100 000 females) was lower than the average standardized rate for the world (43 per 100 000 females) and Asian countries including Japan, South Korea (both 52 per 100 000 females) and Singapore (65 per 100 000 females).\(^2^\)

The results of the present study showed that the age at diagnosis was significantly lower among women who lived in rural areas. In contrast, Mitchel et al, who studied breast cancer during 1999 in Australia, showed that there was no significant relationship between age at diagnosis and residential areas.\(^2^\)

The incidence rates of breast cancer in the present study was 16.7 to 19.2 per 100 000 females from 2010 to 2014, and these results were lower than a developed country such as the USA, where the average incidence was 124.9 per 100 000 females from 2010 to 2014.\(^2^\)

It seems that, compared with women in developed countries, Iranian women develop breast cancer at younger ages and visit doctors at more advanced stages of the disease. This needs more attention and appropriate screening programs.\(^4^\)

Lifestyle and behavioral and nutritional habits are risk factors for many noncommunicable diseases and cancers, including breast cancer.\(^2^\) In the last two decades, breast cancer has rapidly increased in the less developed regions of the world.\(^5^\) Likewise, in the present study, breast cancer incidence showed an increasing trend, and this needs proper interventions including screening and periodical breast examinations.

A total of 1 426 (96.8%) BC cases undergoing mastectomy were pathologically staged. Of this number, 39.1% were stage III. The majority of cases were in stages III and IV combined (62.8%) (Figure 3). Only of 47 (3.2%) cases were residual cancers.

A total of 202 (13.7%) of the BC cases undergoing mastectomy were incompletely excised.

In the present study, although breast cancer mortality increased from 1 to 4.1 per 100 000 females from 2008 to 2014, that rate was still less than developed countries such as England and Wales.\(^2^\) The mortality rate in England showed a decreasing trend from 2002 to 2006, but the adjusted mortality rate for different age groups was 30 per 100 000 females, which is much more than Iran.\(^2^\) Also, according to the US National Cancer Institute, the mean breast cancer mortality rate in the US was 21.2 per 100 000 females from 2010 to 2014,\(^2^\) while we found a breast cancer mortality rate of 1.9 to 4.1 per 100 000 females over the same period in Kerman province—although underestimations may have occurred due to low-quality data collection in Iran.

The results of the present study showed that although the mean age of death among women who lived in the rural areas was lower, this difference was not significant. In Europe in 2014, the mortality rate decreased by 9% compared with 2009,\(^2^\) while, Taghavi et al. reported that breast cancer mortality had increased from 1995 to 2004 in Iran, with the increase being greater for women younger than 50 years.\(^2^\) According to Rastad et al., lack of breast cancer awareness, not considering themselves at risk, fear of being diagnosed with cancer, and incorrect diagnosis and relief by physicians were among the most important factors for the delay in seeking treatment for breast cancer in Iran.\(^2^\)

Considering the growing trend in breast cancer mortality and lower age of breast cancer death in Kerman province, as well as the results of previous studies,\(^9^\) more appropriate measures should be taken to improve early diagnosis by enabling physicians and increasing patient awareness about breast cancer symptoms.

A limitation of this study was its missing data. About half of the cases had missing data on stage and grade.

In conclusion, breast cancer incidence is growing in Kerman province, and this needs appropriate interventions including screening, early detection, enabling physicians, and empowering patients to identify breast cancer symptoms. Also, providing greater access to facilities for early diagnosis and treatment of breast cancer patients in rural areas should also be considered.

Funding

This project was supported by Kerman University of Medical Sciences.

Conflict of interest

The authors have no conflict of interest.

Acknowledgment

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assistance with data collection.

References
26. Armstrong T, Bonita R. Capacity building for an integrated noncommunicable disease risk factor


ARTICLE INFO

Methods:
This was a retrospective review from January 2002 to December 2014.

Conclusion:
The current study found that 87.7% of the women with breast had moderate to poor prognosis at the time of diagnosis. Patients are found to present late when the disease is advanced.

Background:
Making prognosis and identifying the patients at higher risk of mortality are important issues in breast cancer (BC) treatment. The aim of this study was to stratify BC case receiving mastectomy into prognostic risk categories using the Nottingham Prognostic Index (NPI).

Results:
Approximately 35% of all BCs diagnosed in our institution had undergone mastectomy. The mean age was 51.9 years. The mean size of the primary breast tumor was 5.8 cm and showed significant association with the histologic grade (P = 0.001), nodal involvement (P < 0.001), positive tumor margins (P = 0.027), and the cancer stage (P < 0.001). Based on the NPI sores, 1.5% of the cases would have an excellent prognosis, 10.8% a good prognosis, 55.8% a moderate prognosis, and 31.9% a poor prognosis.

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Key words:
Nottingham Prognostic Index, stratification, Ghanian women, breast cancer, mastectomy

ABSTRACT

Background: Making prognosis and identifying the patients at higher risk of mortality are important issues in breast cancer (BC) treatment. The aim of this study was to stratify BC case receiving mastectomy into prognostic risk categories using the Nottingham Prognostic Index (NPI).

Methods: This was a retrospective review from January 2002 to December 2014.

Results: Approximately 35% of all BCs diagnosed in our institution had undergone mastectomy. The mean age was 51.9 years. The mean size of the primary breast tumor was 5.8 cm and showed significant association with the histologic grade (P = 0.001), nodal involvement (P < 0.001), positive tumor margins (P = 0.027), and the cancer stage (P < 0.001). Based on the NPI sores, 1.5% of the cases would have an excellent prognosis, 10.8% a good prognosis, 55.8% a moderate prognosis, and 31.9% a poor prognosis.

Conclusion: The current study found that 87.7% of the women with breast had moderate to poor prognosis at the time of diagnosis. Patients are found to present late when the disease is advanced.

Introduction
Breast cancer (BC) is the most frequently diagnosed cancer in women globally and in Ghana. Previous institution-based publications on BC in Ghana and other African countries have found the disease to be common among younger women, with advanced stage at presentation. Unfortunately, none of the previous BC publications from Ghana included data on prognostic grouping of Ghanian women diagnosed with the disease. A number of putative prognostic factors for human BC have been reported by the American Joint Committee on Cancer (AJCC). Recognizing the prognostic factors enables greater accuracy in predicting the outcome of BC treatment. The universally acknowledged prognostic factors for BCs are the age at diagnosis, tumor size, histologic type, tumor grade, lymph node status, metastases to other organs, estrogen and progesterone receptor expression, HER2 expression, and the proliferation index. Combining these factors is of greater clinical value than considering each in isolation, and this is the basis for a number of schemes that are used to group patients into various risk categories. One of such schemes is the Nottingham Prognostic Index (NPI), which we used to group Ghanian women with BC into prognostic categories. The aim of this study was to stratify BCs undergoing modified radical mastectomy into prognostic risk categories using the NPI.

Methods

Study Design
This was a retrospective study covering the period from 2002 to 2014. Data were collected from the Department of Pathology, School of Biomedical Sciences, Korle-Bu Teaching Hospital, Accra, Ghana.
Sciences, College of Health Sciences of the University of Ghana located at Korle-Bu. This institution reports between 5,000 and 8,000 histology cases yearly.

**Inclusion Criteria**
1) All female BCs.
2) Well-fixed mastectomy specimens with axillary clearance.

**Exclusion Criteria**
1) All male breast cancers.
2) Poorly fixed female mastectomy specimens.
3) Receiving neoadjuvant chemotherapy prior to mastectomy without residual malignant cells.

**Ethical consideration/informed consent**
Permission to publish the data in this manuscript was granted by the head of the Department of Pathology.

**Data Collection**
Histopathology reports and slides of confirmed BC cases included in the study were reviewed. Of the total number, BC cases who had undergone mastectomy were selected and the clinicopathological characteristics of BCs were collected.

A positive tumor margin in this study was defined as the presence of malignant cells within 2 mm of the resection margins.

The study did not include the following prognostic factors: estrogen receptor, progesterone receptor, and HER2 expression, which are not routinely performed but on request, and proliferation indices, which are not performed at all in our institution.

BCs were classified according to the World Health Organization histology classification of breast tumors (WHO 2003). Histologic grading of breast tumors was performed according to the modification of the Bloom-Richardson system by Elston and Ellis.

The cancer stage was determined using the TNM system (pathological) developed by AJCC, which uses the size of the primary tumor (T), nodal involvement (N), and presence of metastasis (M).

**Calculation of the Nottingham Prognostic Index (NPI)**
The NPI is one of the most widely used prognostic indices for patients with invasive BC. The NPI was developed in 1982 to help in the management of primary operable BC. Multivariate analysis identified 3 factors to be significant: tumor grade, the number of lymph nodes involved, and the size of the tumor. These are viewed as the strongest independent predictors of outcome and make up the formula to calculate the prognostic score. Cutoff points were applied to divide patients into prognostic categories of excellent, good, moderate, and poor, and these correlate strongly with the survival rate. Since the development of the NPI, attempts have been made by other researchers to improve its prognostic power, with limited success. Some people have suggested modifying the variables used to determine the index or combining additional markers with it, and others have used different statistical approaches. However, to date, the original NPI remains the gold standard for stratifying BC patients into prognostic categories.

BCs that met the following criteria were included in the calculation of the NPI:
1. Must be graded using the modified Bloom-Richardson grading system.
2. Must have stated gross primary tumor size (cm).
3. Must have lymph nodes retrieved from the axillary content.

The NPI was calculated as follows:

- Lymph node involvement (L) was scored as 1-3 (No nodes: 1, 1-3 nodes: 2, and > 3 nodes: 3).
- The NPI was calculated using the formula: $\text{NPI} = G + L + (S \times 0.2)$.

BC cases were put into prognostic categories and 5-year survival rates based on their NPI scores.

**Data Analyses**
Data were entered into the SPSS software version 18 (IBM, Chicago, USA). Frequency distributions and descriptive statistics were calculated. The associations between tumor variables were determined using Spearman’s correlation coefficient. Finally, BCs were categorized into prognostic groupings using the NPI. P values of < 0.05 were considered significant.

**Results**

**Clinicopathological Characteristics of Bcs**
During the period under review 4,175 BCs were diagnosed in our institution, of which 1,473 (35.3%) received a mastectomy. There was a gradual rise in the proportion of mastectomy-receiving BC cases over the period (Figure 1).

The age of patients ranged from 14 to 94 years (M = 51.9 y, SD = 12.5), with the modal age group of 40 to 49 years (27.9%) (Figure 2).

The most common primary presentation of BC was a painless palpable lump (N = 1467, 99.6%). A total of 669 (47.6%) women with palpable lumps had skin involvements. The duration of symptoms at presentation was available for 911 (61.8%) patients, of which 79.6% had reported after 3 months of illness (Table 1).

The size of primary tumors ranged from 0.5 to 24 cm, with a mean of 5.8 cm (SD = 3.9). Half of the tumors were larger than 5.0 cm (T3) in diameter. This was followed by 595 (40.4%) tumors that were larger than 2.0 cm, but less than or equal to 5.0 cm (T2), and 141 (9.6%) tumors that were 2.0 cm or less (T1).

The major categories of BCs in this study were epithelial (97.2%), mesenchymal (1.0%), and fibroepithelial (0.95%). The great majority of the BC...
Table 1. Symptoms and duration of BC at presentation

<table>
<thead>
<tr>
<th>Variable</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary complaint</td>
<td></td>
</tr>
<tr>
<td>Nipple discharge</td>
<td>6 (0.4%)</td>
</tr>
<tr>
<td>Palpable breast lump</td>
<td>1467 (99.6%)</td>
</tr>
<tr>
<td>Laterality</td>
<td></td>
</tr>
<tr>
<td>Right breast</td>
<td>746 (50.9%)</td>
</tr>
<tr>
<td>Left breast</td>
<td>713 (48.6%)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>8 (0.5%)</td>
</tr>
<tr>
<td>Additional symptoms</td>
<td></td>
</tr>
<tr>
<td>Skin involvement</td>
<td>699 (45.4%)</td>
</tr>
<tr>
<td>Other</td>
<td>25 (1.7%)</td>
</tr>
<tr>
<td>Duration of symptoms at presentation (months)</td>
<td></td>
</tr>
<tr>
<td>1-3</td>
<td>186 (20.4%)</td>
</tr>
<tr>
<td>4-6</td>
<td>172 (18.9%)</td>
</tr>
<tr>
<td>7-9</td>
<td>108 (11.9%)</td>
</tr>
<tr>
<td>10-12</td>
<td>224 (24.6%)</td>
</tr>
<tr>
<td>13-24</td>
<td>134 (14.7%)</td>
</tr>
<tr>
<td>&gt; 24</td>
<td>87 (9.5%)</td>
</tr>
</tbody>
</table>

Figure 1. Trend in BC cases receiving mastectomy at Korle-Bu teaching hospital

Figure 2. Age distribution of BCs during the period of review
Table 2. Histological types of BC

<table>
<thead>
<tr>
<th>Histological types of breast cancers</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epithelial</strong></td>
<td></td>
</tr>
<tr>
<td>Invasive ductal carcinoma, not otherwise specified (IDC-NOS)</td>
<td>1432 (97.21%)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>37 (2.51%)</td>
</tr>
<tr>
<td>Lobular</td>
<td>31 (2.10%)</td>
</tr>
<tr>
<td>Ductal carcinoma in situ (DCIS)</td>
<td>17 (1.15%)</td>
</tr>
<tr>
<td>Papillary</td>
<td>17 (1.15%)</td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>5 (0.33%)</td>
</tr>
<tr>
<td>Medullary</td>
<td>9 (0.7%)</td>
</tr>
<tr>
<td>Apocrine</td>
<td>5 (0.33%)</td>
</tr>
<tr>
<td>Cribriform</td>
<td>4 (0.27%)</td>
</tr>
<tr>
<td>Tubular</td>
<td>3 (0.20%)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>4 (0.27%)</td>
</tr>
<tr>
<td>secretory</td>
<td>1 (0.06%)</td>
</tr>
<tr>
<td>Inflammatory</td>
<td>1 (0.06%)</td>
</tr>
<tr>
<td>Sweat gland</td>
<td>1 (0.06%)</td>
</tr>
<tr>
<td><strong>Mesenchymal</strong></td>
<td></td>
</tr>
<tr>
<td>Metaplastic</td>
<td>15 (1.01%)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>10 (0.67%)</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>3 (0.20%)</td>
</tr>
<tr>
<td>malignant peripheral nerve sheath tumor (MPNST)</td>
<td>1 (0.06%)</td>
</tr>
<tr>
<td><strong>Fibroepithelial</strong></td>
<td></td>
</tr>
<tr>
<td>Malignant phyllodes tumor</td>
<td>14 (0.95%)</td>
</tr>
<tr>
<td><strong>Tumors of the nipple</strong></td>
<td></td>
</tr>
<tr>
<td>Paget’s disease</td>
<td>11 (0.74%)</td>
</tr>
<tr>
<td><strong>Lymphoma</strong></td>
<td>1 (0.06%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1473</td>
</tr>
</tbody>
</table>

Subtypes were invasive ductal carcinoma, not otherwise specified (IDC-NOS) (88.0%) (Table 2). A total of 1,458 (99%) cases undergoing mastectomy had Bloom-Richardson grading. Of this number, 42% were grade 2, 38.1% grade 3, and 19.9% grade 1. The number of cases with nodal involvement was 976 (66.3%), with the mean number of nodes involved being 3.4 (SD = 4.2, range: 1-42). Many (44%) of these cases had between 1 and 3 positive lymph nodes (Figure 3). In all, 56% had 4 or more positive lymph nodes. A total of 1,426 (96.8%) BC cases undergoing mastectomy were pathologically staged. Of this number, 39.1% were stage III. The majority of cases were in stages III and IV combined (62.8%) (Figure 3). Only of 47 (3.2%) cases were residual cancers. A total of 202 (13.7%) of the BC cases undergoing mastectomy were incompletely excised.

![Figure 3. TNM staging of BC cases](image-url)
Table 3. Association between BC prognosis indicators (By Spearman's Correlation)

<table>
<thead>
<tr>
<th>Tumor Size</th>
<th>Histologic type</th>
<th>Tumor grade</th>
<th>Positive LN</th>
<th>Tumor Stage</th>
<th>Positive margins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.003</td>
<td>0.001</td>
<td>0.000</td>
<td>0.000</td>
<td>0.027</td>
</tr>
<tr>
<td>0.003</td>
<td>-</td>
<td>0.441</td>
<td>0.300</td>
<td>0.503</td>
<td>0.350</td>
</tr>
<tr>
<td>0.001</td>
<td>0.441</td>
<td>-</td>
<td>0.002</td>
<td>0.000</td>
<td>0.125</td>
</tr>
<tr>
<td>0.000</td>
<td>0.300</td>
<td>0.002</td>
<td>-</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>0.000</td>
<td>0.503</td>
<td>0.000</td>
<td>0.003</td>
<td>-</td>
<td>0.001</td>
</tr>
<tr>
<td>0.027</td>
<td>0.350</td>
<td>0.125</td>
<td>0.003</td>
<td>0.001</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4. Stratification of breast cancer cases (N = 1,451) into prognostic categories using the NPI

<table>
<thead>
<tr>
<th>NPI</th>
<th>N (%)</th>
<th>Prognosis</th>
<th>Expected 5-year survival rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0-2.4</td>
<td>22 (1.5%)</td>
<td>Excellent</td>
<td>93%</td>
</tr>
<tr>
<td>2.4-3.4</td>
<td>157 (10.8%)</td>
<td>Good</td>
<td>85%</td>
</tr>
<tr>
<td>3.4-5.4</td>
<td>810 (55.8%)</td>
<td>Moderate</td>
<td>70%</td>
</tr>
<tr>
<td>&gt;5.4</td>
<td>462 (31.9%)</td>
<td>Poor</td>
<td>50%</td>
</tr>
</tbody>
</table>

**Associations Between Tumor Variables**

The size of the primary tumor was significantly correlated with histologic type (P = 0.002), tumor grade (P = 0.001), positive lymph nodes (P < 0.001), positive tumor margins (P = 0.027) and the tumor stage (P < 0.001). There were also significant positive associations between other tumor variables (Table 3).

**Nottingham Prognostic Index Calculation**

A total of 1,451 (98.5%) of the cases receiving mastectomy met the criteria for the calculation of the NPI. More than half (55.8%) had NPIs within between 3.5 and 5.4, which translates into a moderate prognosis and an expected 5-year survival rate of 70%. Only 1.5% had an excellent prognosis and an expected 5-year survival rate of 93% (Table 4). The remaining 87.7% had moderate to poor prognosis.

**Discussion**

Breast cancer is the most common malignancy among women in developed and developing countries, such as Ghana. In the current study, 35.3% of all histologically confirmed BCs had received mastectomy, with 51% of the women being younger than 50 years. This value seems to suggest that BC is still common among young Ghanaians, as is the case with other African countries. Patient’s age at histological diagnosis is an independent prognostic factor: the younger the age the poorer the outcome after treatment. This have been the fate of the women in the current study.

Women commonly presented with palpable breast lumps, with 47.9% having skin involvements. This seems to suggest that women with BC in current study only present to a health facility when the disease is obvious. This may be due to the fact that there is no population-based BC screening program in the country, so lesions are not detected until they become palpable. BC women in our study are not used to self-examination of their breasts, therefore they detect their BC only when the tumor is obviously large to be felt accidentally. The current findings are in accord with some institution-based studies in Africa, which found that the disease was common in young women, and that women with BC presented late, with large advanced tumors with skin involvement.

The great majority (79.6%) of women with BC presented late (after 12 weeks) to health facilities, with advanced diseases. Late presentation of BC to health facilities in Ghana has been attributed to a prior visit to prayer camps, stigmatization of women with the disease, the wait-and-see attitude of patients, ignorance about the disease, financial hardship, and the hostile attitude of hospital workers and the hospital environment. Studies in Nigerian, Tunisian, and South African women similarly found the presentation of the disease to be late. Literature from other parts of the world for the past 30 years indicates that survival is worse for women with longer duration of symptoms. Furthermore, the current findings are similar to the studies in the UK in the early 80s and late 90s (when routine BC screening was not commonplace), which found that most women with BC presented late with symptoms of the advanced disease and that there was a need for early detection and treatment. It is expected that early diagnosis and treatment would lead to a reduction in disease severity or mortality. For instance, Burgees et al., who defined patient delay as 12 weeks of symptoms before the first visit to the general practitioner (GP), found that 90% of their patients delayed ≥ 12 weeks in seeing the GP, and that patient delay was associated with clinical tumor sizes greater than 4 cm (P = 0.0002) and a higher incidence of locally advanced and metastatic disease (P = 0.001). There is evidence, however, that the duration of symptoms may not be associated with survival. Dennis et al and Fisher et al found that about 20% of the patients visited the GP at about 4 to 8 weeks after symptoms onset and concluded that survival after the symptoms have appeared is not related to the duration of the symptomatic period but...
to established pathological criteria such as rate of growth, the tumor size, lymph node involvement, the number and location of lymph nodes involved, blood vessel invasion, and the presence of systemic metastases. These apparently conflicting results may be explained by differences in the cutoffs used to define delay.

The mean primary tumors size in our study was large (5.8 cm) and showed significant positive associations with the histological grade (P = 0.001), nodal involvement (P < 0.001), and the tumor stage (P < 0.001). The size of primary breast tumor is an independent prognostic factor and, together with nodal involvement, is used for staging BC. Studies have shown that women with large primary are more likely to have positive lymph nodes and hence poor prognosis. In the present study, 50% of the women had tumor sizes of greater than 5 cm, indicating that the majority of the women had a poor outcome and low survival rate. This, however, differs from Wo et al who showed that very small tumors can equally have extensive lymph node involvement and hence poor survival rate.

Invasive ductal carcinoma, not otherwise specified (IDC-NOS) was the most common histological subtype of BC in this study similar to other studies. The grade of a breast tumor is directly related to prognosis, and the 5-year survival. The higher the tumor grade, the lower the 5-year survival. The great majority (80.1%) of the cases in this study were of high histological grade, and this may translate into a poor prognosis and a low 5-year survival rate. This finding is similar to the 76% prevalence of grade 3 tumors in Ghanaian women in the study of Stark et al, who compared Ghanaian women with African American and white women diagnosed with BC.

The number of involved lymph nodes is a very important prognostic factor for invasive cancers. It is associated with a lower survival rate, high recurrence rate, decreased time to recurrence, and treatment failure. Disease-free survival (DFS) and overall survival diminish with each additional positive axillary node. It has been shown that about 70% of patients with nodal involvement will develop recurrence after mastectomy, and patients with 4 or more lymph nodes involved have a worse prognosis compared with those having fewer nodes involved. Survival rate decreases with increased nodal involvement. With no nodal involvement, the 10-year DFS rate is between 70% and 80%. This rate declines to 35% to 40% when there are 1 to 3 positive nodes, and to 10% to 15% when ≥ 10 nodes are involved. The current study found that 44% of the cases had 1 to 3 positive lymph nodes, while about 56% had 4 or more positive lymph nodes. This is consistent with Jatoi et al, who found the hazard ratios for patients with 1 to 3 and ≥ 4 positive nodes to be 1.2 (95% CI, 0.8 to 1.9) and 2.5 (95% CI, 1.8 to 3.4), respectively. They concluded that patients with ≥ 4 involved nodes at diagnosis had a significantly poorer outcome after relapse compared with node-negative cases, regardless of the duration of the disease-free period. Our results suggest that nodal metastasis is a marker of an aggressive phenotype as well as being a diagnostic marker at a later point in the natural history of BC. Thus, the majority of women in this study are expected to be at increased risk for recurrence of the disease after mastectomy, treatment failure, and survival rates less than 40%.

The cancer stage at histological diagnosis showed strong positive associations with the histological grade (P < 0.001), and positive tumor margins (P = 0.001). The stage of BC determines the treatment option and the prognosis of tumors after the treatment. The higher the stage at diagnosis, the shorter the 5-year survival rate. The majority of the BC patients in this study had stage III or IV cancers at the time of histological diagnosis and potentially a poor outcome of the disease, even with treatment, and a low 5-year survival rate. Furthermore, the large percentage of advanced-stage BCs in this study differs from the results of the study of Walters et al in Canada, where they found that 82.9% of the BC patients were in stage I or II, while only 17.1% had stage III or IV cancer.

In this study, positive tumor margin frequency of 13.7% was found in mastectomy specimens. This value is close to the 12% for total mastectomies in Sheikh et al, but lower than the 20% in the study of Walters et al. These women are therefore potentially at risk of developing local recurrence and systemic disease.

The NPI is one of the most widely used prognostic indices for the management of patients with primary operable invasive BC. It is the gold standard for stratifying BC patients into prognostic categories as excellent, good, moderate, and poor, which correlate strongly with the survival rate. Data on the survival of BC patients after definitive diagnosis and treatment is nonexistent in Ghana. Available data from some African countries with limited resources for BC screening, diagnosis, and treatment showed varied results compared with the United States of America and Canada, where effective screening and treatment and cancer registries are present. In the current study, based on the NPI scores, 31.9% of the women would have a poor prognosis, with an expected 5-year survival rate of 50%; 55.8% would have a moderate prognosis, with an expected 5-year survival of 70%; and only 13.3% would have an excellent prognosis, with an expected 5-year survival of 93%. The proportion of women with poor prognosis (31.9%) in this study is higher than the values observed in the Gambia (12.5%) and Nigeria (24.5% and 25.6%), but performs less than some African countries such as Uganda (56.4%) and South Africa (64%,
black women). Furthermore, the finding from the current study also compared far less favorably with values such as 80% for South African white women, 81% for the Republic of Korea, 86% for Canada, and 88% for the United States of America. In conclusion, the current study found that 87.7% of the BC cases had moderate to poor prognosis at the time of diagnosis. Patients were found to present late when the disease is advanced.

Acknowledgment
We would like to thank all the residents and the biomedical staff of the institution for their support.

Conflict of Interest
No conflict of interest.

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Conclusion:
A multimodality approach with neoadjuvant chemotherapy can lead to high rates of breast conservation in women with primary occult breast cancer. This approach appears to be oncologically safe.

Methods:
We conducted a retrospective review of women diagnosed with isolated metastatic adenocarcinoma to the axilla histologically consistent with breast adenocarcinoma without evidence of a primary breast tumor. Medical records were analyzed to gather pertinent information regarding diagnostic workup, treatment, recurrence, and survival.

Results:
We identified seven patients treated in our institution between 2012 and 2017 who met the criteria for primary occult breast cancer. The median age at diagnosis was 63 years old (range 42-71). Subtypes by immunohistochemistry (IHC) were HER-2 positive (3 pts), triple negative (2 pts), and hormone receptor positive/HER-2 negative (2 pts). All patients received neoadjuvant chemotherapy and axillary surgery without mastectomy followed by adjuvant radiotherapy to the breast and regional nodes. Hormone receptor positive patients received adjuvant endocrine therapy. At a median follow-up of 3.5 years, all patients were alive with no local or regional recurrence of disease while one patient developed distant metastases.

Background:
Occult primary breast cancer is a presentation of breast cancer involving lymph nodes without an identified primary tumor within the breast. Mastectomy with axillary node dissection has been the traditional management. However, reported oncologic outcomes with mastectomy have been similar to those with breast conserving therapy (axillary surgery and radiotherapy). With the increased sensitivity of MRI and its routine use in the workup of occult breast cancer, the role of mastectomy for occult breast cancer is now even less clear. We report our institutional experience of neoadjuvant chemotherapy followed by axillary surgery and radiotherapy for women with occult breast cancer.

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Conclusion:
A multimodality approach with neoadjuvant chemotherapy can lead to high rates of breast conservation in women with primary occult breast cancer. This approach appears to be oncologically safe.
become the standard of care in the workup of OPBC and can identify a primary breast lesion in up to 70% of women with mammographically occult tumors. As such, the incidence of true OPBC has decreased significantly with improved imaging. Given the rarity of true OPBC, there is limited data to guide optimal management in this setting. Several retrospective case series and a population study have evaluated the impact of surgical extent and radiotherapy for OPBC. These have consistently demonstrated equivalent oncologic outcomes between mastectomy compared to breast conservation with radiotherapy. Despite this, the majority of patients diagnosed with OPBC are treated with modified radical mastectomy. Currently, optimal management in several aspects of OPBC remain to be defined. These include surgical management of the breast, extent of axillary surgery, and timing of systemic therapy.

We report our clinical approach and treatment outcomes in management of patients with OPBC treated at a single institution. Based on extrapolation from studies in node positive patients with intact primary tumors, we have adopted a standardized treatment algorithm for OPBC involving increased emphasis on optimal systemic therapy and radiotherapy with omission of mastectomy. Our aim is to demonstrate how a multidisciplinary treatment plan can decrease treatment morbidity without compromising oncologic outcomes in women with OPBC.

Methods
We performed an institutional review board approved, retrospective review of patients diagnosed with OPBC between October 2012 and July 2017 at Banner University Medical Center in Tucson, Arizona. Patients were identified by querying electronic medical records. Inclusion criteria were as follow: diagnosis of adenocarcinoma involving axillary lymph nodes, biopsy pathologically consistent with breast origin, absence of disease within the breast on mammogram and MRI and consistent with breast origin, absence of disease in axillary lymph nodes, biopsy pathologically excluded. Medical records were analyzed to gather data including: method of diagnosis, imaging workup, tumor characteristics, systemic therapy, radiation, surgery, surveillance imaging and disease status at last date of contact.

Results
Patient Characteristics
Seven patients were identified with primary occult breast cancer treated between 2012 and 2017. Patient characteristics are summarized in Table 1. The median age at diagnosis was 63 years old. (range 42-71). Method of detection was either screening mammogram (57.1%) or self-palpation (42.9%). Six out of seven patients (85.7%) had clinical stage IIA occult breast cancer while one patient had stage IIIA (14.3%). Molecular subtype as approximated by IHC was human epidermal growth factor (HER-2) positive (three patients), triple negative (two patients), and hormone receptor positive / HER-2 negative (two patients). All patients underwent mammography and dedicated breast MRI to evaluate for a breast primary. To evaluate for distant organ involvement, all patients had a metastatic workup with either PET CT (85.7%) or a CT chest/abdomen/pelvis and bone scan (14.3%). Median follow-up time from diagnosis to last contact was 41.1 months.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>N(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>58 (42-71)</td>
</tr>
<tr>
<td>Mean (Range)</td>
<td>63</td>
</tr>
<tr>
<td>Method of Detection</td>
<td></td>
</tr>
<tr>
<td>Self palpation</td>
<td>3 (42.9%)</td>
</tr>
<tr>
<td>Screening mammogram</td>
<td>4 (57.1%)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>IIA</td>
<td>6 (85.7%)</td>
</tr>
<tr>
<td>IIIA</td>
<td>1 (14.3%)</td>
</tr>
<tr>
<td>Clinical Stage</td>
<td></td>
</tr>
<tr>
<td>cT0N1M0</td>
<td>6 (85.7%)</td>
</tr>
<tr>
<td>cTN2M0</td>
<td>1 (14.3%)</td>
</tr>
<tr>
<td>Pathologic Stage</td>
<td></td>
</tr>
<tr>
<td>ypTtXN0M0</td>
<td>4 (57.1%)</td>
</tr>
<tr>
<td>ypTtXN1M0</td>
<td>2 (28.6%)</td>
</tr>
<tr>
<td>ypTtXN2M0</td>
<td>1 (14.3%)</td>
</tr>
<tr>
<td>Tumor Subtype</td>
<td></td>
</tr>
<tr>
<td>Luminal B</td>
<td>2 (28.6%)</td>
</tr>
<tr>
<td>Triple Negative</td>
<td>2 (28.6%)</td>
</tr>
<tr>
<td>HER2+</td>
<td>3 (42.9%)</td>
</tr>
<tr>
<td>Investigations</td>
<td></td>
</tr>
<tr>
<td>Mammogram, MRI, PET-CT</td>
<td>6 (85.7%)</td>
</tr>
<tr>
<td>Mammogram, MRI, CT C/A/P</td>
<td>1 (14.3%)</td>
</tr>
</tbody>
</table>

Treatment
Patients were discussed at a multidisciplinary treatment planning conference where workup and treatment strategy was agreed upon before initiation of any therapy. All patients received neoadjuvant chemotherapy, axillary surgery, and radiotherapy to the breast and regional nodes. Four patients received dose-dense doxorubicin and cyclophosphamide (ddAC) followed by weekly paclitaxel. Three of these patients completed 4 cycles of ddAC. One patient stopped ddAC after 2 cycles due to adverse events. All four received 12 cycles of weekly paclitaxel following AC. Two patients with HER-2 positive disease received Taxol and Trastuzumab while the third received Paclitaxel and Trastuzumab followed by ddAC. Both patients with ER positive tumors received adjuvant endocrine therapy with anastrozole. Following neoadjuvant chemotherapy, six out of seven patients received an axillary lymph node dissection (ALND) while one patient (triple negative with clinical complete response to...
Occult breast cancer is a rare but well-defined presentation of breast cancer characterized by lymph node involvement with carcinoma of breast origin but no identifiable primary tumor within the breast. Pathogenesis is unclear, but it is generally felt to represent lymphatic dissemination from either an unidentified, occult cancer within the breast or due to dissemination from a primary tumor within the breast which has subsequently regressed. Mastectomy with axillary node dissection has been the traditional management for OPBC; primarily out of concern for sub-clinical or otherwise undetected disease within the breast. Over the last decade, the sensitivity of breast imaging (with MRI and tomosynthesis) has significantly improved the detection of small-volume breast lesions. Prior studies in OPBC have demonstrated that MRI can detect clinically and mammographically occult lesions with detection rates ranging from 43-86%.9,14,16

The largest reported single institution series evaluating the utility of MRI in OPBC comes from Memorial Sloan Kettering Cancer Center. Olson et al. published their initial institutional results in 40 women with clinically and mammographically occult axillary adenocarcinoma. MRI identified a primary breast lesion in 70% of patients. In their series, 47% of patients underwent breast conservation. Five patients with negative MRI underwent axillary dissection and radiotherapy. Among five women with negative MRI who went on to mastectomy, one patient had an invasive tumor identified on final pathology. The authors note that the MRI performed at that time was inadequate by current standards and the region containing the tumor was outside the scan field-of-view.16 Buchanan et al. reported on an updated series from the same institution on 69 patients. MRI was able to identify a breast primary in 49% of patients. Among women with negative MRI who went on to mastectomy, 25% were found to have invasive tumor in the surgical specimen with one patient having a 3cm, non-enhancing IDC. Ten patients with negative breast MRI were treated with breast conservation with ALND and breast radiotherapy and all were free of local disease at a median follow up of 4.5 years.14

De Bresser et al. conducted a systematic review and identified 8 retrospective studies which evaluated the ability of breast MRI to detect breast tumors in patients with clinically and mammographically OPBC.15 Breast MRI was found to have 90% sensitivity with the ability to detect a previously unidentified breast cancer in more than two-thirds of patients. Breast MRI had much lower specificity ranging from 22-50%.

All patients received adjuvant radiotherapy to the whole breast (50Gy) and supraclavicular fossa (45Gy) in 25 fractions using a mono-isocentric, three-field technique. Supraclavicular fields included the full level 1-3 axilla in five patients. In two patients (who had axillary dissection with limited residual disease), the dissected axilla was not included in the supraclavicular field.

### Outcomes

Four patients (57.1%) had a pathologic compete response within the axilla following neoadjuvant chemotherapy (Hormone receptor positive=1, TNBC=1, HER-2 positive=2). Six of seven patients have continued with annual surveillance breast MRI in addition to annual breast tomosynthesis. At a median follow up of 41.3 months from diagnosis, all patients remain free of local or regional recurrence. One patient with HER-2 positive pleomorphic infiltrating lobular carcinoma developed osseous metastases at 57.4 months from diagnosis.

### Discussion

Occult breast cancer is a rare but well-defined presentation of breast cancer characterized by lymph node involvement with carcinoma of breast origin but no identifiable primary tumor within the breast. Pathogenesis is unclear, but it is generally felt to represent lymphatic dissemination from either an unidentified, occult cancer within the breast or due to dissemination from a primary tumor within the breast which has subsequently regressed. Mastectomy with axillary node dissection has been the traditional management for OPBC; primarily out of concern for sub-clinical or otherwise undetected disease within the breast. Over the last decade, the sensitivity of breast imaging (with MRI and tomosynthesis) has significantly improved the detection of small-volume breast lesions. Prior studies in OPBC have demonstrated that MRI can detect clinically and mammographically occult lesions with detection rates ranging from 43-86%.9,14,16

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authors concluded that MRI has clear utility in the workup of mammographically occult breast cancer. This study did not specifically report on outcomes of women with negative MRI treated with breast conservation.

The recognition that radiotherapy can eradicate microscopic residual disease within the breast following resection of gross disease provides rationale for applying the same approach in the occult primary setting. Following the publication of multiple large randomized trials demonstrating equivalence between mastectomy and lumpectomy plus radiotherapy, breast conservation became well-established as the standard of care for operable breast cancer. These studies demonstrated that breast conserving therapy provides equivalent disease-free and overall survival compared to mastectomy. In studies that mandated negative surgical margins, adequate axillary staging and tumors smaller than 5 cm, lumpectomy plus radiation have also demonstrated equivalent local control to mastectomy.

While there are no prospective studies to guide management of OPBC, numerous retrospective series have demonstrated equivalent oncologic outcomes between mastectomy and breast conserving therapy (axillary dissection and radiation). The results of these studies have consistently shown that patients with occult breast cancer receiving ALND followed by breast radiotherapy have similar outcomes to patients who underwent mastectomy. In contrast, patients treated with axillary surgery alone without radiotherapy have consistently had worse outcomes.

Yang et al. published the largest reported single institution study of breast conservation for patients with mammographically occult breast cancer. The authors compared outcomes of 214 mammographically occult breast cancer patients treated with BCT at Yale University between 1973 and 2003 and 2168 mammographically positive breast cancer and found that in breast recurrence rates after breast-conserving therapy and adjuvant radiation therapy were not significantly different between the groups. Likewise, at 10 years, there was no difference in overall survival, cause-specific survival, and distant relapse between patients with occult breast cancer and mammographically positive breast cancer. Receipt of BCT was an independent predictor of nodal relapse-free survival. The authors note that regional nodal irradiation was reserved for patients who had additional risk factors for recurrence such as high nodal burden. This may have been associated with the increased rate of nodal recurrences in the BCT arm. Among patients with in-breast recurrences, patients with initially mammographically occult breast cancers were more likely to have mammographically occult disease at the time of recurrence (32%) compared to patients with initially mammographically positive disease (13%). These findings support the use of MRI for surveillance in women with OPBC treated with BCT.

Walker et al. utilized the Surveillance, Epidemiology, and End Results (SEER database) to identify 750 patients diagnosed with T0N+ disease between 1983 and 2006. 202 patients received ALND plus radiotherapy while 268 received mastectomy. Cause specific survival at 10 years was 74.6% and was equivalent for patients treated with axillary dissection plus radiotherapy or mastectomy. Likewise, 10-year overall survival was equivalent between mastectomy and ALND plus radiotherapy at 64.9%. In contrast, 10-year OS following ALND alone without radiotherapy was significantly worse at 58.5% (n=126; log-rank P = .02). While this study lacks locoregional recurrence information (a limitation of the SEER dataset), it does support the long-term oncologic efficacy of breast conservation and radiotherapy for OPBC. Taken together with single institution series, these findings suggest that the same treatment paradigm for known-primary breast cancer yields equivalent oncologic outcomes when applied to OPBC. This is the current approach recommended in NCCN guidelines.

Neoadjuvant systemic therapy has become standard management for patients with node positive breast cancer. As such, there is a strong rationale for neoadjuvant therapy in women presenting with OPBC. Rueh et al. reported on outcomes in 36 patients with OPBC treated at MD Anderson Cancer Center. In this series, 27 patients were treated with BCT. 70% of patients received neoadjuvant systemic therapy. At a median follow up of 64 months, the authors reported no local or regional recurrences and one distant recurrence. This series is also notable in that 92% of patients were staged with MRI. Our findings are consistent with those of these authors and demonstrate equivalent outcomes with a similar treatment algorithm.

Optimal axillary management for patients with intact primary breast cancer continues to evolve. Specifically, the necessity for completion axillary dissection in patients with small volume axillary involvement is being questioned. In clinically node negative but sentinel node positive patients, axillary dissection conveys a significantly higher rate of lymphedema compared to axillary radiotherapy without improving oncologic outcomes. Ongoing randomized studies are evaluating the role of completion axillary dissection in patients with clinically positive axillary nodes who convert to clinically negative following neoadjuvant chemotherapy. In our practice, sentinel node biopsy is routinely performed in women who initially presented with clinically positive axillary nodes who have converted to clinically negative following neoadjuvant chemotherapy. Patients who are found to be pathologically node negative at time of sentinel node procedure are then offered axillary radiotherapy in lieu of full axillary dissection.

One patient in our series had a negative sentinel node biopsy after chemotherapy and did not receive
completion axillary dissection. As axillary techniques become further refined, incorporation into the management of OPBC should further reduce the rates of treatment-associated toxicity.

Like any retrospective study, this case series has several limitations. Due to the rarity of occult breast cancer, our study has a small sample size. Likewise, the extended period of time over which patients were treated leads to changes in practice patterns as evidenced by the omission of axillary dissection in patients with pathologic complete response in our current practice. A series such as this can also include medical record errors. Lastly, our follow-up is still relatively short given the long latency period for breast cancer recurrence. Nonetheless, our case series is a valuable addition to the literature in that all patients had occult disease on MRI and all patients were treated with neoadjuvant chemotherapy, regarding the treatment and management of patients with primary occult breast cancer.

Given the increased sensitivity of MRI over mammography, patients diagnosed with OPBC in the current era are less likely to have gross breast involvement than patients diagnosed without MRI. As such, the therapeutic role of mastectomy for OPBC in the modern era is questionable. In our series, all patients underwent diagnostic workup with negative dedicated breast MRI in addition to digital or 3D mammography. Our series highlights the importance of a prospective, multidisciplinary approach for the management of OPBC. All patients were discussed at a multidisciplinary treatment planning conference where workup and treatment strategy were discussed before initiation of any therapy. All patients underwent neoadjuvant chemotherapy followed by axillary surgery and radiotherapy. Patients have continued routine surveillance with MRI and mammogram. At a median follow-up of 41.1 months from diagnosis, all patients remain alive and no patients have had recurrence within the breast or regional nodes. Our results are consistent with other series and support the oncologic validity of this combined-modality approach for OPBC.

Conflict of Interest
The authors have nothing to disclose.

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A Breast Cancer Patient Presenting for the First Time With a Meningioma-like Intracranial Mass

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ABSTRACT

Background: Breast cancer metastases to the central nervous system are more commonly multiple intracranial lesions. Rarely, in the case of single extradural metastasis, it is clinically and radiologically challenging and important to differentiate other solitary extradural masses such as meningioma. Moreover, according to the literature, it seems that there is a relationship between breast cancer and meningioma.

Case Presentation: We report a 54-year-old female presenting with a sudden onset of headache and seizure whose MRI showed an extra-axial intracranial mass in her brain. The patient underwent operative removal of the tumor with the clinical and radiologic diagnosis of meningioma. The pathological finding was suspected to be breast cancer metastasis. Future evaluation of her breast showed tissue distortion in the left breast and the pathologic diagnosis of the breast lesion was invasive ductal carcinoma. In a follow-up CT scan, there were multiple liver and lung metastases.

Conclusion: It is crucial for physicians with various specialties to be aware of different possibilities in the setting of a single extra-axial brain mass.

Introduction

Meningioma, as the most common meningeal tumor, is an extra-axial, nonglial neoplasm that originates from meningocytes. It constitutes up to 20% of primary brain tumors. There is a female predominance, and it usually occurs in the fifth and sixth decades of life—similar to the peak incidence age for breast cancer. The incidence of meningioma is 1.57 to 1.90 times greater in breast cancer patients compared with the healthy population. A hormonal relationship between breast cancer and meningioma is suggested.

Breast cancer metastases to central nervous system usually present as multiple intra-axial masses, but sometimes isolated dural tumors may be misdiagnosed as meningioma. In cases without the diagnosis of primary breast tumor, isolated enhancing mass in skull base is in favor of meningioma rather than isolated brain metastasis, but, in the presence of primary breast cancer, metastasis from primary tumor should also be considered as differential diagnosis.

Generally, intracranial metastasis is seen in approximately 17% of patients with malignant primary tumor; however, with improved cancer treatments and the resultant prolonged survival rates, this number will increase. While 25% of intracranial metastases have some degree of meningeal involvement, isolated meningeal metastasis is seen in 1%. In addition, in cases without known clinical evidence of primary cancer, it is even rarer. Here we report a case of a patient with a single extra-axial dural-based mass of unknown origin in the brain.
Case Presentation
A 54-year-old woman referred to Imam Khomeini Hospital of Tehran University of Medical Science with a chief complaint of a sudden onset of headache and seizure. Her brain MRI showed an extra-axial intracranial mass (Figure 1). She underwent surgery and was clinically diagnosed with meningioma. On pathologic examination, sheets of atypical epithelial cells with pleomorphic vesicular nuclei and eosinophilic cytoplasm occasionally arranged in tubular structures were identified. Comedo-type necrosis and frequent mitotic figures, all in favor of metastatic carcinoma, were also seen. Immunohistochemistry revealed positive reactions with CK7, GATA3, GCDFP-15, and HER2 (3+). Tests for ER, PR, CK20, CDX2, TTF1, and Napsin-A were negative. Ki67 showed 30% proliferative activity. Overall findings were compatible with metastatic carcinoma of breast origin (Figure 2). We were consulted for breast evaluation. She did not have a family history of breast cancer and had a breast ultrasound with the benign result from 3 months ago. In our evaluation, we found a tissue distortion in the central upper part of the left breast and lymph nodes with a pathologic appearance in left axilla (Figure 3). We performed core needle biopsy of breast distortion, and the result of pathology was indicative of invasive ductal carcinoma with the hormonal profile the same as a metastatic brain tumor.

The patient underwent brain radiotherapy. After a while, her thoracic and abdominal computed tomography (CT) scan showed multiple lung and liver metastases.

Figure 1. Brain MRI with contrast injection in the patient with a sudden onset of headache and seizure 1a (axial), 1b (sagittal), and 1c (coronal) images show an extra-axial dural-based avid, homogenous, enhancing mass in left side anterior skull base accompanied by a dural tail, compression on nearby parenchyma, and edema.

Figure 2. (A) and (B), microscopic examination of hematoxylin- and eosin-stained sections reveals sheets of neoplastic epithelial cells with eosinophilic cytoplasm and pleomorphic vesicular nuclei occasionally arranged in glandular structures. Central comedo-type necrosis is also seen. Immunohistochemistry reveals positive immune reaction with CK7(C), GATA3 (D), and GCDFP15 (E).
Then, chemotherapy was started for her by the oncology team, which is continued till now.

**Discussion**

When dealing with an extra-axial intracranial mass lesion, it is crucial to consider differential diagnoses like metastases to the skull or dura other than more common primary masses such as meningioma, or subdural hematoma or collection. In imaging, dural-based metastatic masses may present as nodular or even elongated and plaque-like lesions. The surrounding cerebral cortex may be compressed, and edema incited in the underlying parenchyma. Dural tail sign (the enhancement of the meninges adjacent to tumor) on CT scan and MRI was once considered as a specific sign for meningioma; however, it is known that this sign also can be present in meningeval metastases.

Meningioma is benign and curable and accounts for up to 20% of all intracranial tumors, while brain metastases from breast cancer have an ominous prognosis despite surgery, radiation, and chemotherapy.

As the physical appearance of these tumors can be quite similar, surgeons may not distinguish a metastasis from meningioma during surgery, as was the case for our patient, and pathology will lead to diagnosis.

It has been reported that breast cancer is the second most common cause of intracranial metastasis after lung cancer. Metastatic pulmonary carcinoma was excluded in our case immunohistochemically. Most patients with breast cancer metastasis to the central nervous system either have synchronous multiple organ metastatic disease or soon develop it.

The role of hormones in meningioma has been already proposed since meningiomas enlarge during pregnancy and express estrogen and progesterone receptors.

Schmidt et al. reported two women with breast carcinoma who presented with clinical presentations of sphenocavernous syndrome with MRI that was misdiagnosed as basal skull meningioma, but who were found to have metastatic breast carcinoma following biopsy of the lesions. Like our patient, those cases had no history of breast malignancy.

Interestingly, some patients are reported in the literature with metastasis of breast carcinoma to meningioma. It is reported that HER2-positive breast cancer cells preferentially like to deposit in the patient’s meningioma. This may be due to the high vascularity, low metabolic rate, and hormonal status of meningioma. However, in such cases, there is usually a known history of breast cancer.

Seckin et al. reported a 72-year-old woman presenting with nausea and vomiting, headache, reduced mentation, and amnesia initially misdiagnosed as meningioma. In this case, however, the patient also had a history of breast mass leading to metastasis.

Computed tomography, MRI, and angiography may be somewhat beneficial for the differentiation of metastasis from other lesions. A hematoma or collection does not enhance and has a related history most of the times. A meningioma is dural based and shows homogenous avid enhancement, whereas a metastatic tumor is not homogenously enhanced. Gadolinium-enhanced MRI is known as the most sensitive imaging technique for the diagnosis of neural axis metastases and enhanced MRI is even superior to the enhanced CT for both brain parenchymal and leptomeningeal diseases due to its higher soft tissue contrast, higher sensitivity to the contrast enhancement, the direct multiplanar capability, and the lack of the artifacts related to the bone. It particularly helps to reveal the lesions in the posterior fossa and brainstem, where the presence of beam-hardening artifacts may be problematic in CT scan. The MRI is also superior to the CT scan for identifying multiple lesions, which is useful in differential diagnosis. Sometimes other advanced
techniques like magnetic resonance perfusion imaging are needed to differentiate between lesions. Dural metastases and meningiomas have different vasculature and perfusion characteristics which are assessed using dynamic perfusion MRI.

As Conclusion, it is important for physicians to be aware of different diagnosis in the setting of a single extra-axial brain mass

Conflict of Interest
The authors have nothing to declare

References
One of the most significant findings of the 1990s was the discovery of the BRCA gene mutation, which was a major advancement in the prevention of breast cancer. Since the BRCA breakthrough, screening for breast cancer susceptibility genes has become of great interest to high-risk patients and their family members.\(^1\) BRCA1 and BRCA2 are two major genes responsible for about 5% to 10% of all breast cancer cases and 10% to 18% of ovarian cancer cases. Carriers of BRCA1/2 mutation could have an elevated risk of 69% to 72% for breast cancer compared with the average-risk women (12%). The risk of ovarian cancer incidence is also much higher in women with a BRCA1/2 mutation than in the normal population (17%-44% vs 1.5%).\(^2\) Today, female carriers of a BRCA1 or BRCA2 mutation (both affected and unaffected) are offered various choices of lifesaving preventive care, including prophylactic surgery, chemoprevention, and intensive surveillance, aimed at reducing their risk of developing breast or ovarian cancer. Nonetheless, in reality, finding the right choice is far more complicated.

The limitation in risk quantification is one of the key areas of challenge. Currently, carriers of BRCA mutation variants known as “definitely pathogenic” are commonly offered full high-risk surveillance, whereas decision making for carriers of variants with little or unknown risk for breast cancer remains unanswered.\(^3,4\)

Bilateral prophylactic mastectomy, which is known as the single most effective preventive option, reduces the risk of breast cancer by about 90%.\(^5\) Similarly, prophylactic bilateral salpingo-oophorectomy (PBSO) has been estimated to substantially decrease the risk of ovarian cancer in BRCA carriers.\(^6\) However, age is very critical for prophylactic surgery. It has been demonstrated that unaffected carriers younger than 50 years gain more benefit from prophylactic procedures such as mastectomy than the others.\(^7\) Also, it has been estimated that PBSO in premenopausal BRCA carriers may reduce their risk of breast cancer by 50%.\(^8\)

The effect of the inherited mutation on the overall survival of a breast cancer patient is also a challenging issue. While many studies have shown that prophylactic procedures will be beneficial and increase the long-term survival, a recent study called POSH (Prospective Outcomes in Sporadic versus Hereditary breast cancer) did indicate that there was no significant difference in survival between BRCA1/2 carriers and non-carriers with young age at onset and at the time points of 2, 5, and 10 years after the first diagnosis and concluded that immediate bilateral mastectomy had no advantage in the overall survival of patients in at least 10 years’ time period from the first diagnosis.\(^9\) Another study showed that BRCA carriers undergoing breast-conserving therapy had a higher risk of local recurrence after 5, 10, and 15 years compared with the carriers who underwent a mastectomy.\(^10\) A cohort study also showed that early-stage (I or II) breast cancer patients with a BRCA mutation who received bilateral mastectomy had longer survival rates compared with those undergoing unilateral mastectomy.\(^11\)

None of the risk-reducing strategies is fully eliminating the risk of developing cancer and yet comes with potential complications. Risk-reducing surgeries of a woman who is within her childbearing time period have implications for her fertility or breastfeeding and also body image. Oophorectomy also causes early menopause and imposes several issues including gain weight, osteoporosis and heart diseases.\(^12,13\)

Intensive surveillance has been designed to facilitate early detection of breast cancer in high-risk
women, however, adherence is commonly required which may impose regular expenditure. The false positive test result is also another issue coming with psychological costs adding more distress to the individual.

Chemoprevention by estrogen modulators tamoxifen and raloxifene decreased the risk of breast cancer by up to 70% and 76%, respectively, but had no effects on estrogen-negative breast cancer patients. While other chemoprevention agents are yet to be approved, existing cancer medications exert adverse effects including increased risk of endometrial cancer and venous thrombosis.

Given these considerations, there is no straightforward task as the best risk-reducing practice for a BRCA mutation carrier. What is clear is that the risk of cancer in mutation carriers increases over time, but this does not mean to push patients to act immediately. Recent findings are emphasizing that carriers who have a pathogenic germline mutation in a susceptibility gene benefit from prophylactic surgeries; however, the best practice should be individualized and taken in to account according to each patient’s type of mutation, age, tumor prognosis, consideration of the short-term and long-term risks, as well as the patient preferences.

References
Displaced Epithelium in Breast Pathology: A Review

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ABSTRACT

**Background:** Although iatrogenic displacement of epithelial cells after breast instrumentation is a well-documented phenomenon, it is usually underdiagnosed. Misinterpretation of this issue results in overtreatment of patients in some instances. Additionally, the hazard of tumor seeding and dissemination after needling is a concern to both clinicians and patients. Both issues are addressed in this narrative review.

**Methods:** We searched PubMed for abstracts of English-language publications using keywords “needle track/tract” and “displaced epithelium/epithelial displacement/iatrogenic displacement,” which resulted in 439 records restricted to human subjects. We read all the abstracts and selected 27 manuscripts with the most relevance.

**Results:** There are some histopathologic features that can be useful in differentiating between epithelial displacement and real invasion/metastasis. The risk of seeding or metastasis after needling is shown to be trivial. Factors militating against the growth of dislodged cells are also discussed.

**Conclusion:** Epithelial displacement is an important issue in breast pathology that should be considered in every patient with a history of breast instrumentation.

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**Introduction**

Displacement of tissue may occur following any type of intervention or surgical procedure in different organs. Both epithelial and mesenchymal components can be displaced. Implantation of carcinoma cells in the needle tract during percutaneous biopsy has been reported in cancers such as renal cell carcinoma, thyroid carcinoma, hepatocellular carcinoma, small cell carcinoma of lung and breast carcinoma.

In breast tissue, displacement of epithelial component, both benign and malignant, may result in a misdiagnosis of invasive carcinoma or metastasis. Therefore, pathologists and other practitioners in the field of breast diseases should be cognizant of this phenomenon.

Displacement of epithelium is common after needling procedures, including percutaneous core needle biopsy that is commonly used for evaluation of suspected breast lesions. It occurs in any type of breast lesion. In one study, 22 out of 64 excised breast specimens showed displaced neoplastic cells in needle tracks. In a recent systematic review, displacement rate in a total of 927 cases from nine studies on surgically excised breast specimens ranged from 2% to 63%. This wide range can be attributed to the type of breast lesion (more common in papillary lesions), time interval between needling and surgery, type of procedure, and needle gauge. Although both patients and clinicians may be concerned about the proliferation of displaced malignant cell resulting in tumor recurrence at needling site or distant organs, the risk of this so-called “seeding” is only 0.2% to 0.7%.

Epithelial displacement is important from two points of view including correct histopathologic diagnosis and hazard of needle track seeding or even metastasis. Therefore, both issues are addressed in this review.
Methods
We searched PubMed for English-language publications related to human subjects using the following keywords: “needle track,” “needle tract,” “displaced epithelium,” “epithelial displacement,” and “iatrogenic displacement,” which yielded 439 records. We read all the abstracts and selected 27 papers with the most relevance. The full texts of the selected papers were obtained. We used both the selected papers and our experience to write the narrative review. The images are all taken from our cases in the Cancer Institute of Tehran University of Medical Sciences.

Results
Definition of displaced epithelium and how it differs from real invasion or metastasis
When the epithelium is dislocated mechanically by surgical or radiological interventions, it is called displaced epithelium (DE). This iatrogenic phenomenon should be distinguished from local invasion or metastasis. In the latter situations, the neoplastic cells that have gained the ability to invade the stroma or vascular channels are dislodged from the main bulk of in situ carcinoma without requiring any external mechanical force. In both situations, some atypical cells are evident in the stroma, regional lymph nodes, or even distant sites such as bone marrow. Therefore, distinguishing between these two entities may be a great challenge for the histopathologist.

Procedures resulting in DE
Any type of instrumentation can result in DE, including fine needle aspiration, core needle biopsy, wire localization of lesions, vacuum-assisted breast biopsy (VABB), injections for sentinel lymph node identification, and incisional or excisional biopsies. DE has also been reported after liposuction in a male patient with gynecomastia.

The least damaging method is fine needle aspiration, where a 22-G needle is employed for entering the tumor. Needles with a larger gauge, such as 11- or 14-G, used for tissue diagnosis, result in more frequent DE.

Having a very low rate of false negative results, VABB has a sensitivity and specificity superior to core needle biopsy. In three studies, no malignant cell displacement was identified after this procedure. In these studies, 28, 23, and 21 VABBs were performed using 11-G, 14-G, and 11-G needles, respectively.

Factors influencing the frequency of DE
The number and volume of identified tumor cells in needle track is inversely related to the intervening time between core needle biopsy and excision of the lesion. Two main determinants of neoplastic cell displacement along needle tracks are the short term between core needle biopsy and excisional surgery and the histology of the tumor.

In benign and malignant papillary lesions, epithelial displacement occurs more commonly in comparison with other neoplasms of the breast because of the fragility of these lesions. In one study in 2005, 50 out of 53 cases with epithelial displacement had an underlying papillary lesion. These epithelia were dislocated to the stroma surrounding the papillary lesions and adjacent lymph-vascular channels imitating invasion. These displaced epithelia can be even transferred to axillary lymph nodes.

Clinical significance of DE
The trivial risk of development of clinical tumor recurrence at the needle biopsy site despite the high frequency of DE suggests that DE has little clinical significance. Radiotherapy is probably sufficient for eradicating isolated tumor cells in the needle track. The study of Boutin et al. on mesothelioma patients revealed metastasis in the needle track in 8 out of 20 patients who had not received radiation, while none of the twenty patients receiving radiotherapy developed metastasis. Thurfjell et al. showed local recurrence in 11% of patients with breast carcinoma who had undergone breast-conserving surgery (33/303). Recurrence due to seeding in the needle track was suspected in 3 cases (3/33), none of whom had received postoperative radiotherapy. Therefore, radiation of breast tissue, which is often performed after lumpectomy, obviates the mentioned risk of local recurrence. Additionally, the needle track is often removed during the surgical excision of malignant tumors. A study on a large number of patients showed that core needle biopsy or fine-needle aspiration, for diagnosis of a malignant breast tumor, does not result in an increased risk of sentinel lymph node metastasis.

The major issue is the risk of misinterpretation of DE by the pathologist who reviews the patient’s excised material.

Histopathologic features of the needle track
Displaced epithelia are most commonly identified at the site of needle track. The salient histopathologic features of needle track are as follows:

• Old or recent hemorrhage, depending on the interval between biopsy and excision (Figure 1).
• Hemosiderin deposition, either within the macrophages or in the stroma
• Granulation tissue formation, fibrosis, and chronic inflammation (Figure 2)
• Fat necrosis
• Foreign body reaction
• The presence of a large number of macrophages as identified by immunostaining for CD68 (Figure 3)
• The inflammation, hemorrhage, and fibrosis are arranged in a linear/elongated fashion (Figure 4)
Figure 1. Hemorrhage at needling site (H&E, ×40).

Figure 2. Intraductal papilloma with features of needle site. Note the hemorrhage, granulation tissue, and fibrosis (H&E, ×40)

Figure 3. A. Intraductal papilloma in a case showing displaced epithelium. Note the hemorrhage at needle track site (H&E, ×100); B. Immunostaining for CD68 highlights the macrophages at needling site (×100).
**Figure 4.** Needle track. A part of an intraductal papilloma is seen at the right side of the image. The elongated fibrosis and inflammation highlight the path of the needle biopsy performed a few weeks before (H&E, ×40).

**Figure 5.** Displaced epithelium in a fibrotic stroma. Small cords or nests of displaced epithelium are evident in an extensively fibrotic stroma. The displaced epithelium is arranged in a rather linear fashion (H&E, ×100).

**Histopathologic features of DE and how it can be distinguished from authentic invasion or metastasis**

DE should be suspected when we observe a major intraductal component (particularly if this component is a papillary proliferation) or a small presumably invasive tumor. The surgeon or radiologist should notify the pathologists, if there has been any history of prior biopsy or other instrumentations.

How can DE be differentiated from genuine invasive carcinoma? Apart from being located along needle track with aforementioned histopathologic features, the following characteristics may be helpful:

- The displaced epithelial cells are usually arranged in a linear pattern (Figure 5).
- The displaced cells show some degree of degeneration. It depends on the time interval between needling and excision. The longer this interval is, the more degenerative changes are identified. Changes. The degenerated cells have pyknotic nuclei with increased intensity of cytoplasmic eosinophilia (Figure 6). If the interval between needling and biopsy is long enough, the displaced cells become necrotic or are completely removed by macrophages.
- The cells are morphologically similar to the intraductal component.
- Immunostaining for cytokeratin (Figures 7 and 8) and CD68 may be helpful in distinguishing between DE and epithelioid histiocytes (Figures 3 and 6).
- Immunostaining for myoepithelial markers and high–molecular weight cytokeratin may be utilized when the intraductal component is a benign proliferation with preserved myoepithelial cells. However, the absence of staining for these markers does not preclude the
Some displaced epithelial cells may show squamous metaplasia, resulting in its being mistaken as adenosquamous cell carcinoma. Sometimes the displaced epithelia are seen in lymphatic channels. There are even reports about the transfer of the displaced epithelia to the lymph nodes along the lymphatic channels. Fibrosis related to needling, and not a desmoplastic reaction around invasive carcinoma, is very extensive. Therefore, the displaced epithelia usually constitute a small fraction of an extensive reaction (Figure 3).

Some displaced epithelial cells may show squamous metaplasia, resulting in its being mistaken as adenosquamous cell carcinoma. Displacement of the epithelium to regional lymph nodes and even remote organs such as bone marrow is on record. Since most of these cases are misdiagnosed as metastasis by pathologists, the exact frequency of these findings cannot be determined. Some features useful for distinguishing between DE and real metastasis include:

- The displaced cells are usually in the form of isolated cells or small clusters.
- They are usually evenly distributed in the lymph node sinus and do not form expansile masses or nodules.
- They are accompanied by red blood cells, macrophages, and giant cells.
- The invasive component is very small (for instance, when we have a microinvasive carcinoma).
- The grade of tumor and its type are less compatible with metastasis, e.g., a low-grade tumor or a papillary or mucinous carcinoma (in these situations, the likelihood of lymph node metastasis is very low, and if we see epithelial cells in lymph nodes, it should be interpreted cautiously).
- The biomarker results (ER, PR, and HER2) are different from the primary tumor.
- The tumor is morphologically different from the invasive carcinoma.
- DE is evident in lymphatic channels of the breast tissue.

Figure 6. A. Displaced epithelium: some cells show degenerative changes characterized by pyknotic nuclei and increased eosinophilia of cytoplasm (white arrows); B. The displaced epithelium is highlighted by cytokeratin AE1/AE3; C. No staining for P63.
Displacement of epithelium often occurs after needle biopsy of a breast lesion. Any epithelial cell can be displaced, including benign intraductal proliferations, in situ carcinoma, and invasive carcinoma. The major diagnostic problem is related to the displacement of intraductal proliferative cells (both benign and malignant) to the stroma or lymphatic channels, which results in misdiagnosis as an infiltrating tumor. Displacement of an invasive carcinoma along the needle track usually poses no diagnostic problem for the histopathologist. Its clinical significance has been discussed elsewhere in the manuscript. Occasionally, the displaced epithelium, dislodged into the lymph-vascular channels, may be carried to the regional lymph node, resulting in detection of isolated tumor cells in sentinel lymph nodes.

Diagnosis of DE may be a great challenge for pathologists. Sometimes, it is overcalled as invasive carcinoma.

According to previous studies, the incidence of tumor seeding and recurrence at the site of needle track is low relative to the frequency of displaced tumor cells at this site. This finding may be explained based on cancer stem cell theory and the characteristics of malignant tumors. All tumors have two basic components including parenchyma and stroma. The stroma is host-derived and crucial for the growth of neoplasms because it supplies blood and essential factors. In tumors, normal interactions between cells and extracellular matrix are altered and disrupted so that tumor cells can detach from each other easily. Tumor cells, as well as host-derived stromal cells, can secrete proteolytic enzymes that can degrade interstitial connective tissue, facilitating the movement of and, eventually, local invasion by tumor cells.

During diagnostic procedures such as core needle biopsy or aspiration and excisional biopsy, tumor cells can be dislodged and displaced in needle track. Detached tumor cells can also enter lymphatic channels via these procedures and, at least theoretically,
colonize other sites far from the main lesion.\textsuperscript{32,39} How can the low rate of local recurrence at biopsy site or lymph node metastasis be explained despite the presence of tumor cells in lymph nodes?

Cancer cells do not survive alone but recruit normal host-derived cells to serve as contributing members by producing tumor microenvironment. Tissue microenvironment is necessary for the growth of the tumor and also the development of other characteristics of malignancy such as invasion and metastasis. The cells that contribute to the production of tumor microenvironment are cancer-associated fibroblasts, angiogenic vascular cells, and infiltrating immune cells.\textsuperscript{53}

Alternatively, according to cancer stem cell theory, all tumors contain a population of cells that show biologic properties similar to those of normal adult stem cells. These stem cells are responsible, if not crucial, for maintenance of malignant tumors.\textsuperscript{31,34}

Moreover, like normal stem cells, they require a specialized microenvironment named “stem cell niche” for regulation and progression of their growth. It represents a dynamic compartment by means of which additional components including endothelial, immune, and stromal cells as well as soluble factors help develop the environment necessary for both self-renewal and differentiation of stem cells.\textsuperscript{52,55}

Almost all tumors, even at early phases, release cancer cells into blood, but a clinically evident metastatic disease occurs after a long period of latency. This observation suggests that metastatic colonization is an inefficient process and that most cancer cells die in blood. The investigations that show circulating tumor cells outnumber the overt metastatic lesion support this observation.\textsuperscript{38}

Disseminated tumor cells need both stem cells and specialized microenvironment to survive. Experimental studies have provided evidence that signals from primary tumor can result in the development of a microenvironment in a distant homing organ. Thus, the so-called “premetastatic niche” is provided before the arrival of circulating tumor cells.

The low frequency of needle track seeding or metastasis can be due to the absence of cancer stem cells or appropriate microenvironment in the new location. However, this proposal should be confirmed by further investigations.

Although the frequency of seeding in needle track is very low, it is a well-documented entity.\textsuperscript{26,29,37} The risk factors for the development of seeding in the track of breast needleling were evaluated in a study by Santiago et al.\textsuperscript{18} They showed that the major risk factors included grade 3 infiltrating ductal carcinomas, triple-negative carcinomas, use of non coaxial needles, and multiple insertions. The frequency of seeding in this study was less than 0.2% (8 of 4010 core needle biopsies) and the mean time between core needle biopsy and development of seeding was 60.8 days.

Undoubtedly, a large number of cases of displaced epithelia are signed out as invasive carcinoma or metastasis by pathologists all over the world. Accordingly, the unexpectedly good prognosis in some cases of presumed invasive carcinoma with regional lymph node metastasis can be attributable to the overcalling of a noninvasive lesion.\textsuperscript{33,58,59}

As conclusion, we should be aware of the possibility of epithelial displacement after needle aspiration or biopsy to differentiate dislodged epithelial cells from infiltrating tumor cells and lymphatic invasion. Degenerative alteration of neoplastic cell clusters and the absence of desmoplastic stroma around them indicate epithelial displacement.

\textbf{References}


Recurrence multiple fibroadenomas: History of a case presented in MDT meeting with clinical discussion and decision making

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ABSTRACT

Background: Fibroadenoma is a common benign breast disorder in young women which has a low risk of malignant transformation. Most fibroadenomas present as a single mass, but the presence of multiple fibroadenomas can be seen in 15–20% of patients, with average number of 3–4 masses in one breast. In different studies and reports, various treatment modalities-including observation and follow up, surgery, radiofrequency ablation, etc- have been proposed, though the best management for these patients are not determined yet.

Case presentation: We present the case of 33-year-old female with history of multiple bilateral benign breast lesions with a presumptive diagnosis of fibroadenomas. She had three previous surgical excisions in the past 14 years. Her case was presented to a breast MDT meeting to obtain a recommendation on appropriate management.

Question: The proposed a question in MDT concerned the best and most appropriate management plan for the patient; Does she require further surgical excisions? And if not, how should she be followed?

Conclusion: After reviewing past medical history, physical examination, and all documents regarding the patient, MDT members recommended that the patient should be managed with close follow up with physical examination and ultrasound every 6 months. The necessity of further surgical intervention would be determined according to any new findings.

Introduction

Fibroadenoma is a common benign breast disorder occurring in almost 20% of women. It mostly occurs in women of child-bearing age, especially those under 30. The risk of malignant transformation is very low in fibroadenomas; but epithelial hyperplasia, atypical hyperplasia (0.81%), invasive and insitu ductal and lobular carcinoma has been seen rarely in fibroadenomas. Patients with strong positive family history of breast cancer and complex fibroadenomas need greater attention, as the risk of malignant transformation is higher in these subgroups of patients.

Ultrasound is a reliable diagnostic test for fibroadenomas. Generally, when clinical and ultrasound features are consistent with diagnosis of fibroadenoma, no tissue biopsy is needed and it is managed either conservatively or excised; but if there is no certainty, biopsy -often core needle biopsy- is helpful in making definite diagnosis. Fibroadenomas are often managed by observation with clinical control and imaging (usually ultrasound), or surgical excision according to the size and features of the lesion and its risk of malignant transformation, and patient characteristics and preferences.

When considering a patient with presumed fibroadenoma based on clinical and imaging findings, one important thing to deal with is how to accurately differentiate it from phyllodes tumor. Phyllodes tumor...
of the breast is an uncommon fibroepithelial neoplasm which is classified into benign, borderline, and malignant types according to histologic features. Although in some studies special features of imaging modalities (like size, shape, and margin of the mass in mammography; size, shape, margin, echo pattern, and vascularization of the mass in ultrasound; internal cystic areas on MRI) are reported to be helpful to ascertain imaging-histological concordance and guide clinicians to make appropriate decisions about correct follow-up or the necessity of biopsy or excision, differentiating fibroadenoma from phyllodes tumor based on clinical, radiologic and even pathologic findings is not always easy.

Most fibroadenomas present as a single mass, but the presence of multiple fibroadenomas can be seen in 15–20% of patients. Average number of masses in cases of multiple fibroadenomas has been reported to be 3–4 in one breast, and occurrence of more than five fibroadenomas is reported to be much less common. Unlike women with a single fibroadenoma, most of the patients with multiple fibroadenomas have a strong family history of these lesions. The etiology of this entity has not been clearly known yet. Possibilities like taking oral contraceptive pills, imbalance of estrogen levels in the body, hypersensitivity of local breast tissue to estrogen, dietary factors, or inherited predisposition has been proposed in different studies.

Patients with multiple fibroadenomas usually undergo several diagnostic imaging, tissue biopsies and surgical excisions during their life, all showing same benign results. Therefore, this disorder poses a conundrum for the surgeon, as it is difficult to avoid unnecessary imaging and procedures while not missing any malignant lesions. In different studies and reports, various treatment modalities -including observation and follow up, surgery, radiofrequency ablation, ...- have been proposed, though the best management for these patients has not been determined yet.

Herein, the medical records of a 33-year old woman discussed in Multidisciplinary Team Discussion (MDT), is presented. The patient had the history of multiple bilateral benign type breast lesions in favor of fibroadenoma and three previous excisions during the past 14 years. She was suffering from the recurrence of lesions in the same area of the breast, as well as the other quadrants of both breasts. The medical records of the patient are presented in MDT session for clinical discussion and decision making concerning appropriate management.

Case presentation
A 33-year-old female was referred to our breast clinic with complaint of multiple bilateral breast masses for the past 14 years. She had no positive family history of breast or ovarian cancer, she was married and nulliparous. In her physical examination a 25mm mobile mass was palpated in UIQ of the right breast. She had undergone multiple previous imaging tests (including 6 ultrasound exams and two breast MRIs). All the imaging modalities reported multiple benign looking masses in both breasts. She had three surgical excisions, two with pathology of fibroadenoma, and the last one (which had been done 2 years before the current presentation) with pathology of benign fibroepithelial lesion reported to be benign phyllodes tumor in pathology review. On her recent ultrasound, multiple hypoechoic lesions with benign features in both breasts was reported, five masses in the left breast (the biggest one 20 mm in 7 o’clock), and three in the right (the biggest one was 21mm in 1 o’clock). The report of core needle biopsy for the recent lesions in both sides was compatible with fibroadenoma. She did not take oral contraceptive pills or any other medications. Her menstrual cycles were regular and there were no findings on gynecologic exam. The patient’s history was presented in multidisciplinary team meeting of breast unit in the surgery department, Tehran University of medical sciences to decide appropriate management.

Question
The proposed question was about the most appropriate management for the patient as the further step: Does she need further surgical excisions? And if not, how should she be followed up?

Discussion
Fibroadenoma is a common benign breast disorder mostly seen in young women under 30. They rarely progress into malignant lesions. There are several treatment options for fibroadenomas including observation with clinical and imaging control and surgery. It is postulated that fibroadenomas may be safely followed, if volume growth rate is less than 16% in those younger than 50 years and less than 13% per month in those 50 years or older. A mean size change of 20% for a 6-month interval reported to be acceptable for all ages. It was concluded that non-operative management of fibroadenomas seems to be safe. Surgical resection is another effective and appropriate treatment, but the permanent surgical scar can cause psychological issues for the patients, especially younger ones. The surgical treatment of choice for fibroadenoma is breast lumpectomy, however, multiple fibroadenomas may pose a particular challenge. Other proposed treatments include minimally invasive treatment such as vacuum assisted breast biopsy applied for mass resection and thermal ablation treatment with radiofrequency ablation (RFA), microwave ablation, and laser ablation.

Most fibroadenomas present as a single mass; however, the presence of multiple fibroadenomas can be seen in 15–20% of patients, with average
number of 3-4 masses in each breast. The best management for patients with multiple fibroadenomas is not determined yet. Most patients undergo several imaging and diagnostic procedures, as well as surgical excisions during their life which causes psychologic and cosmetic distress for them. In different studies and reports, various treatment modalities have been proposed for this group of patients.

Pasta et al. have reported a case of a 22-year-old woman undergone surgical treatment many times to remove multiple mammary fibroadenomas. They proposed that the periodic radiological exams would be the proper management, and the surgical treatment should be reserved only for rapidly growing lesions. Based on natural history of fibroadenomas, the method of observation, follow-up and implementing surgical resection only under certain conditions—such as large tumor size and inability to rule out malignancy—has been claimed to be the most common advice in other studies.

As mentioned above, the treatment of choice for fibroadenoma is the surgical excision, but multiple fibroadenomas may pose a particular challenge and necessitate more extensive surgeries e.g. oncoplastic techniques for reconstruction of the breast shape. In one study by Camara et al., Riberi technique of reduction mammoplasty modified by Rezai was used for selective resection of more than seventy fibroadenomas on two patients. In this technique, remodeling of the breast like a mastopexy through the inferior pedicle technique was done. The authors concluded that multiple fibroadenomas of the breast can be removed safely with these techniques.

In another study by Lai HW et al., 20 patients with multiple fibroadenomas (average number of 3.3 masses in each patient) underwent surgical excision with round block technique; they reported low complications rate and good aesthetic results and concluded that the round block technique is a useful oncoplastic procedure for the management of multicentric fibroadenomas excised at the same time.

The effect of some new non-surgical techniques in treatment of multiple fibroadenomas has been investigated in some studies. In one report by Povoski, a female patient with 16 breast lesions (eight within each breast), presumed on ultrasound to be fibroadenomas, underwent ultrasound-guided vacuum-assisted breast biopsy of 14 of these 16 lesions during a total of 11 separate procedures performed during seven separate sessions over a ten and fifteen days. The patient discontinued taking OCP after the third treatment session. In addition, two of these 16 breast lesions were removed by surgical excision. A pathologic diagnosis of fibroadenoma and/or fibroadenomatosis was confirmed for all lesions. The rate of recurrence in this study was extremely low; with an interval follow-up ultrasound at least 8 months following treatment, showing the absence of any residual lesions or recurrences at the site of the 16 original breast lesions. The authors finally concluded that this approach is highly recommended in similar appropriately selected patients.

In another study by Li et al. the safety and efficacy of ultrasound (US)-guided percutaneous radiofrequency ablation (RFA) for multiple breast fibroadenomas was evaluated. Sixty-five patients with multiple breast fibroadenomas underwent US-guided percutaneous RFA under general anesthesia. Contrast-enhanced US (CEUS) was used immediately after operation to determine whether the tumor was ablated completely or not. The complete ablation rate (CAR) and the change of focal volume were evaluated by CEUS at the first and third months after operation. The number of lesions in all patients was reported as high as 256 nodules. Complete ablation was achieved for 251 nodules (98.04%) after the first month. The volume reduction rate was 39.06% and 75.99% at the first and the third month after operation, respectively, of which 45 nodules were completely absorbed (17.58%). The authors reported no complications such as skin burns, hemorrhage, hematoma, or nipple discharge during and after RFA. They concluded that regarding the advantages of high CAR, mild injury, rapid recovery, and cosmetic outcome, RFA has the potential to become the preferred method in the treatment of breast fibroadenoma, especially for multiple fibroadenomas.

In a study by Dhar et al. the effect of Centchroman (Ormeloxifene) -a novel non-steroidal selective antiestrogen- on the treatment of mastalgia and fibroadenoma was evaluated. Sixty patients with mastalgia or breast fibroadenoma (42 and 18 patients, respectively) who were 35 years old and younger were included in this study. They received centchroman 30 mg on alternate days for 3 months and were followed up for 6 months. Results of clinical examination, visual analog scale (VAS) for pain relief, and ultrasonography for the size of breast mass were recorded. Fibroadenoma size ranged from 1.5 to 5 cm, single or multiple in one or both breasts. In the fibroadenoma group, there was a mixed response, with complete disappearance in 40%, partial regression in 20%, and no response in the remaining 40%. There were very few side effects. The authors concluded that Centchroman is safe for the treatment of fibroadenoma.

In conclusion, management of multiple fibroadenomas can be a complicated clinical situation especially for recurrent lesion, and there is no universal consensus about the best management yet. More prospective investigation is needed to reach a standard management plan. It seems that consideration of history, physical examination, imaging and any previous pathologies of each patient and making decision individually, probably with help
of a MDT discussions, is the best option for now.

**Multidisciplinary team (MDT) Recommendation**

The patient history, imagings, and pathologic reports was presented to MDT. The imaging and pathology reports was reviewed by two other pathologists and radiologists. According to the expert opinion of the breast radiologists, all masses seen in the last ultrasound and MRI were looking to be benign and neither malignant features nor significant changes in the recent masses were found in the follow up MRI and ultrasonography exams. After review of all documents, MDT members recommended that close follow up could be continued by means of physical examination and ultrasound every 6 months. The necessity of further surgery would be determined according to the possible new findings or mass enlargements in the future.

**Ethical Consideration**

Medical ethics considerations were fully observed according to the protocol delivered by ethics committee of surgery department at Tehran University of Medical Sciences (TUMS).

**Conflict of Interest**

The authors declare that they have no conflict of interest.

**References**

7. Dhar A, Srivastava A. Role of centchroman in regression of mastalgia and fibroadenoma.
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ABSTRACT

Background: Breast surgery for women newly diagnosed with breast cancer is associated with poor postoperative quality of life (QOL). The aim of this study was to determine the effectiveness of a programmed discharge planning in improving overall QOL and its physical, emotional, social, and spiritual domains.

Method: In this quasi-experimental study, we evaluated the newly diagnosed breast cancer women undergoing breast-conserving therapy in two groups, an experimental group (n = 35) and a control group (n = 34). The experimental group received programmed discharge planning at the time of hospital admission until six weeks after discharge. The control group received routine hospital care. Participants completed the QOL questionnaires before and after the intervention. The data were analyzed using the independent-samples t test, Fisher exact test, and Mann-Whitney U.

Results: Before the intervention, there were no significant differences between the control and experimental groups in overall QOL or its physical, emotional, social, and spiritual domains. The data analysis after intervention showed significant improvement in QOL in the experimental group as compared with the control group. The changes in the scores of various domains of QOL were statistically significant (P < 0.01).

Conclusion: This study emphasizes that programmed discharge planning is useful for improving QOL after breast-conserving surgery. Our finding could be applied to breast cancer patients with radiation therapy or chemotherapy.
Discharge planning and QOL in BC

between continuity of care and patient satisfaction, health-related quality of life, reduced reanimation rate, urgent care use, and health care costs have been demonstrated previously.

Discharge planning is an essential component of continuity of care. Planning the discharge has demonstrated positive outcomes for hospitalized patients, including decreased early unplanned readmissions and costs for providing health services.

Recent data suggest that newly diagnosed breast cancer patients who are discharged early from the hospital need an effective follow-up plan. Although early discharge is safe and feasible, postoperative follow-up plan should be initiated as soon as possible after surgery. It also could help improve patients’ quality of life (QOL). Quality of life is now considered an important end point in the treatment of breast cancer patients. These patients might benefit from programmed discharge planning.

The aim of this study was to determine the effectiveness of a programmed discharge planning in improving overall QOL and its physical, emotional, social, and spiritual domains in breast cancer patients undergoing breast-conserving therapy (BCT). The findings of this investigation can provide the basic information required for the development of a systematic discharge planning service.

Methods

This quasi-experimental study was conducted at Imam Khomeini Hospital of Tehran University of Medical Sciences between December 2010 and May 2011.

All subjects of the study were identified at diagnosis through the results of the histopathological reports. Inclusion criteria were as follows: being a female aged ≥ 20 years, having a newly diagnosed stage I/II breast cancer, being an initial BCT candidate, having no history of mental or physical illness, being reachable via telephone, living in Tehran, have not participated in a patient education program, have not undergone immediate mastectomy after BCT.

Patients were contacted after being referred to the breast cancer clinic. The data were collected through interviews with patients and the review of their medical records. The patients were invited to participate in the study if they met the inclusion criteria.

Demographic and medical data for the participations were recorded. This questionnaire contained items such as age, marital status, employment, education (illiterate, able to read and write, primary, high school, college, and above), cancer stage, and axillary lymph node dissection (removed nodes).

Two measurements of quality of life were obtained, the first after the diagnosis and the second at the end of the study period.

The sample size for the study was calculated based on QOL end points from previous studies. Using a 95% confidence interval and 80% power, we calculated a sample size of n = 36 in each group. Patients were randomly assigned to 2 groups. The approval by the institutional ethics committee (approval No. S/624/130) and the informed consent from participants were obtained.

Quality of life was assessed using a 51-item questionnaire comprising physical (16 items), emotional (17 items), social (11 items), and spiritual (7 items) domains. The overall QOL was computed by calculating the scores of all the questions. We used the SF-36 questionnaire for physical domain, and the Quality of Life Instrument-Breast Cancer Patient Version(QOL-BC) for emotional, social and spiritual domains. The total score ranged between 0 and 204, with lower scores corresponding to better QOL.

The questionnaire content validity was determined by 10 experts in the breast cancer field, and its reliability was determined by calculating Cronbach’s alpha, which was 0.77. Internal consistency and validity were assessed by a pilot study on 15 patients with newly diagnosed breast cancer not included in the study.

The participants in the experimental group completed the patient care needs assessment checklist, telephone consultation form, and home visit form during the intervention. These forms were developed by researchers based on the information in the literature and after interviewing 5 experts in the area of breast cancer.

The patients in the discharge-planning program were provided with preoperative education, postoperative education, two home visits, and telephone consultations. Individual patients’ needs were considered in all consultations.

A nursing instruction and counseling were provided in every step based on the patients’ individual needs. After patients were discharged, we provided appropriate support and instruction for each patient during two home visits. On each visit, the specific patient needs were identified using the checklist, and then the first author gave instructions on such matters as changing the dressing, managing drains, exercise interventions for upper limb, etc. The first visit was made within the first week after discharge, the second in the sixth week after discharge. Telephone consultations were performed on six occasions with 1-week intervals to address potential problems at times other than during home visits.

Patients in the control group received the hospital routine discharge plan. Two interviews were done in this group: the first one was held upon their admissions to collect data about demographic and medical characteristics, and the second took place 6 weeks after discharge to complete the QOL questionnaire.

Table 1. Baseline characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Experimental group (n = 36)</th>
<th>Control group (n = 36)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>3 (8.6)</td>
<td>6 (17.6)</td>
<td>0.39*</td>
</tr>
<tr>
<td>35-55</td>
<td>26 (74.3)</td>
<td>26 (76.5)</td>
<td></td>
</tr>
<tr>
<td>&gt;55</td>
<td>6 (17.1)</td>
<td>2 (5.9)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>46.2 (8.7)</td>
<td>44.3 (9.2)</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>27 (77.1)</td>
<td>29 (85.3)</td>
<td>0.71**</td>
</tr>
<tr>
<td>Single</td>
<td>3 (8.6)</td>
<td>3 (8.8)</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>4 (11.4)</td>
<td>1 (2.9)</td>
<td></td>
</tr>
<tr>
<td>Widowed</td>
<td>1 (2.9)</td>
<td>1 (2.9)</td>
<td></td>
</tr>
<tr>
<td>Employment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>9 (25.7)</td>
<td>10 (29.4)</td>
<td>0.73***</td>
</tr>
<tr>
<td>Housewife</td>
<td>26 (74.3)</td>
<td>24 (69.6)</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>6 (17.1)</td>
<td>4 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Able to read and write</td>
<td>4 (11.4)</td>
<td>4 (11.4)</td>
<td></td>
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<tr>
<td>Primary</td>
<td>16 (45.7)</td>
<td>14 (41.2)</td>
<td>0.46****</td>
</tr>
<tr>
<td>High school</td>
<td>6 (17.1)</td>
<td>10 (29.4)</td>
<td></td>
</tr>
<tr>
<td>Collage and above</td>
<td>3 (8.6)</td>
<td>2 (5.9)</td>
<td></td>
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<tr>
<td>Cancer stage</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Stage I</td>
<td>14 (40.0)</td>
<td>13 (38.2)</td>
<td>0.88**</td>
</tr>
<tr>
<td>stage II</td>
<td>21 (60.0)</td>
<td>21 (61.8)</td>
<td></td>
</tr>
<tr>
<td>Axillary lymph node dissection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>27 (77.1)</td>
<td>24 (70.6)</td>
<td>0.53***</td>
</tr>
<tr>
<td>No</td>
<td>8 (22.9)</td>
<td>10 (29.4)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

*Independent t test, ** Fisher exact test, *** Chi-square test, **** Mann-Whitney U test

Data analysis
The Statistical Package for the Social Sciences was used for data analysis. Descriptive statistics were used to summarize the demographic and medical characteristics. The Independent t test was used to compare the mean scores on QOL domains between the groups. A significance level of $\alpha = 0.05$ was used in statistical analyses.

Results
A total of 72 women with breast cancer met the inclusion criteria for the study, with 36 in each group. One woman in the experimental group and 2 in the control group withdrew from the study because of mastectomy and moving to another city, respectively.

Table 1 presents descriptive data. Most participants aged 35-55 years. The average age of each group was $44.3 \pm 9.2$ (control group, mean $\pm$ SD) and $46.2 \pm 8.7$ (experimental group, mean $\pm$ SD).

Twenty-nine women (85.3%) in the control group and 27 (77.1%) in the experimental group were married. Most participants had secondary education in both groups (41.2% in the control group, 45.7% in the experimental group). Twenty-four patients (70.6%) in the control group and 26 (74.3%) in the experimental group were unemployed.

Many of the patients in this study had stage II breast cancer (61.8% in the control group, 60% in the experimental group).

Most participants also had axillary lymph node management (65.7% in the control group, 62.8% of the experimental group). There were no significant differences between the groups in age at diagnosis, marital status, employment, education, cancer stage, and axillary lymph node dissection.

Women in the experimental group were followed up for six weeks according to their discharge plan. Table 2 shows the comparison of before- and after-intervention QOL scores between the groups.

There was no significant difference in physical, emotional, social, spiritual domain scores or in overall QOL between the groups before the intervention. All QOL domains significantly improved in the experimental group after the intervention compared with the control group (overall QOL: 101.48 vs 46.6; physical domain: 31.8 vs 12.8; emotional domain: 38.1 vs 17.8; social domain: 24.3 vs 11.9; and spiritual domain: 7.1 vs 4.1; $P < 0.01$ for all variables).

Discussion
The global burden of breast cancer continues to increase largely. This issue is a threat to public health throughout the world. However, breast cancer and its treatment can also result in a wide range of physical and psychological problems that exert a negative impact upon patients’ QOL.

In general, length of stay in hospital after breast cancer surgery has decreased during the past decades.9
Appropriate follow-up is necessary after early discharge from hospital following surgery for breast cancer. Nursing care planning for patients with breast cancer undergoing surgery can continue after being discharged from hospital. One of the main objectives of continuity of care is to keep the relationships between patients and health care provider.29 Early discharge from hospital in breast cancer patients needs special follow-up. This may help the patient to complete her long treatment process.

In the current study, we examined the effect of programmed discharge planning including telephone consultation and home visits after breast-conserving surgery on QOL. Before the intervention, both groups had almost the same levels of physical, emotional, social, and spiritual well-being. However, mean scores of all domains of QOL improved significantly in the experimental group 6 weeks after discharge from the hospital, indicating the effectiveness of discharge planning and continuity of care in promoting QOL of women after breast cancer surgery.

No significant changes were found in QOL of the patients in the control group at the end of the study. A reason for this could be that the patients in the control group were discharged after BCT without receiving special nursing cares except for some brochures. As a result, the QOL scores in the control group did not improve by the end of the study.

In a study of patients with coronary artery bypass graft, QOL subscales improved when the patients were provided with postoperative training and counseling sessions over 6 weeks after discharge from the hospital.21 In other study conducted in Egypt by Mounir et al., a discharge-planning program had a very significant effect on QOL and knowledge of geriatric patients with acute myocardial infarction.22

Our finding is especially important for directing the attention toward initiating discharge planning in hospitals. An important element in this study was that the intervention was developed to address the individual needs of the participants. In contrast to the usual patient discharge plan, which was the same for all breast cancer patients after surgery, this intervention focused on the individual’s needs. We found that the focus of the discharge plan on physical, emotional, social, and spiritual well-being of individual patients was an essential factor contributing to success in this population.

In conclusion, effective discharge planning can play an essential role in improving QOL in newly diagnosed breast cancer patients after discharge from the hospital. Supporting patients during hospitalization and after discharge has a positive effect on postoperative patient QOL.

Addressing specific patient needs through an individualized discharge plan may be particularly cost-effective as it increases the self-care knowledge and patient satisfaction and reduces hospital readmission rate and complications after discharge.23,24

Discharge planning is important for clinicians and women with breast cancer to optimize long-term QOL.

**Conflict of Interest**
The authors declare that they have no conflict of interest.

**References**


ARTICLE INFO

Methods: Two hundred sixty patients receiving different types of breast cancer surgery for therapeutic and reconstructive purposes were enrolled in this study. They were divided into three groups according to the anesthesia risk assessment. Group 1 consisted of low-risk patients (ASA I) who received small surgeries such as lumpectomy. Patients with intermediate risk of anesthesia (ASA II) or those who underwent breast cancer and axillary surgery with overnight admission (ASA I or II) were considered as group 2. Group 3 comprised the patients with higher risk for anesthesia (ASA class III) regardless of the surgery type or those in any ASA class who were about to undergo advanced and prolonged surgeries such as breast reconstruction with free or pedicle flaps.

Results: Two hundred sixty-eight surgical interventions were done in 260 patients. There were 106, 107, and 47 patients in groups 1, 2, and 3, respectively. In group 1, five patients out of 106 were admitted in the hospital for 24 hours after surgery and the remaining 101 patients were discharged from the hospital in a few hours after the operation when they were fully conscious and could tolerate the diet completely. All 107 patients in group 2 were admitted in the hospital for a few days after the operation, though the vast majority of them (98 patients) discharged from the hospital the day after surgery. In the last group, 6 out of 47 patients showed the signs of surgical complications such as partial flap ischemia in the postoperative period, mostly after TRAM or DIEP flap breast reconstruction surgery.

Conclusion: The findings of this study support the idea that breast surgeries can be done in an ambulatory situation with no considerable risk. In contrast, all medical and anesthetic considerations should be taken into account in more complex surgeries, especially when they are applied in high-risk patients.

Background: There are various factors affecting the effectiveness of the treatment of breast cancer patients. Although the disease pathology, along with surgery and other therapeutic modalities, plays the principal role in patient outcomes, anesthesia still plays an important role in the success of treatment. This study was designed to show the effects of anesthetic plans on risk classification and assessment in breast cancer surgeries.

Methods: Two hundred sixty patients receiving different types of breast cancer surgery for therapeutic and reconstructive purposes were enrolled in this study. They were divided into three groups according to the anesthesia risk assessment. Group 1 consisted of low-risk patients (ASA I) who received small surgeries such as lumpectomy. Patients with intermediate risk of anesthesia (ASA II) or those who underwent breast cancer and axillary surgery with overnight admission (ASA I or II) were considered as group 2. Group 3 comprised the patients with higher risk for anesthesia (ASA class III) regardless of the surgery type or those in any ASA class who were about to undergo advanced and prolonged surgeries such as breast reconstruction with free or pedicle flaps.

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Conclusion: The findings of this study support the idea that breast surgeries can be done in an ambulatory situation with no considerable risk. In contrast, all medical and anesthetic considerations should be taken into account in more complex surgeries, especially when they are applied in high-risk patients.
especially those affecting the respiratory, cardiac, or immune system. Also, the type of disease is important in anesthesia risk of the patients. Surgery for the treatment of cancer is a good example demonstrating the importance of the type of disease in perioperative care of the patients. Cancer patients may have a higher risk in the perioperative period compared with other patients. As an example, cancer patients have higher-than-normal numbers of platelets and clotting factors in their blood. On the other hand, some treatment modalities applied in the management of breast cancer patients may increase the risk of postoperative complications such as developing a blood clot. Chemotherapy, with or without targeted therapy, and tamoxifen can increase the risk of thromboembolic events.\cite{3} Research indicates that there is an association between thrombosis and malignancy.\cite{3}

Reoperation can independently increase the risk of anesthesia. One of the most common reasons for reoperation in breast cancer patients is thrombotic events in autologous breast reconstruction. The pattern of early thrombosis is variable and is governed by the complex interplay between tumor factors, cancer treatment medications, and patient factors. The trauma of major surgery and extensive surgical dissections in the trunk may itself play an important role in increasing thrombotic events after autologous breast reconstruction.\cite{4}

In the modern era, with the intensive preoperative monitoring of the patients, the risk of anesthesia and preoperative complications has decreased considerably. Early postoperative mobilization and the earlier discharge of the patient from the hospital are important in the prevention of thromboembolic events and the complications.\cite{1} Among all cancers that need surgery, breast cancer has the most varied extent of the surgery—from small, short-duration surgeries to extensive, long operations affecting a considerable area of the trunk with microscopic vascular anastomoses. This study was designed to show the anesthesia complications in three study groups.

### Methods
Two hundred sixty patients receiving different types of breast cancer surgery for therapeutics and reconstructive purposes were enrolled in this study from April 2017 to July 2018 in Imam Khomeini referral hospital. Patients who were candidates for surgery were referred to preoperative anesthesia clinic of the hospital as part of the surgery preparation process. They were assessed in the anesthesiology clinic and the anesthesia risk assessment sheets were filled up for every individual patient.

The patients were divided into three groups according to the protocol of the study. The study protocol was approved by the ethics committee of the Surgery Department of the university. Group 1 consisted of the patients who were planned for small breast surgeries such as lumpectomy. The patients in this group had low anesthesia risks (ASA I). Patients with intermediate risk of anesthesia (ASA II) and those who underwent breast cancer and axillary surgery, except for sentinel LN biopsy, and needed overnight admission (whether ASA I or II) were considered as group 2. Group 3 was comprised of patients who had a high risk of anesthesia (ASA III) or were going to receive advanced and prolonged surgeries such as autologous breast reconstruction regardless ASA classification (I, II, or III).

Anesthesia was induced by intravenous infusion of propofol 1% and remifentanil (total intravenous anesthesia) in 70 cases, and 95 patients were anesthetized with inhaled anesthetics sevoflurane and isoflurane with intermittent inhalation of fentanyl and sufentanil. In all the cases, muscle relaxation was induced by atracurium besilate or cisatracurium. Optimal ventilation and oxygenation along with the monitoring of blood gases, acid-base balance, and metabolic balance, i.e., blood sugar and osmolality, temperature monitoring, and avoidance of hypothermia in both groups were monitored. Depth of anesthesia was controlled using a bispectral index (BIS) monitoring system. Because of the particular importance of proper perfusion of the flaps and their vascular anastomosis, inotropic drugs and crystalloid fluids were used in some cases as required. Postoperative care including hemodynamic monitoring, urinary output record, and postoperative pain management with opiate and non-opiate drugs were done.

### Results
Two hundred sixty-eight surgical interventions were done in 260 patients. Age distribution of the patients is presented in Table 1. The most frequent operation was breast-conserving therapy with axillary staging. Table 2 shows the distribution of patients in different study groups and the different types of surgeries.

There were 106, 107, and 47 patients in groups 1, 2, and 3 respectively. In group 1, five patients out of 106 stayed in the hospital for the next 24 hours after surgery, and the remaining 101 patients were discharged from the hospital within a few hours after the operation when they were fully conscious and could tolerate the PO diet completely. All the 107 patients in group 2 were admitted to the hospital for a few days after the operation, though the vast majority of them (98 patients) were discharged from the hospital the day after surgery. Three patients were hospitalized more than 1 day because of insurance and administrative issues, and the remaining 6 patients stayed at the hospital for more prolonged monitoring and analgesic medications. There were no anesthesia complications in group 1 or 2.

In the last group, 6 out of 47 patients showed signs...
of surgical complications such as partial flap ischemia in the postoperative period mostly after TRAM or DIEP flap breast reconstruction surgery. Although 4 patients recovered with conservative management, two patients were reoperated and discharged after 8 to 10 days of intensive medical and anesthesia care. There was one mortality in the third group due to comorbidity in a patient who underwent mastectomy without axillary lymph node dissection. She suffered from recurrent ulcerative breast cancer lesions that were not responsive to systemic chemotherapy. She has been diagnosed with breast cancer 4 years before and treated with surgery followed by chemotherapy, radiotherapy, and hormonal therapy in 2013. Despite the use of appropriate anticoagulation in the preoperative period, the patient showed the signs of diffuse pulmonary thromboembolism, confirmed with CT angiography, which resulted in cardiac arrest. The patient was not responsive to cardiopulmonary resuscitation. Another patient had progressive respiratory distress and tachycardia on the first day after surgery. She was rechecked for flap perfusion and vascular grafts circulation, and perfusion scan was done in order to rule out diffuse emboli. The result was diffuse thromboembolism and arterial gas disturbance. The patient immediately received respiratory support treatment using anticoagulants so that her life, as well as the flap, would be saved. However, the revascularization for DIEP flap failed, and the second operation ended up with flap resection.

the other cases had skin color changes and coldness due to hypoperfusion but were restored with conservative treatment.

Discussion
Preoperative evaluation and risk assessment are essential to anesthesia management. In this study, we categorized the patients who were candidates for any kind of breast surgery into three groups. While there was no complication in group 1 and 2, group 3 had a different outcome because the patients in this group were at a greater risk of morbidity and mortality compared with group 1 and 2. The patients were categorized in this group according to one or more of the following conditions: 1. suffering from an underlying disease which made comorbidity; 2. undergoing more complex and longer surgeries, which cause higher physiologic stress during the surgery; and 3. receiving chemotherapy before treatment. One mortality in the study group occurred in a patient with severe morbidity, although all other morbidities were in the subgroup of TRAM or DIEP flap reconstructions. For example, DIEP flap, which is used for breast reconstruction in a selected number of patients, is a prolonged procedure involving microvascular anastomoses. Furthermore, the extent of the dissection in this operation causes hypothermia in the patient. These patients are at greater risk, especially when the blood flow of the flap is questionable. The ischemia in the flap induces

Table 1. Distribution of breast cancer patients by age

<table>
<thead>
<tr>
<th>age</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>12 (4.8%)</td>
</tr>
<tr>
<td>20-30</td>
<td>27 (10.8%)</td>
</tr>
<tr>
<td>31-40</td>
<td>46 (18.5%)</td>
</tr>
<tr>
<td>41-50</td>
<td>81 (32.6%)</td>
</tr>
<tr>
<td>51-60</td>
<td>51 (20.5%)</td>
</tr>
<tr>
<td>61-70</td>
<td>19 (7.6%)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>12 (4.8%)</td>
</tr>
<tr>
<td>total</td>
<td>248 (100.0%)</td>
</tr>
</tbody>
</table>

Table 2. Surgery type and study classification

<table>
<thead>
<tr>
<th>Type of Surgery</th>
<th>Number of interventions (N = 268)</th>
<th>Study group 1* (N = 106)</th>
<th>Study group 2* (N = 107)</th>
<th>Study group 3* (N = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumpectomy</td>
<td>66</td>
<td>61</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>BCS** ± OBS*** ± ASLNB</td>
<td>50</td>
<td>15</td>
<td>29</td>
<td>6</td>
</tr>
<tr>
<td>BCS ± OBS + ALND</td>
<td>26</td>
<td>0</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td>Mastectomy ± ALND/SLNB</td>
<td>40</td>
<td>0</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>Nipple-areola complex reconstruction</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Revision of the previous breast reconstruction</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Mastectomy and early reconstruction with implant/expander</td>
<td>28</td>
<td>0</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>Autologous breast reconstruction ± mastectomy</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Bilateral Mastectomy ± implant reconstruction</td>
<td>9</td>
<td>0</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Other</td>
<td>17</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Number of patients
** Breast-conserving surgery
*** Oncoplastic breast surgery
cytotoxic products in the ischemic tissue, which then enter the systemic circulation and negatively affect the cardiovascular function of the patients who are already under considerable physiologic stress after a long surgery. On the other hand, the tissue perfusion is crucial in these patients in the postoperative period. The mean arterial pressure should be maintained within $\pm 20$ to $\pm 30$ mm Hg of the baseline. Sufficient fluid therapy, electrolyte balance, normothermia, and correction of anemia with blood transfusion are the basics to maintain the physiologic status of the patient in a stable condition and to establish flap perfusion.

In the recent decade, the indications and use of preoperative chemotherapy have been developed considerably. Because of vascular toxicity, chemotherapy leads to endothelial dysfunction, with loss of vasodilatation effects and suppressed anti-inflammatory and vascular reparative functions. Those changes exacerbate ischemia, and hypothermia worsens the situation of the patients.\textsuperscript{10}

The extent of surgical dissection also plays an important role in increasing postoperative complications and morbidity. Dissection of the extensive surface areas of the trunk, including chest, abdomen, and -in some situations- thighs, would increase insensible loss and lead to hypothermia during surgery as well as in the postoperative period. These factors increase the risk of thrombosis in vascular grafts and diffuse thromboembolism in some cases. Sufficient oxygenation, perfect ventilation with acid-base balance during surgery, and monitoring the electrolytes are necessary.\textsuperscript{11-14} We had two thromboembolic events in our series, and one of them led to mortality. Prophylactic anticoagulant therapy should be considered in the surgical and anesthetic plan.\textsuperscript{15-16} There were one morbidity and one mortality in this study. These complications happened despite risk assessment and appropriate preparation of the patients. It should be always taken into consideration that these kinds of complications can be life-threatening. We recommend considering ICU care for the patients at higher risk of complications, especially when the operation is long and complex with extensive dissection.

There were no complications in groups 1 and 2. Taking enough care of the hemodynamic function and tight monitoring of ideal oxygenation helped us to avoid further complications. Six patients remained hospitalized for more than one day in group 2 because of severe pain in the site of operation. It seems that enough analgesic medication can also prevent the hospitalization of patients. Opiate and non-opiate medications should be advised meticulously in the early postoperative period.\textsuperscript{17-19}

It seems that most breast surgeries can be done in an ambulatory situation with no considerable risk. In contrast, all medical and anesthetic considerations should be taken into account in more complex surgeries, especially when they are performed on high-risk patients.

**Conflict of Interest**

The authors have none to disclose.

**References**

Article Info

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Abstract

Background: Breast cancer is considered a chronic disease owing to the increases in survival rate. Thus, better body image and patient satisfaction with the surgery have become more important factors to be considered when choosing the surgical approach. The aim of this study was to compare body image and patient satisfaction following three different approaches.

Methods: We evaluated 183 consecutive patients who had undergone three different surgeries including breast-conserving surgery (BCS), mastectomy, or mastectomy followed by reconstruction (M-R). Body image was evaluated using the BICI questionnaire, and patient satisfaction was rated using a multiple-choice question and a scale ranging from 1 to 10.

Results: A significantly better body image was observed in the M-R and BCS groups compared with mastectomy (P = 0.02). In body image subscale analysis, social functioning scores were higher in the M-R and BCS groups than in the mastectomy group (P = 0.01), but no differences were obtained between surgery groups in appearance dissatisfaction subscale. Patients were more satisfied with BCS than the other two surgeries (P = 0.008).

Conclusion: Based on the results of this study, it could be proposed that both oncoplastic BCS and implant reconstruction could provide patients with acceptable body image, while BCS could bring about better satisfaction with the surgery. Reconstruction may be an alternative for the patients to improve body image and satisfaction when BCS is not applicable.

Introduction

Breast cancer is diagnosed at a more advanced stage in developing countries, and the incidence is also increasing. More and more patients will need breast surgery in the near future in low- and middle-income countries including I.R. Iran. Nowadays, the most frequent surgery for breast cancer patients, especially at early stages, is breast-conserving surgery (BCS). Although surgical approach toward breast cancer favors more conserving procedures, mastectomy is sometimes inevitable and is performed in 20%-30% of cases. It was shown that survival rates were not different between mastectomy and other less invasive approaches. In spite of these findings, some studies demonstrated increasing rates of mastectomy, mostly chosen by the patients. This may be due to the fear of recurrence or perception of...
improved survival. Breast reconstruction, a proper alternative for patients who are candidates for mastectomy, was first described in the 1970s, and multiple surgical techniques are now available, making interactive decision making for reconstruction essential for patients and doctors.16

Why perform breast reconstruction? Improved appearance satisfaction, positive effects on body image, better sexual function, and better long-term health may be among the reasons to opt for breast reconstruction. With improved breast cancer survival rates, breast cancer is classified as a chronic disease. Thus, other factors such as quality of life, satisfaction, body image, and sexuality become important besides the treatment of the disease itself.31,32

Body image, which is defined as a multidimensional construct that extends beyond the evaluation of an individual’s appearance and entails an individual’s perception of his or her body, may be altered postoperatively and affect patients’ psychosocial functioning.34

Patient satisfaction is influenced by different factors such as overall outcome and specific breast features (size, shape, and symmetry).7 Also, factors other than the procedure itself, including personality traits, appearance investment, pain, scars, missing a nipple, and recovery time, may cause dissatisfaction.5

Breast reconstruction is thought to be a coping strategy to prevail over body image changes after mastectomy. Whether patient satisfaction is higher with reconstruction or breast conservation is still a question. The goal of this study was to compare patient satisfaction and body image of patients undergoing mastectomy, BCS, or mastectomy followed by reconstruction. To our knowledge, this is the first study to compare satisfaction and body image among these techniques in I.R. Iran.

Methods

Subjects

A series of 183 consecutive patients who had undergone breast cancer surgery between August 2016 and September 2017 at Breast Cancer Research Center (BCRC; Motamed Cancer Institute, Tehran, Iran) or Imam Khomeini Hospital Complex (IKHC; Tehran, Iran) were enrolled. The decision to choose the type of surgery had been taken through an interactive process involving a multidisciplinary team of physicians and the patients, with the final decision remaining with the patient. Patients were eligible to enter the study if they had undergone mastectomy, oncoplastic BCS, or mastectomy followed by implant reconstruction (M-R, either immediate or delayed) and had finished the treatment at least 3 months before enrollment in the study. The end of the treatment was marked by the last session of radiotherapy if applicable. Otherwise, the time of completing the treatment and the start of follow-up was considered the treatment completion time. Written informed consent was obtained from each patient, and the data were collected anonymously. This was an observational study and researchers made no controlled or randomized intervention. Patients completed the Persian version of Body Image Concern Inventory (BICI) during a follow-up visit. Demographic and clinical data were extracted from patients’ records in BCRC and IKHC. The local ethics committee approved all the study protocols (IR.ACECR.IBCRC.REC.1394.7).

Questionnaire

The BICI questionnaire is a 19-item multiple-choice, self-report instrument to evaluate the patient’s body image. Patients were asked to rate each item about how often they had the described behavior or feeling on a 5-point Likert scale from 1 (never) to 5 (always). The total score ranged from 19 to 95, with higher scores corresponding to greater concern regarding body image (12 of 86). During answering the questionnaire, the patient was supervised by a research assistant to provide any additional information and make sure that no item was left unrated or rated twice. Six patients were illiterate, so a research assistant read the questions and marked the answers for them. This questionnaire consists of two subscales, namely, appearance dissatisfaction (items 1, 3, 5, 8, 9, 14-19) and social functioning interference (items 2, 4, 6, 7, 10-13). The mean Spearman-Brown coefficient was calculated 0.62 and Cronbach’s alpha was 0.93. Sajadinejad et al. reported an overall Cronbach’s alpha coefficient of 0.84, and alpha coefficients of 0.84 and 0.74 for appearance dissatisfaction and social functioning interference, respectively, in the Persian version of BICI.35

Patient Satisfaction With the surgery

We used a multiple-choice question to evaluate patients’ satisfaction with the surgery: how satisfied are you with the cosmetic result of your operation? The patients could choose from among very satisfied, moderately satisfied, moderately dissatisfied, slightly satisfied, and not satisfied at all. This was used in a similar study.39 We also asked the participants to rate their satisfaction with the surgery on a scale of 0 (least satisfaction) to 10 (most satisfaction).

Data Analysis

Distributions of body image and satisfaction score were not normal based on the Kolmogorov-Smirnov test. Quantitative data were presented as mean and standard deviation. The one-way ANOVA test was used to show differences in scale variable between groups. The Fisher exact test was used to represent differences in categorical variables between the three surgery groups. Statistical comparisons for body image score and satisfaction (scale score) were made using Kruskal-Wallis one-way
ANOVA, and differences between each pair of groups were shown using the Dunn’s post hoc test. We then used a multiple linear regression model to predict satisfaction score (scale score) and body image score based on surgery groups and adjusted the model for age, time after treatment, education, and socioeconomic status. Breast-conserving surgery was assumed as the reference group. We used binary logistic regression to predict satisfaction (categorical score) based on the type of surgery (“very satisfied” and “moderately” were grouped as satisfied and “slightly” and “not satisfied at all” were grouped as unsatisfied). Tests were two-tailed and P values ≤ 0.05 was considered significant. All analyses were performed on SPSS v.16.

Results
We enrolled 183 consecutive females with breast cancer who had undergone mastectomy, breast-conserving surgery (BCS), or mastectomy followed by reconstruction (M-R) and were seen post-operative as their routine follow-up. Seventy patients (38.3%) had undergone mastectomy, 70 (38.3%) BCS, and 43 (23.5%) reconstruction. The mean (SD) age of the participants at the time of surgery was 44.31 ± 8.12 years (range: 25-62 y). The mastectomy group was significantly older than the M-R group (46.5 vs 41.5 y, P = 0.005). The mean follow-up was 43.52 (Range: 3-288) months. The characteristics of the three groups are presented in Table 1.

Table 1. Comparison of patients' characteristic between surgery groups

<table>
<thead>
<tr>
<th></th>
<th>BCS (%)</th>
<th>Mastectomy (%)</th>
<th>M-R (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>62 (88.6)</td>
<td>56 (83.6)</td>
<td>37 (88.1)</td>
<td>0.669</td>
</tr>
<tr>
<td>Single</td>
<td>8 (11.4)</td>
<td>11 (16.4)</td>
<td>5 (11.9)</td>
<td></td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>University ≤ Diploma</td>
<td>21 (30.0)</td>
<td>15 (23.1)</td>
<td>16 (41.0)</td>
<td>0.158</td>
</tr>
<tr>
<td>University ≥ Diploma</td>
<td>49 (70.0)</td>
<td>50 (76.9)</td>
<td>23 (59.0)</td>
<td></td>
</tr>
<tr>
<td>Economic status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>6 (9.5)</td>
<td>8 (11.8)</td>
<td>21 (48.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Moderate</td>
<td>57 (90.5)</td>
<td>60 (88.2)</td>
<td>22 (51.2)</td>
<td></td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clerk</td>
<td>17 (24.3)</td>
<td>26 (37.1)</td>
<td>11 (25.6)</td>
<td>0.218</td>
</tr>
<tr>
<td>Housewife</td>
<td>53 (75.7)</td>
<td>44 (62.9)</td>
<td>32 (74.4)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>43.8 (7.7)</td>
<td>46.5 (8.5)</td>
<td>41.5 (7.2)</td>
<td>0.006</td>
</tr>
<tr>
<td>Time after treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>41.3 (39.0)</td>
<td>50.2 (44.3)</td>
<td>35.3 (29.3)</td>
<td>0.146</td>
</tr>
</tbody>
</table>

Abbreviations: a, breast-conserving surgery; b, mastectomy followed by reconstruction

Table 2. Comparison of body image score and satisfaction in surgery groups

<table>
<thead>
<tr>
<th></th>
<th>BCS (%)</th>
<th>Mastectomy (%)</th>
<th>M-R (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body image</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (1, 3 Q)</td>
<td>32 (23-41.25)</td>
<td>36.5 (29-45.25)</td>
<td>30 (26-42)</td>
<td>0.027</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>33.80 (11.93)</td>
<td>38.57 (12.78)</td>
<td>33.30 (11.25)</td>
<td></td>
</tr>
<tr>
<td>Mean rank</td>
<td>84.66</td>
<td>105.30</td>
<td>82.30</td>
<td></td>
</tr>
<tr>
<td>Social function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (1, 3 Q)</td>
<td>16 (11-20.25)</td>
<td>17 (14-22)</td>
<td>15 (11-19)</td>
<td>0.013</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>15.87 (6.09)</td>
<td>18.21 (5.71)</td>
<td>15.30 (5.54)</td>
<td></td>
</tr>
<tr>
<td>Mean rank</td>
<td>84.36</td>
<td>106.51</td>
<td>80.81</td>
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<tr>
<td>Appearance dissatisfaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (1, 3 Q)</td>
<td>17 (11-7.52)</td>
<td>18 (14-25)</td>
<td>17 (13-23)</td>
<td>0.098</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>17.93 (6.82)</td>
<td>20.36 (7.89)</td>
<td>18.00 (6.44)</td>
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<tr>
<td>Mean rank</td>
<td>84.41</td>
<td>102.61</td>
<td>87.68</td>
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<tr>
<td>Satisfaction with surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (1, 3 Q)</td>
<td>10 (10-10)</td>
<td>10 (8.75-10)</td>
<td>10 (7.5-10)</td>
<td>0.008</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>9.49 (1.34)</td>
<td>9.08 (1.65)</td>
<td>8.89 (1.63)</td>
<td></td>
</tr>
<tr>
<td>Mean rank</td>
<td>104.19</td>
<td>87.38</td>
<td>79.69</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: a, breast-conserving surgery; b, mastectomy followed by reconstruction
social functioning score. The mean total BI score of patients was 33.61 (18-70). The mean body image score in the BCS was 33.80 (11.93), in the mastectomy group was 38.57 (12.78), and in the M-R group was 33.30 (11.25). A significant difference was seen among the three groups in BI (P = 0.027), with the lowest BI score being seen in the mastectomy group. The difference in social functioning was also significant, with the mastectomy group scoring lower than the other two groups (P = 0.013). No statistical difference, however, was observed in appearance dissatisfaction among the three groups (P = 0.098). The information regarding body image scores is shown in Table 2. Pairwise differences are presented in Figures 2 and 3.

![Figure 1. Comparison of body image scores between surgery groups](image1.png)

![Figure 2. Comparison of social function score between surgery groups](image2.png)

![Figure 3. Comparison of satisfaction score between surgery groups](image3.png)
Patient satisfaction after BCS surgery

We then performed a multivariable linear regression and adjusted the regression for age, marital status, education, employment, and economic status. Breast-conserving surgery was assumed as the reference group. The results are shown in Table 3. The mastectomy group had significantly lower scores on body image and social function. However, scores on BI and its subscales showed no significant difference between the BCS and M-R groups.

As a supplementary analysis, we divided the patients into two groups; the first was patients who were surveyed within 6 months after the last treatment, and the second included those who were surveyed after 6 months. There was no significant difference in the mean body image score between the two groups (P = 0.30).

Patient satisfaction

We evaluated patient satisfaction with the surgery in two ways. First on a scale of 1-10, and second using a multiple-choice question. The satisfaction score was significantly different between the three groups (P = 0.008). That was the highest in the BCS group (9.49 ± 1.34) and the lowest in the M-R group (8.89 ± 1.63). There was no significant difference in satisfaction score between the mastectomy and M-R groups (P = 0.36). However, the difference was significant between the BCS and the other two groups (P = 0.024 for the mastectomy and P = 0.004 for the M-R group). These results are illustrated in Figure 3. In the multiple-choice question on satisfaction, there was a significant difference between the groups (P = 0.049). The BCS group had the highest number of “very satisfied” answers (91.4%), and in pairwise comparisons, there was a significant difference just between the BCS and M-R groups (91.4% vs 74.4%, P = 0.015). However, when considering “very satisfied” and “moderately” answers as satisfied and the other ones as dissatisfied, there was no significant difference in the percentage of satisfied patients between the surgery groups (BCS: 97.2%; mastectomy: 94.2%, and M-R: 95.3%). In the binary logistic model, none of the surgical techniques could increase the risk of dissatisfaction (BCS assumed reference; OR: 0.413 and 0.620; 95% CI: 0.08-2.74 and 0.08-4.46 for Mastectomy and M-R, respectively). In the supplementary analysis, there was no statistical difference in satisfaction score between patients who were surveyed within 6 months after the last treatment and those surveyed after 6 months (P = 0.32).

Discussion

The results of this study showed that among the three frequent surgeries for breast cancer, mastectomy was associated with the highest dysmorphic appearance concern, while BCS and mastectomy followed by reconstruction led to the same BI score. Mastectomy patients scored significantly lower on the social functioning subscale of the BICI compared with the other two groups; however, appearance dissatisfaction was not significantly different among the three groups. At the same time, BCS was associated with a greater satisfaction with surgery in comparison with mastectomy or M-R.

Breast cancer surgery approach has changed during recent years. As the number of breast cancer survivors increases, we should consider breast cancer a chronic disease. Chronic disease may potentially worsen the overall health of patients by limiting their functional status, productivity, and quality of life. Thus, factors besides treatment options will bear importance for these patients, including body image and satisfaction after surgery.

The findings of our study indicated that the mastectomy group had a lower body image score compared with the BCS or M-R group. The latter groups were identical regarding BI. This finding is contrary to that of Fang et al., who found that women receiving BCS had a significantly better overall body image. They declared that losing a breast could cause loss of body integrity. Thus, patients with reconstruction perceive themselves as deficient despite satisfaction with shape and appearance. Moreover, it was shown that reconstruction would contribute to a better body image than mastectomy alone, which is consistent with our finding. Another study, by Al-Ghazal et al., showed a significantly better body image and less psychosocial problems after BCS compared with breast reconstruction. The

| Table 3. Information regarding linear regression for predicting body image and satisfaction |
|-----------------|-----------------|-----------------|-----------------|
| Type of surgery | Body image      | Appearance       | Social function |
|                 |                 | dissatisfaction  |                 |
| Mastectomy      |                 |                 |                 |
| B               | 4.684           | 2.425           | 2.259           |
| B̂              | 0.189           | 0.164           | 0.189           |
| Significance    | 0.036           | 0.069           | 0.034           |
| M-R             |                 |                 |                 |
| B               | -1.306          | -0.502          | -1.303          |
| B̂              | -0.064          | -0.030          | -0.096          |
| Significance    | 0.514           | 0.762           | 0.324           |
| R²              | 0.045           | 0.029           | 0.58            |

Breast-conserving surgery is assumed as the reference group. The regression models are adjusted for age, marital status, education, economic status, and employment status. M-R, mastectomy followed by reconstruction.
rational behind this finding was that BCS is less extensive and disfiguring, and as the most important factor in determining the type of treatment is the concern about adverse effects such as disfigurement. BCS brought better body image.

Previous studies comparing reconstruction and mastectomy have reported different results. Some studies found no significant differences between reconstruction and mastectomy in body image, while others, in line with our findings, reported better body image after reconstruction. There are several possible explanations for this inconsistency. The scale to measure body image differs widely in these studies. Some studies measure satisfaction with body image, while others designed their own scale. Another explanation is that different women choose different surgery types, i.e., patients choosing reconstruction might differ from others in terms of preoperative body image and perception of their appearance. Preoperative body image was a predictor of a better general body image in the long term. Another important factor in body image is the conservation of nipple-areola complex, which was shown to improve body image score.

Another variable that has been suggested to influence BI is the time after treatment. It was shown that if BI was evaluated during the first 6 months after treatment, the problems with body image would increase, although there are studies that point to the contrary. The mean follow-up time in our study was 43 months and we found no difference in body image of patients with less than <6 months versus >6 months of follow-up.

In addition, we should consider radiotherapy after BCS and the complications such as fibrosis, infection, and skin edema as factors which may be associated with body image. The effects of age, marital status, and socioeconomic status on body image were investigated in previous studies, with conflicting results. Nevertheless, we controlled for them in the linear regression model and matched the three groups based on these variables.

Our study showed that mastectomy patients had significantly low scores on overall BICI scale and social functioning subscale. However, no significant difference was observed between the surgery groups in appearance dissatisfaction. We should be aware of a particular domain of body image called body stigma, which emphasizes the loss of body integrity. Although breast appearance may be acceptable to a woman, the integrity may be considered lost. Therefore, none of these surgeries may solve this problem. The use of propensity score may help to group the patients by demographic and clinical factors without randomization. However, we were unable to use this score in our analysis. There are other factors apart from those we considered in our study that can influence body image. These factors, including self-esteem, may be confounding, and we were unable to control them.

We found that the BCS group had the highest satisfaction with surgery. Satisfaction evaluation is believed to be completely different from the point of view of patients and physicians. In our study, we just evaluated satisfaction from the patients’ point of view. Although radiotherapy was reported to have the greatest impact on the cosmetic outcome, the BCS group had the highest satisfaction in our study. Our findings are consistent with a study by Al-Ghazal et al. who performed oncoplastic BCS, which uses the reconstructive technique simultaneously. This may increase satisfaction and should be considered. A study by Kaviani et al. showed no differences in QoL between BCS and oncoplastic breast surgery (OBS). However, OBS was considered cosmetically more acceptable in one study with acceptable oncologic outcomes. Nicholson et al. showed higher satisfaction in patients receiving reconstruction versus BCS. They inferred that it is because the reconstruction patients are highly motivated individuals for whom cosmetic outcome is important and also the level of choice they had in the nature of their treatment.

We didn’t evaluate patients’ preoperative satisfaction with their breast appearance. However, it has been shown that patients with higher satisfaction prior to surgery were more likely to be satisfied with reconstructed breast.

Although the reconstruction group had the lowest satisfaction score among the three groups in our study, there was no statistically significant difference. A study by Ng et al. reported a significant difference in satisfaction between mastectomy and reconstruction. A majority (64%) of their reconstructions were autologous, while all of our participants had received implant reconstruction. The type of reconstruction may account for this difference. In the binary logistic model, there were no differences between the surgery groups when dichotomizing the satisfaction outcome as satisfied or unsatisfied. The difference between this dichotomous result and the multiple-choice satisfaction measure may show that a yes/no question may not be a good scale to measure satisfaction and that further scales should be designed to measure satisfaction.

A limitation of the present study is that we did not perform a preoperative evaluation of body image and satisfaction, and preoperative status may have altered postoperative results. As we know, the grouping of patients is not randomized in such studies, and a multidisciplinary team decides the surgery approach. Thus, the use of propensity score may help to group the patients by demographic and clinical factors without randomization. However, we were unable to use this score in our analysis. There are other factors apart from those we considered in our study that can influence body image. These factors, including self-esteem, may be confounding, and we were unable to control them.
We cannot generalize these conclusions to all breast cancer patients. Another limitation was the lower number of reconstruction patients because of limitations for this type of surgery, such as lack of insurance coverage and higher costs. As Conclusion, based on the results of this study, it could be proposed that BCS and mastectomy with reconstruction are associated with better body image and social functioning compared with simple mastectomy. Breast-conserving surgery could bring about better patient satisfaction with the surgery. Time is an important factor in determining satisfaction; however, evaluation of satisfaction should be made in different time points during follow-up.

Conflict of Interest
The authors have none to declare.

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The mean age of participants was 46.7 years. Their health literacy was inadequate (18.7) and their decisional conflict was average (51.79). There was also an inverse and significant relationship between health literacy and decisional conflict (P < 0.001, r = -0.81).

Conclusion:
Increasing health literacy could reduce decisional conflict. It requires training individuals to be able to access credible and reliable sources of information. This training can be provided through doctors, treatment staff, and public and social media.

Background:
Patient participation in medical decisions is essential and requires sufficient knowledge and awareness. Thus, the aim of this study was to investigate the relationship between health literacy and the participation of breast cancer patients in their medical decisions in Shiraz, Iran.

Methods:
This was a descriptive-analytical study conducted on 196 women with breast cancer in 2016-2017. Data were collected using the standardized Health Literacy for Iranian Adults (HELIA) and the Decisional Conflict Scale. The data were analyzed using descriptive statistics and inferential methods (t test, Pearson correlation, ANOVA, and Kruskal-Wallis) on SPSS 21.

Results:
The mean age of participants was 46.7 years. Their health literacy was inadequate (18.7) and their decisional conflict was average (51.79). There was also an inverse and significant relationship between health literacy and decisional conflict (P < 0.001, r = -0.81).

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Introduction
Clinical governance is one of the tools used to improve the quality of health care in different countries. One of the goals of establishing clinical governance is to promote the participation of patients and communities in therapeutic processes. In fact, patient participation is considered a legal right and an international standard for health care. During the past 30 years, the concept of patient participation in medical decision making has developed from informed consent to the exchange of information between physicians and patients, which not only includes the benefits and risks of treatment options, but also includes the preferences and values of patients and physicians. Recently, patient participation has become a necessary policy in many countries and health systems all over the world.

Collaborative decision making is considered the key to the quality of patient-centered care, resulting in better health outcomes for patients. In general, the goal of informed and coordinated participation of patients and physicians in treatment decision making is to involve the patients and physicians in the knowledge, values, preferences, and intentions of each other. As a result, the decisions taken will be more consistent with the patients’ values, conditions, and preferences, which in turn may increase the quality of life and access to health objectives.
Several studies have also shown that shared decision making would lead to a more effective physician-patient relationship and a common understanding of the treatment objectives, as well as reducing the decisional conflicts, and bringing about better psychological adjustment, and higher levels of satisfaction.1,2,3 Cuevas et al. mentioned the lower costs of treatment as one of the benefits of patient participation in decision making.4 Furthermore, patient participation in decision making was reported to increase their commitment to health behaviors.4,5 Ghiavandyan et al., too, concluded that the participation of patients in medical decision making would improve their awareness and confidence to start their treatment.6

In this regard, behavioral changes through health literacy to have an active role in medical decision making are possible.7 In fact, health literacy is a prerequisite for making informed health care decisions.7,8 The World Health Organization considers health literacy a major health determinant.8 By definition, health literacy is a broad range of cognitive and social skills that empower people to accept, understand, and use information to enhance their health, well-being, and participation in their health-care decision making.9,10,11,12 Therefore, health literacy is one of the influential factors in guiding patients in terms of the information they need for their health care. Besides, since health literacy addresses all aspects of health care such as prevention, screening, diagnosis, and treatment, it is considered the basis for health care delivery systems.13 According to reports, nearly 80 million Americans do not have adequate health literacy.14 In Iran, according to the results of the national survey of 20,571 Iranian citizens, about half of the people had limited health literacy.15

On the other hand, the level of health literacy can affect cancer outcomes.16 Today, the cause of over 12% of deaths in all countries is cancer.17 Iran has an annual incidence of 70,000 new cancer cases, which can potentially double in the next two decades due to aging.18 Of these cancers, breast cancer is the most common one and the second leading cause of death in women.19,20 According to the World Health Organization, this rate is increasing by 1.8% to 2%.19 In Iran, breast cancer is the most common cancer in women, with an incidence rate increase of about 28.3 new cases per 100,000 women a year.21 Considering the issues raised and insufficient studies in this field in Iran, the present study aimed to investigate the relationship between health literacy and the rate of patient participation in medical decision making among patients with breast cancer in the city of Shiraz, Iran, in 2016-2017.

**Methods**

**Participants**

This was a descriptive-analytical study with a cross-sectional design that was conducted on women with breast cancer in Shiraz, Iran, in 2016-2017. The inclusion criteria were being 18 to 65 years old, being a newly diagnosed case, having the ability to communicate (lack of mental, hearing, visual, or speech disorders), willing to answer the questions, giving informed consent to participate in the study, and having visit with their doctors on how to treat their disease. At first, because of lack of similar articles, a sample of 40 cases was considered as a pilot. Then, based on a type I error of 0.05, a type II error of 0.2, and the correlation between the patients’ health literacy and participation in their medical decisions obtained from the pilot study (r = 0.122), the sample size was determined to be 160 patients. The calculated sample size was increased to 196 to account for an estimated non-response rate of 20%. Due to the lack of cooperation of the private sector, the patients were selected only from public centers. There were two public centers dealing with breast cancer counseling and treatment in the city of Shiraz. One of the centers was excluded from the study because it provided only outpatient visits, and the patients would be referred to another center if the diagnosis of breast cancer was confirmed. The other center was for hospitalized patients where surgeries were done two days a week. Using the inclusion criteria, three trained interviewers interviewed the patients one day prior to their surgery and completed the questionnaire.

**Instruments**

Health literacy was measured using the Health Literacy for Iranian Adults (HELIA) questionnaire. The questionnaire consists of two sections including demographic characteristics and 33 questions in five domains of reading (4 questions), access (6 questions), comprehension (7 questions), assessment (7 questions), and decision making (12 questions). The validity and reliability of the questionnaire were determined by Montazeri et al (Cronbach’s alpha coefficient range: 0.72 to 0.89).22 Izadirad and Zareban, too, evaluated its validity and reliability (Cronbach’s alpha coefficient: 0.92).23 The items were rated on a 5-point scale (1 = totally disagree, and 5 = totally agree). The scores were then transferred to 0-100 scale so that scores 0-50 indicated inadequate health literacy, 50.1-66 indicated almost inadequate health literacy, 66.1-84 represented sufficient health literacy, and 84.1-100 represented excellent health literacy.

Patient participation in medical decision making was evaluated using the Decisional Conflict Scale, which has been identified in various studies as a standard for assessment of the uncertainty and factors influencing the decision-making process in health care.24-26 The questionnaire measures an individual’s uncertainty about a course of action and the factors contributing to uncertainty through 16 questions on a scale from 0 (totally agree) to 4
(totally disagree). The uncertainty factors comprise five groups: a feeling of awareness, individual values, support, uncertainty, and effective decision making. Total score ranges from 0 (the highest certainty about the best decision taken) to 100 (the highest uncertainty about the best decision taken). The questionnaire was translated and validated by Mousavizadeh et al., with the Cronbach’s alpha being 0.94.4

Data Analysis
Descriptive statistics (percentage, frequency distribution, mean, and standard deviation) were used to provide a summary of participant characteristics, and nonparametric (Kruskal-Wallis) and parametric (t test, ANOVA, and Pearson correlation) tests were used to analyze the differences between groups. The nonparametric test was used if the data on a particular variable had not a normal distribution. Analyses were performed using SPSS 21.

Results

Demographic Information
A total of 196 breast cancer patients with the mean age of 46.7 ± 9.92 years were enrolled in the study. In terms of education, 37.5% had elementary, 30.5% had a middle school degree, 18.5% had a high school certificate, and 13.5% had a university education. The majority of patients (81%) were housewives, with retired and employed patients making up the remaining 6.5% and 12.5% of the study sample, respectively. Regarding the access of the study population to the sources of health and illness information, 36% got information from doctors and health-care staff, 21.4% used radio and television, 17% used the Internet, 12.46% got information from their friends and acquaintances, 6.07% used booklets, pamphlets, and brochures, 3.8% used newspapers and magazines, and 0.32% used the interactive voice dialer (IVR)(a technology that allows a computer to interact with humans through the use of voice and DTMF (Dual-tone multi-frequency signaling) tones input via a keypad), and 2.88% did not know how to get the information they needed.

Health Literacy
The mean health literacy score of the patients under study was 18.70 ± 7.34. The mean scores on health literacy subscales were 33.45 ± 14.5 (decision making), 19.59 ± 9.8 (comprehension), 16.76 ± 7.1 (access), 11.72 ± 5.5 (reading), and 11.54 ± 4.7 (evaluation). The results of the Kruskal-Wallis test did not show a significant relationship between health literacy and education levels (P = 0.06). Furthermore, the ANOVA test did not show any significant relationships between health literacy and employment status (P = 0.8), the t test showed that general health literacy had no significant relationship with information-gathering methods (P = 0.98). No significant relationship was observed between health literacy and age (P = 0.09).

Decisional Conflict
The overall mean decisional conflict score was 51.79 ± 29.1, with the scores on subscales being 53.72 ± 32.1 (uncertainty), 53.3 ± 34.9 (support), 52.77 ± 35.8 (effective decision making), 50.67 ± 30.7 (value), and 49.64 ± 29.0 (awareness). There was no significant relationship between decisional conflict and age, job, education, and information-gathering methods (P = 0.3, P = 0.81, P = 0.95, P = 0.3).

Health Literacy and Decisional Conflict
There was a significant inverse relationship between health literacy and decisional conflict in general (r = -0.81, P < 0.001). Pairwise analyses of the correlations between the dimensions of health literacy and decisional conflict are shown in Table 1. A similar inverse relationship was observed for each pairwise comparison at a 99% confidence level.

Discussion
The main objective of the present study was to investigate the relationship between health literacy and the rate of participation in one’s medical decisions making among breast cancer patients in Shiraz, Iran. The results of this study showed an inadequate level of health literacy in the women with breast cancer (18.70) which was consistent with the results of a study by Sahrayi et al. on adults in Karaj (24.2%) and that of Izadirad and Zareban carried

| Table 1. Correlations between health literacy dimensions and decisional conflict dimensions |
|----------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Decisional conflict                  | Health literacy | Reading         | Access          | Comprehension   | Assessment      |
| Awareness                             | R = -0.661a     | R = -0.534a     | R = -0.66a      | R = -0.549a     | R = -0.594a     |
| Personal values                       | R = -0.655a     | R = -0.536a     | R = -0.726a     | R = -0.513a     | R = -0.668a     |
| Support                               | R = -0.867a     | R = -0.516a     | R = -0.787a     | R = -0.504a     | R = -0.724a     |
| Uncertainty                           | R = -0.665a     | R = -0.516a     | R = -0.771a     | R = -0.554a     | R = -0.723a     |
| Effective decision making             | R = -0.696a     | R = -0.537a     | R = -0.825a     | R = -0.553a     | R = -0.793a     |

P < 0.001
out in Baluchestan (34%).\textsuperscript{24} However, it was inconsistent with the results of a research by Haghighi \textit{et al.} on women with breast cancer in Tehran (38.8%).\textsuperscript{23} The reason for this inconsistency can be the difference in the populations studied. The participants in our study were newly diagnosed patients, while in the study of Haghighi \textit{et al.},\textsuperscript{24} the participants were patients living with breast cancer and were more likely to have obtained knowledge during the treatment period.

In the present study, nearly one-third of the participants received their health and disease information from doctors and health-care staff (36%), and through radio and television (21%). In the studies by Sahrayi \textit{et al.} and Izadirad and Zareban, radio and television (42.5% and 19%) and health-care staff (37% and 49.8%) were the most important sources of getting health information, which is consistent with the results of the present study.\textsuperscript{24,25} In a systematic review, Hur \textit{et al.} concluded that learning and health literacy could be improved by using multimedia tools and teaching in a simple language.\textsuperscript{26} Another study suggested that a simple and understandable language used by the providers could be useful for patients’ effective communications and better understanding.\textsuperscript{27} The aforementioned issues indicate the importance of knowledge and awareness of the providers about health information and how to transfer them to patients, and also the importance of the mass media. In this regard, policy makers can plan to increase health literacy through the use of popular technologies.

The decisional conflict was reported at a moderate level (51.79) in this study, indicating the patients’ uncertainty about their medical decision making. In a study by Essink \textit{et al.} in 2016 to assess decision making knowledge and awareness of the patients with colon cancer, a low decisional conflict was reported (21.12).\textsuperscript{28} Besides, in the study by Jukkala \textit{et al.} in 2013 on women with breast cancer, decisional conflict was 48.5% and at a moderate level,\textsuperscript{29} which is consistent with the present study. The conflict and hesitation could be due to the patients’ uncertainty about their best medical decisions.\textsuperscript{30} Uncertainty might also be due to the patients’ inadequate awareness and knowledge of the disease and therapies, and it is recommended that service providers spend more time counseling their patients in order to reduce this uncertainty.

Finally, there was an inverse and significant relationship between decisional conflict and health literacy ($r = -0.81$, $P < 0.001$), which is consistent with the study done by Tagai on patients with colon cancer ($P < 0.001$),\textsuperscript{4} the one by Doyle \textit{et al.} on patients with AIDS ($P = 0.05$),\textsuperscript{31} that of Essink \textit{et al.} in colon cancer screening ($P = 0.05$), and also the study by Jukkala \textit{et al.} on women with breast cancer ($P < 0.001$).\textsuperscript{32}

High levels of uncertainty and concern for decision making can be caused by inadequate health literacy.\textsuperscript{33} Low health literacy can be due to the patients’ inadequate knowledge of their disease. This inadequate knowledge might lead to uncertainty in the patients and they might feel that the treatment was imposed on them and was not based on their preferences. In fact, they may think they made a useless choice that would ultimately lead to regret over the decision.\textsuperscript{34}

Given that low health literacy causes conflict and uncertainty in patient decision making, it can be concluded that factors such as low ability to read, understand, and evaluate health information, the lack of access to reliable information, the lack of adequate knowledge of the benefits, risks, and side effects of the treatment, a perceived lack of support or the feeling of being under pressure, and the lack of attention paid to the patients’ personal preferences and values are matters of concern and regret of the patients about their choice. Therefore, health literacy increases the ability of the patient, and subsequently the patient participation in decision making.\textsuperscript{35} Hence, in order to increase the patients’ health literacy and reduce their doubts in decision making, and considering patient-centeredness of the health care, it is suggested that required information along with methods of access to reliable information be provided to patients by the medical staff, and that patients be counseled about the disease and the treatment methods in a simple and understandable language, especially when there are more than one choice. This can lead to a better patient-doctor communication and mutual trust between them, increased patient commitment to health-care and treatment outcomes, increased quality of life, increased satisfaction, reduced decisional conflict, and reduced costs of the health care system.

The present study showed that increased health literacy could lead to reduced decisional conflict. Given the low level of health literacy observed in this study and the role of health-care providers and media in providing health information, it is suggested that these sectors should receive incentives to provide more comprehensive information to patients. Also, the use of mass media programs a simple and understandable way can help.

One limitation of this research was the lack of access to patients in private clinics, which reduced the study generalizability to some extent. It is suggested to consider the socioeconomic conditions that can affect patient decision-making, and take into account the use of educational packages and assess the patients’ health literacy before and after using the packages.

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**Conflict of Interests**
The authors declare no competing interest.

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We present a case of warfarin induced necrosis of the breast in a 38-year-old patient, treated with simple mastectomy.

Case presentation
A 38-year-old female presented to the breast clinic of Imam Khomeini Hospital with the complaint of a necrotic ulcer on her left breast for 10 days. She had a history of admission for deep vein thrombosis (DVT) of the left lower limb one month earlier. The deep vein thrombosis occurred following long-term use of oral contraceptive pills (OCP). She received intravenous heparin followed by warfarin. She was discharged from the hospital with a daily dose of 10 mg of warfarin and with an INR of 2.1.

On admission for breast necrosis, no fever was detected but the patient had a foul-smelling necrosis involving most of her left breast (Figure 1). Blood work showed INR, activated thromboplastin time (aPTT), and platelets of 1.32, 38 seconds, and 460,000 per cubic milliliter, respectively. Hematology consultation recommended discontinuation of warfarin and administration of Rivaroxaban.

Considering the extent of breast necrosis (Figure 1), mastectomy was recommended. Before surgery, Rivaroxaban was discontinued, and heparin and
Skin and subcutaneous tissue necrosis is a rare complication of warfarin, occurring in 0.1-0.01% of patients receiving oral anticoagulation. Women are more commonly affected with a female to male ratio of 3:1. The areas with increased subcutaneous fat (abdomen, thighs, buttocks, legs, and breasts) are more susceptible to necrosis. Breast is affected in about 10-15% of the patients. It usually affects one breast, but bilateral breast necrosis is also reported.

The symptoms start 1 to 10 days after initiation of warfarin, and the majority of cases occur within 3 to 6 days. However, there are reports of late-onset symptoms several years after commencement of therapy. The initial manifestations of breast necrosis are paresthesia, pressure, and discomfort in the affected area. Later, erythematous or hemorrhagic skin lesions appear and will develop into blisters and bullae, leading to skin necrosis and scar formation.

Further hematologic evaluation showed protein C deficiency. Pathology report confirmed breast parenchyma with diffuse and extensive necrosis and acute inflammation.

**Discussion**

Skin and subcutaneous tissue necrosis is a rare complication of warfarin, occurring in 0.1-0.01% of patients receiving oral anticoagulation. Women are more commonly affected with a female to male ratio of 3:1. The areas with increased subcutaneous fat (abdomen, thighs, buttocks, legs, and breasts) are more susceptible to necrosis. Breast is affected in about 10-15% of the patients. It usually affects one breast, but bilateral breast necrosis is also reported.

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anticoagulant protein C and protein S. Protein C and protein S have shorter half-life than vitamin K dependent clotting factors. Consequently, the patient develops a transient hypercoagulable state at the beginning of warfarin therapy and needs another form of anticoagulation, such as heparin, for 48-72 hours to prevent clot formation.\textsuperscript{1,3,8,15}

Management of breast necrosis following oral anticoagulation therapy with warfarin, starts with discontinuation of warfarin. However, withdrawing warfarin will not affect the course of the established skin lesions.\textsuperscript{4,5,16} Vitamin K and fresh-frozen plasma are administered to replenish protein C levels,\textsuperscript{7} and anticoagulation should be continued by administering intravenous heparin or the non-vitamin K antagonist oral anticoagulants (NOACs) such as Dabigatran or Rivaroxaban.\textsuperscript{17} The necrotic lesions should be managed surgically with debridement and appropriate wound care.

In conclusion, fulminant painful purpura and erythematous skin lesions in patients receiving warfarin should be suspected as warfarin induced necrosis. Immediate discontinuation of warfarin, administration of vitamin K, and fresh-frozen plasma is recommended. The skin lesion should be managed with appropriate surgical debridement and wound care.

**Ethical Consideration**

The patient announced her consent for using her data for this case report.

**Conflict of Interest**

The authors have no conflict of interest to disclose.

**References**


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1. Open Access

Archives of Breast Cancer (ABC) provides immediate open access to its contents with the goal of contributing to a greater global exchange of knowledge. Through open access publication of ABC, we aim to broaden the free and full-access reach of the users all over the world to the published articles. All the published material in ABC are, under a Creative Commons license, free to view and reuse with attribution by anyone in the world via the internet.

2. What do we publish?

2.1 Aims & scope

ABC is an open access, peer-reviewed journal that publishes articles on all aspects of breast cancer research, including the pathophysiology, prevention, early detection, diagnosis, treatment, molecular and cellular biology, genetics, epidemiology, psychological issues, rehabilitation, and quality of life. Although the main focus of the journal is breast cancer, important topics on benign breast diseases and breast health, such as breastfeeding, will be considered for publication.

2.2 Article types

- Original article

These are reports of original experiment/research conducted by the authors. Original research must add to the body of knowledge on the subject and should adhere to ethical principles throughout the procedure. Studies
Original articles should not exceed 5,500 words including abstract, references, figure legends, and tables. The number of tables, figures, and references should be appropriate to the manuscript content. Authors whose first language is not English are advised to have their manuscripts checked carefully before submission.Abbreviations of standard SI units of measurement should be used. Sections of an original article are: (1) title page, (2) abstract, (3) keywords, (4) introduction, (5) methods, (6) results, (7) discussion, (8) acknowledgments, (9) conflicts of interest, (10) references, (11) figure and video legends, (12) tables, (13) figures, and (14) videos whenever applicable.

- **Short communication**
  A small-scale study that includes important new information may be published as a short communication. It usually carries an abstract of up to 150 words, a body of up to 800 words, up to 2 tables or figures, and essential references. Sections of a short communication paper are: (1) title page, (2) abstract, (3) keywords, (4) introduction, (5) methods, (6) results, (7) discussion, (8) acknowledgments, (9) conflicts of interest, (10) references, (11) figure and video legends, (12) tables, (13) figures, and (14) videos whenever applicable.

- **Commentary**
  Commentaries discuss the findings, implications, and/or outcomes of specific research or wider research on a general topic. They elaborate on or offer original ideas about a specific paper or a widely-researched subject. Commentaries differ from reviews in that they represent the author's original ideas and suggestions instead of reporting and comparing the previous research. Sections of a commentary include (1) title page, (2) keywords, (3) text, (4) acknowledgments, (5) conflicts of interest, and (6) references.

- **Editorial**
  Editorials are usually written by the editor in chief, deputy editor in chief, an associate editor, or a guest editor and are intended to represent the official opinion of the journal (or the guest editor) or introduce supplements, special issues, or new ideas relevant to the journal. In limited circumstances, individuals other than the individuals listed here may propose an editorial topic if they wish. Sections of an editorial include (1) title page, (2) keywords, (3) main manuscript (4) acknowledgments, (5) conflicts of interest, (6) references.

- **Letter to the editor**
  Letters commenting on, questioning, or criticizing articles recently (within the past 4 issues) published in ABC expressing views on relevant topics will be considered for publication. Sections of a letter to the editor are (1) title page, (2) keywords, (3) text, (4) acknowledgments, (5) conflicts of interest, and (6) references.

- **Case report and case series**
  Case reports include case report/studies of patient(s) and describe a novel approach or add important insights into mechanisms, diagnosis, or treatment of a disease. Case reports are limited to 1,500 words including references. A case report manuscript should include (1) title page, (2) structured abstract (including background, case presentation, and conclusion), (3) keywords, (4) introduction, (5) case presentation, and (7) discussion. It should also contain discussion, acknowledgments, references, and illustrations (if applicable) as explained for the original articles. All ethical considerations according to the Committee on Publication Ethics (COPE) guidelines, especially the informed consent in case reports, should be included at the end of the text just before the references.

- **Review article**
  A review article provides a detailed, thorough examination and summary of the literature on a specific topic. Authors are encouraged to contact the editor in chief (akaviani@archbreastcancer.com) before preparing an unsolicited review article to avoid duplication of other works already in progress. Review articles are limited to 7,000 words, including references, and should include the following sections: (1) title page, (2) structured abstract, (3) keywords, (4) introduction, (5) methods (search strategy), (6) results, (7) discussion, (8) acknowledgments, (9) conflicts of interest, (10) references, (11) figure legends, (12) tables, and (13) figures, whenever applicable.

- **Clinical experience**
  In this type of manuscripts, the authors explore a debate in the clinical dilemma by presenting one or more
patients whose records are presented in the tumor board or multidisciplinary team (MDT) session of the hospital. The clinical decision which is made in the sessions are argued in this type of the manuscripts according to the scientific evidence.

Section of review article: (1) title page, (2) abstract, (3) keywords, (4) introduction, (5) case presentation, (6) question, (7) discussion, (8) acknowledgments, (9) conflicts of interest, (10) references, (11) figure legends, (12) tables, and (13) figures whenever it would be applicable.

2.3 Writing your paper

2.3.1 General format
All manuscripts should be prepared using MS Word (in .doc or .docx format). Submissions in the form of PDF files are not accepted. Manuscripts should be double-spaced, including text, tables, legends, and references. Number each page and avoid footnotes; instead, and as sparingly as possible, use parentheses within the text. Use the Tab key once for paragraph indents, Times New Roman typeface for the text and Symbols for Greek and special characters.

2.3.2 Main sections of the manuscript
Papers can be divided into the following sections:

Introduction
- State clearly the main objectives of the study.
- Indicate the main reasons for doing the work.
- A detailed review of the literature is not recommended.
- The content should be accompanied by relevant references.

Methods
- Mention the type of study.
- Describe the methods, tools, and procedures employed with sufficient details to allow others to reproduce the results without the need to communicate with the authors.
- Describe the intervention and control groups, when relevant.
- Mention the type of statistical tests used.
- The rationale for using specific statistical tests, if applicable.
- State statistical significance when appropriate.

ABC complies with the COPE guidelines; therefore, all researchers are asked to read these guidelines to avoid cases of suspected research and publication misconduct (e.g., falsification, fabrication, plagiarism, inappropriate image manipulation, and redundant publication). For more information about COPE, please visit http://www.publicationethics.org.

Also, the authors should state that their study complies with the Declaration of Helsinki, that the locally appointed ethics committee has approved the research protocol, and that informed consent has been obtained from the subjects (or their guardians).

Results
- State findings of the study in the text, tables, or figures, and do not repeat the same findings in tables and figures and the text.
- Be precise and do not include material that is appropriate for the discussion, e.g., explanation of findings.
- Use the metric system/SI units to report scientific quantities and measurements.

Discussion
- State the original and important features of the study. Do not repeat the findings presented in the results section.
- Contain the significance of the findings and the relevance to previously published studies.
- State only the conclusions that are supported by the data.
- Mention the limitations and applications of the results.

2.3.3 Making your article discoverable
From the moment you set about writing the first draft of your manuscript, think about how you can make it more discoverable. The title, keywords and abstract are key to ensuring readers find your article through search engines.
3. Editorial policies

3.1 Peer review policy
Peer review is an integral part of scientific publishing that confirms the validity of the science reported. Peer-reviewers are experts who volunteer their time to help improve the journal manuscripts they review—they offer authors free advice.

Key characteristics of the peer review process are listed below:
- All research articles are reviewed by at least two qualified experts.
- All publication decisions are made by the journal's editor in chief on the basis of the reviews.
- Members of the editorial board lend insight, advice, and guidance to the editor in chief generally and assist decision making on specific submissions.

- Initial manuscript evaluation
All manuscripts submitted to ABC are read by the editorial staff. To save time for authors and reviewers, only those papers that seem most likely to meet our editorial criteria are sent for formal review. Those papers judged by the editors to be of insufficient general interest or otherwise inappropriate are rejected promptly without external review (although these decisions may be based on informal advice from specialists in the field).

- Type of peer review
ABC employs a double-blind review process, where both the reviewer and author remain anonymous throughout the process.

- Selecting peer reviewers
Reviewer selection is critical to the publication process, and we base our choice on many factors, including expertise, reputation, specific recommendations, and our own previous experience of a reviewer's characteristics.
Reviewers should strive to observe the principle of confidentiality with regard to materials submitted to them.

- How long does the review process take?
Typically the manuscript will be reviewed within 4 weeks.

- Final report
A final decision to accept or reject the manuscript will be sent to the author along with any recommendations made by the reviewers and may include verbatim comments by the reviewers.

3.2 Authorship
Only when all the contributing authors give consent should a manuscript be submitted to a journal. The corresponding author should make sure that everyone whose work contributed to the manuscript is acknowledged as a contributing author. Only those who can legitimately claim authorship should be listed as authors. This consists of (a) contributions to the conception or design of the work; or the acquisition, analysis, and interpretation of data for the work; (b) drafting the work or revising it critically; and (c) final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.
When a study is conducted by a large, multicenter group, the group must identify the individuals who can take direct responsibility for the manuscript. These individuals should meet all the criteria mentioned above.
Acquisition of funds, collection of data, or general supervision of the research group alone does not qualify one as an author; however, the contributors not meeting the authorship criteria should be acknowledged. For more information on authorship requirements see the International Committee of Medical Journal Editors (ICMJE) guidelines.

3.3 Acknowledgements
All contributors who do not meet the criteria for authorship as defined previously should be listed in the Acknowledgment section. Examples include a department chairperson who provided only general support, a person who provided purely technical help, or writing assistance.

3.4 Funding
Authors should provide detailed information regarding the source of financial and material support (grant number, etc.) for the research under a separate heading. If no funding is provided for the research, include the
3.5 Declaration of conflicting interests
State all funding sources and the names of companies, manufacturers, or external organizations providing technical or equipment support.
Visit ICMJE recommendations for more guidance on conflict of interest statements.

3.6 Research ethics and patient consent
Medical research involving human subjects must be conducted in accordance with the World Medical Association Declaration of Helsinki.
Manuscripts should conform to the ICMJE Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals. All manuscripts reporting animal or human studies should state in the Methods section that ethical approval has been obtained from (or waived by) an ethics committee or institutional review board. Please make sure of providing the full name of the review committee as well as the approval number.
For research articles, a statement should be included in the Methods section indicating that informed consent, written or verbal, was obtained from participants. Case reports and case series, too, should provide an informed consent statement in the manuscript text. Also, manuscripts are required to state whether written informed consent was obtained for publication of patient information and images.
Do not submit the patient's actual written informed consent with your article, as this in itself is a violation of the patient's confidentiality. It suffices to submit a confirmatory letter along with the manuscript that written informed consent has been obtained from the patient and that the forms are kept by the authors/investigators themselves, for example in a patient's hospital record. See the ICMJE Recommendations for the Protection of Research Participants for more information.
Studies involving animal subjects are required to have the approval of an ethics committee with oversight of the facility in which the studies were conducted. The journal has adopted the Consensus Author Guidelines on Animal Ethics and Welfare for Veterinary Journals published by the International Association of Veterinary Editors.

3.7 Clinical trials
In accordance with the ICMJE requirements, ABC requires clinical trials to be registered in a WHO-approved public trials registry at or before the time of first patient enrolment to be considered for publication. The trial registry name and URL and the registration number must be included at the end of the abstract.

3.8 Reporting guidelines
Authors are encouraged to use the relevant research reporting guidelines for the study type provided by the EQUATOR Network. This will ensure that you provide enough information for editors, peer reviewers, and readers to understand how the research was performed and to judge whether the findings are likely to be reliable.
The key reporting guidelines are:
Randomized controlled trials (RCTs): CONSORT guidelines
Systematic reviews and meta-analyses: PRISMA guidelines and MOOSE guidelines
Observational studies in epidemiology: STROBE guidelines and MOOSE guidelines
Diagnostic accuracy studies: STARD guidelines
Quality improvement studies: SQUIRE guidelines

4. Publishing policies
4.1 Publication ethics
4.1.1 Plagiarism
ABC is powered by the iThenticate software, which is a plagiarism detection service that verifies the originality of the submitted content before publication. If plagiarism is identified, we will follow COPE guidelines.
Plagiarism includes (but is not limited to):
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2) Copying ideas, images, or data from other sources
3) Reusing text from your own previous publications
4) Using an idea from another source with slightly modified language
If plagiarism is detected during the peer review process, the manuscript may be rejected. If plagiarism is detected after publication, we reserve the right to issue a correction or retract the paper, as appropriate.

4.1.2 Prior publication
Any manuscript submitted to ABC must be original, and the manuscript, or substantial parts of it, must not be under consideration by any other journal.
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5. Preparing your manuscript

5.1 Word processing formats
We prefer Microsoft Word format (.DOC or .DOCX) for the submitted manuscripts, although PDF files are also accepted.

5.2 Artwork, figures, and other graphics

5.2.1 Figures
Figures should be limited to the number necessary for clarity and must not duplicate data given in tables or in the text. Submissions should have no more than a total of 8 figures and tables. Any number exceeding this should be designated as supplementary material for online access only. Figures must be suitable for high-quality reproduction and should be submitted in the desired final printed size so that the quality of the figure would not be affected after being printed. Figures should be no larger than 125 (height) × 180 (width) mm (5 × 7 inches) and should be submitted as separate files. Figures should be saved in TIFF format at a resolution of at least 300 pixels per inch at the final printed size for color figures and photographs, and 1200 pixels per inch for black-and-white line drawings. Although some other formats can be converted into TIFF format by the publisher, the conversion may alter the tones, resolution, and contrast of the image. Therefore, PDF is not acceptable as a source file for figures and illustrations.

5.2.2 Videos
Authors can submit videos and computer-generated graphics as well as a slide presentation if they think it is a part of their manuscript. The videos will be refereed as well, and the editors and reviewers may suggest changes. It is essential that the patient be not identifiable in the video unless he or she has provided written permission.
The length of each video clip or computer graphic should not exceed 40 MB. The authors can submit up to 3 videos or computer graphics. A concise legend for each video must be included in the manuscript. Only the videos which are compatible with Windows Media Player and submitted in MPEG-1 or MPEG-2 (.mpg) or QuickTime (.mov) format can be accepted.

5.2.3 Tables
Tables should be presented in a separate file. Provide a short, descriptive title for each table. Please make sure that each table is cited in the text and is numbered using arabic numerals. For tables adopted from previously published works, indicate the original source in the form of a reference at the end of the table caption. Asterisks (*) for significance values and other statistical points should be included under the table.

5.2.4 Figure and video legends
Each figure should have a concise caption (legend) describing accurately what the figure depicts. Figure legends should be mentioned at the end of the manuscript file and not in the figure file. Figure legends should begin with the term Fig. in boldface, followed by the figure number also in boldface (e.g., Fig. 1).

5.3 Reference style
ABC follows the **AMA reference style**. Please review the [AMA guidelines on AMA](#) to ensure your manuscript conforms to this reference style.

If you use EndNote to manage references, you can download the [AMA output file here](#).

The accuracy of reference details is the responsibility of the authors. Personal communications, unpublished observations, and submitted manuscripts are not acceptable references. In the main text, references should be cited consecutively by superscript arabic numerals. The reference list should be organized in numerical order according to the order of appearing in the text.

**Examples of acceptable reference formats are as follow:**


**Reference to a chapter in an edited book:**


**Reference to a chapter in an edited book which has been written by authors in a book edited by others:**


If there are 7 or more authors, list the first 6 ones and then insert "et al."

5.4 English language editing services
ABC offers language editing services to assist authors with the preparation of their manuscripts. These services are offered by third parties and will be available at a charge; however, authors publishing with ABC will qualify for a 50% discount. The cost of the language editing for each manuscript of the journal will differ from 50 to 150 Canadian dollars (CAD) depending on the word count of the article.

5.5 DOI Number
All the published manuscripts will be assigned a digital object identifier (DOI) (Crossref) as soon as the article is accepted and the processing of the article is finished. This unique identifier of the published articles enables identifying and citing articles published online without the volume or issue information (for more information, please refer to [www.doi.org](http://www.doi.org)).

6. Submitting your manuscript

6.1 How to submit your manuscript

Manuscripts should be submitted and tracked to the final decision at [www.archbreastcancer.com](http://www.archbreastcancer.com). Manuscripts that do not comply with the instructions for authors will not be sent for external review.

ABC considers the following types of articles for publication: original research articles, reviews, letters, clinical experiences, case reports, case series, and short communication.

6.2 Cover letter, title page, keywords, and abstracts

Please supply a title, short title, an abstract, and keywords to accompany your article. The title, keywords, and
abstract are key to ensuring readers find your article online through online search engines such as Google.

6.2.1 Cover letter
Manuscripts should be submitted with a letter covering the below-mentioned issues:
- A summary explanation of the importance of the manuscript.
- The points that will be added to the literature by publishing the manuscript.
- Certifying that all coauthors have read and approved the final draft of the manuscript submitted to ABC.

6.2.2 Title page
The title page should include:
- The full title
- Complete name, the highest academic degree(s), and the affiliation of each author, along with their email addresses;
- Corresponding author's name should be followed by an asterisk;
- Mailing address, phone/fax number, and email for correspondence;
- A running title of no more than 20 characters;
- The word count for the abstract and main body of the manuscript.

6.2.3 Abstract
- All abstracts may not exceed 250 words;
- Do not cite references in the abstract;
- The abstract should be self-explanatory without reference to the main text;
- Limit the use of acronyms and abbreviations;
- The structure of the abstract includes Background, Methods, Results, and Conclusions.

6.2.4 Keywords
- Provide 3 to 5 keywords for indexing purposes. Keywords found in the Medical Subject Headings (MeSH) list of Index Medicus are preferable (see www.nlm.nih.gov).

6.3 Information required for completing your submission
You will be asked to provide contact details and academic affiliations for all coauthors via the submission system and identify who is to be the corresponding author. These details must match what appears in your manuscript. The affiliation listed on the manuscript should be the institution where the research was conducted. If an author has moved to a new institution since completing the research, the new affiliation can be included in a manuscript note at the end of the paper. At this stage please ensure you have included all the required statements and declarations and uploaded any supplementary files (including reporting guidelines where relevant).

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7. On acceptance and publication
If your paper is accepted for publication after peer review, the author(s) will be required to sign the Journal Contributor's Publishing Agreement. Once your manuscript files have been checked for ABC production, the corresponding author will need to pay the article processing charge (APC) via a payment link.

7.1 ABC production
The journal production editor will keep you posted about your article's progress throughout the production process. The corresponding author will receive proofs in PDF format and should return them immediately. Authors are required to check their proofs carefully to verify the correctness and accuracy of any information, including names, affiliations, sequence, and contact details, and funding and conflict of interest statements. Please note that any changes to the author list at this stage require all authors to complete and sign a form permitting the change.

7.2 Online publication
Online open access publishing offers many benefits, one of them being higher publishing speed. With no limit on
the number of pages, your paper will be published online in a fully citable form with a DOI number once it completes the production process. At this time, it will be completely free to view and download for all.

7.3 Application for waiver
Authors may submit along with their manuscript a waiver request letter, in which they explain the reasons for their request. The journal's office will process the request and respond in 4 business days. The request can be accepted completely or partially or refused. If the waiver request is accepted completely, the journal will proceed with the peer review processing of the submitted manuscript; otherwise, the author will be informed about the decision. As soon as the corresponding author agrees, the peer review will start.

The journal's policy is to offer waivers or considerable discounts to most of the requests submitted from the limited-resource countries.

7.4 Article processing charge (APC)
The publication fee payable by the corresponding author covers a part of processing and publication charges of the manuscripts. Charges are made based on the final page count of the accepted and edited article and vary depending on the number of printed pages. One printed page of pure text contains approximately 6000 characters; however, the final page count will also depend on the number and size of tables and figures. Articles under 3 pages do not incur a charge. Articles exceeding three pages are charged to the author at 500 CAD. To request for waiver, please see section 7.3.

7.5 Complaints procedure
The best way to reach us is by email. Complaints should ideally be made to the person the complainant is already in contact with over the matter being complained about. If that is not appropriate, please email our managing editor Dr. Mojgan Karbakhsh (mkarbakhsh@sina.tums.ac.ir).
Whenever possible, complaints will be dealt with by the relevant member of the editorial staff. If that person cannot deal with the complaint, he or she will refer it to our managing editor.
All complaints will be addressed within three working days. If possible, a full response will be made within four weeks.
If the complainant is not happy with the resolution, he or she may ask for the complaint to be escalated to the ABC editor in chief, Dr Ahmad Kaviani.

8. Further information
Any correspondence, queries, or additional requests for information on the manuscript submission process should be addressed to ABC editor in chief, Dr Ahmad Kaviani (akaviani@archbreastcancer.com).
ANNOUNCEMENT

As of 2018, ABC will consider the publication of articles under a new category called "CLINICAL EXPERIENCE." These articles are meant to present a discussion of a problematic clinical situation. The manuscripts should be organized under the following headings: Case Presentation, Questions on the Management of Patient, Multidisciplinary Team (MDT) Discussion, Conclusion, and References. ABC invites all the clinicians and MDT coordinators to share their invaluable clinical experiences with their colleagues.

All the manuscripts submitted in 2019 will be assessed and peer-reviewed for publication in every issues in couple of weeks.

For more information regarding the journal scope and style, please visit ABC website at:

ARCHBREASTCANCER.COM