Objective: We evaluated ultrasonographic findings and the corresponding histopathological characteristics of breast cancer patients with Breast Imaging Reporting and Data System (BI-RADS) category 1 mammogram.

Methods: We retrospectively reviewed the ultrasonographic findings and the corresponding histopathological features of 45 breast cancer patients with BI-RADS category 1 mammogram and 537 controls with mammographic abnormalities. We evaluated the ultrasonographic findings including mass shape, periphery, internal and posterior echo pattern, interruption of mammary borders and the distribution of low-echoic lesions, and the corresponding histopathological characteristics including histological classification, hormone receptor and human epidermal growth factor receptor 2 status of invasive ductal carcinoma and ductal carcinoma in situ, histological grade, mitotic counts and lymphovascular invasion in individual cases of BI-RADS category 1 mammograms and compared with those of the control group.

Results: The ultrasonographic characteristics of the BI-RADS category 1 group were characterized by a higher ratio of round shape ($P < 0.001$), non-spiculated periphery ($P = 0.021$), non-interruption of mammary borders ($P < 0.001$) and non-attenuation ($P = 0.011$) compared with the control group. A total of 52.6% of low-echoic lesions were associated with spotted distribution in the BI-RADS 1 group, whereas 25.8% of low-echoic lesions were associated with spotted distribution in the control group ($P = 0.012$). As for histopathological characteristics, there was a statistically higher ratio of triple-negative subtype ($P = 0.021$), and this particular tendency was detected in histological grade 3 in the BI-RADS category 1 group ($P = 0.094$).

Conclusion: We evaluated ultrasonographic findings and the corresponding histopathological characteristics for BI-RADS category 1 mammograms and noted significant differences among these findings in this study. Evaluation of these ultrasonographic and histopathological characteristics may provide a more accurate ultrasonographic screening system for breast cancer in Japanese women.

Key words: breast US – BI-RADS category 1 mammogram – histopathological characteristics

INTRODUCTION

The incidence of breast cancer has increased worldwide, which is partly considered to be due to mass screening programs resulting in the discovery of clinically occult or early breast lesions (1). Early clinical detection of breast cancer through screening has therefore led to the detection of the tumor at a relatively earlier clinical stage. The effectiveness of screening mammography on reduction in mortality by breast cancer has been well established in both Western countries and Japan (2). Mammography has thus become the gold standard for...
detecting breast disorders. Therefore, it has become very important to increase the rate of mammographic screening among the general public toward reducing the breast cancer mortality. However, it is also true that 7.2% of the malignant cases were associated with no mammographic abnormalities (3). In addition, the malignant ratio of 20, 30 and 40 years without mammographic abnormalities was statistically higher than the ratio of the other age groups (3). Ultrasonography (US) has been in general proposed to prove much more effective in the detection of breast cancer if the patient is young, has dense breast or their detected masses are small (4–6). Therefore, it has become very important to improve the quality of US diagnoses.

The effectiveness of ultrasound screening for women aged 40 years has been evaluated in detecting and reducing mortality of the breast cancer in Japan in order to complement this particular pitfall of mammography (7). This study named J-START (The Japan Strategic Anti-cancer Randomized Trial) evaluates the effectiveness of screening mammography with US breast cancer screening compared with mammography alone in 40 years, with a design to study 50,000 women with mammography and US and 50,000 controls with mammography only (7). The participants are scheduled to take a second-round screening with the same modality 2 years onwards (7). The primary endpoints are sensitivity and specificity, and the secondary endpoint as the rate of advanced breast cancer (7). Whether or not breast US screening is adopted in the future large-scale screening therefore largely depends on the results of this research. Considerable efforts will be required to successfully carry out this massive undertaking done in Japanese population.

Strict or rigorous conformity to high quality of interpretation of US finding among those involved in this screening is therefore mandatory for the very success of an US diagnosis in such a large scale. We previously examined the correlation between US findings and the corresponding histopathological features in breast disorders in our previous study (6). There have been relatively few reported studies on assessing US performance and its resolution without any mammographic abnormalities (8). Therefore, in this study, we evaluated US findings and the corresponding histopathological characteristics for breast cancer patients with Breast Imaging Reporting and Data System (BI-RADS) (9) category 1 mammogram.

IMAGING DEVICES AND BREAST TISSUE SPECIMENS

The US examinations were assessed by one of the experienced eight breast specialists in Tohoku University Hospital. The consensus meeting of US was held for 1 week in order to standardize the US examination among these eight doctors. In addition, two of them independently evaluated the US findings in a retrospective manner, without the knowledge of clinical and histopathological information of individual patients. All US evaluations were carried out using Aloka SSD 3500 and ProSound a7 (Aloka Co., Tokyo, Japan) with a 10 MHz transducer.

We stained the corresponding tissue slides of the cases using hematoxylin–eosin (H&E) and immunohistochemistry for estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2). Surgical specimens had been fixed in 10% formaldehyde solution, cut into serial 5 mm-thick slices, embedded in paraffin, cut into 4 μm-thick sections and placed on the glue-coated glass slides. We employed the avidin–streptavidin immunoperoxidase method using the clone 6F11 antibody (Ventana, Tucson, AZ, USA) in automated immunostainer (Benchmark System; Ventana). A standardized immunohistochemistry kit (HercepTest for Immunoenzymatic Staining; Dako, Copenhagen, Denmark) was used for HER2 staining. Histopathological slides were reviewed by two pathologists independently without the knowledge of clinical information. Olympus (Tokyo, Japan) BX50 and 20× objectives were used for the analyses.

IMAGING AND HISTOPATHOLOGICAL ANALYSES

Two or more hardcopy transverse and sagittal plane images of breast lesions were analyzed in this study. We recorded tumor shape, periphery, internal and lateral echo pattern, interruption of mammary borders and the distribution of low-echoic lesions, according to the BI-RADS sonographic classification (9) and the Japan Association of Breast and Thyroid Sonology (JABTS) breast sonographic classification (10). Tumor shape was tentatively classified into round, oval, lobular and irregular (9, 10). Periphery was tentatively classified into circumscribed, obscured, indistinct and spiculated (9, 10). Internal echo was classified into low and heterogeneity or high (9, 10). Lateral echo was also classified into accentuation, no change and attenuation (9, 10). Interruption of mammary borders was classified into interruption, indeterminate and no (9, 10). Distribution of low-echoic lesions was classified into spotted and segmental (9, 10) (Fig. 1).

Two of the experienced pathologists independently evaluated surgical pathology specimens, respectively. Histopathological evaluations were based on World Health Organization (WHO) histological classification of tumor of the breast (11) and Rosen’s breast pathology (12). ER was determined by nuclear staining graded from 0 to 8 using the Allred score, and ER positivity was Grade 3 or more (13). With regard to HER2 evaluation, membranous staining was graded as the following: score 0–1+, 2+ and 3+ (14).

PATIENTS AND METHODS

Patients

We retrospectively reviewed the US findings and their corresponding histopathological features of 45 breast cancer patients with BI-RADS category 1 mammogram and 537 controls with mammographic abnormalities. The patients underwent needle biopsies or surgical resection at the Tohoku University Hospital from January 2006 to December 2010. We received informed consents from all the patients and the protocol for this study was approved by the Ethics Committee at Tohoku University Graduate School of Medicine.
Scoring of 2+ was added fluorescence in situ hybridization (FISH) that was used to calculate the gene copy ratio of HER2-to-CEP17 (the PathVysion HER2 DNA Probe Kit; Abbott, Chicago, IL, USA). Positive is defined as either HER2:CEP17 signal ratio (FISH score) >2.2 (14). Histological grades and mitotic counts were assessed according to the criteria of Elston and Ellis (15). Van Nuys classifications were also assessed for ductal carcinoma in situ and invasive ductal carcinoma (IDC) with predominant intraductal components cases (16,17). We also identified the presence or absence of lymphovascular invasion according to the Rosen’s Breast Pathology (12).

At first, we examined the differences of the patients’ characteristics between these two groups including the distribution of age, menopausal status, past history of the benign proliferative disease, background of detection, clinical stage, breast density of mammography according to the BI-RADS lexicon (9) and surgical strategy as the breast-conserving ratio.

We evaluated the US findings including mass shape, periphery, internal and posterior echo pattern, interruption of mammary borders and the distribution of low-echoic lesions and compared them with histopathological characteristics including histological classification, hormone receptor and HER2 status of IDC, tumor size confirmed by histopathology, histological grade, mitotic counts and lymphovascular invasion of BI-RADS category 1 mammograms. We then compared these findings with those of control group patients.

**Statistical Analyses**

Statistical analyses were performed using StatMate III for Windows ver. 3.18 (ATMS, Tokyo, Japan). The results were considered significant at \( P < 0.05 \).

**RESULTS**

**The Details of Both BI-RADS 1 and Control Groups**

Table 1 summarizes the difference in the patients’ characteristics including the distribution of age, menopausal status, past history of the benign proliferative disease, background of detection, clinical stage, breast density of mammography and surgical strategy. The median ages of the study group and the control group were 48 years (range, 32–84) and 56 years (range, 26–88), respectively \(( P = 0.047 \) ). There was a statistically significant higher ratio of Stages 0 and I, heterogeneously and extremely dense, and conserving surgery in the BI-RADS 1 group \(( P < 0.001, <0.001 \) and 0.002, respectively). However, there was a statistically significant lower ratio of menopause and self-palpation in the BI-RADS 1 group \(( P < 0.001, \) respectively; Table 1).

Table 1. The details of patients

<table>
<thead>
<tr>
<th></th>
<th>BI-RADS 1</th>
<th>Control</th>
<th>( P ) value</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>48 (32–84)</td>
<td>56 (26–88)</td>
<td>0.047</td>
<td>—</td>
</tr>
<tr>
<td>Menopausal ratio</td>
<td>37.8%</td>
<td>63.4%</td>
<td>&lt;0.001</td>
<td>0.31</td>
</tr>
<tr>
<td>Benign proliferative disease</td>
<td>2.2%</td>
<td>9.5%</td>
<td>NS</td>
<td>2.34</td>
</tr>
<tr>
<td>Cause of detection</td>
<td>24.4%</td>
<td>59.4%</td>
<td>&lt;0.001</td>
<td>0.22</td>
</tr>
<tr>
<td>(self-palpation ratio)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage (Stages 0 and I)</td>
<td>93.3%</td>
<td>66.4%</td>
<td>&lt;0.001</td>
<td>7.08</td>
</tr>
<tr>
<td>Heterogeneously and extremely dense ratio</td>
<td>91.1%</td>
<td>39.1%</td>
<td>&lt;0.001</td>
<td>15.97</td>
</tr>
<tr>
<td>Surgical strategy</td>
<td>95.6%</td>
<td>74.6%</td>
<td>0.002</td>
<td>7.82</td>
</tr>
</tbody>
</table>

**Figure 1.** Representative illustrations of the distribution of low-echoic lesions. (A) Spotted and (B) segmental.
THE RATIOS OF MASS CASES AND THE TUMOR SIZE

Twenty-six out of the 45 were US mass cases in the BI-RADS 1 group and 370 out of the 490 were US mass cases in the control group. There was a statistically significant difference between the BI-RADS 1 and control groups ($P = 0.003$). The US tumor size of BI-RADS 1 and control groups was 12.1 mm (range, 3.2–24.9 mm) and 18.5 mm (range, 6.5–150 mm) with statistically significant differences ($P < 0.001$).

EVALUATION OF THE US CHARACTERISTICS

Figure 2 summarizes the results of the numbers and ratios of mass shape (Fig. 2A), periphery (Fig. 2B), internal echo pattern (Fig. 2C), lateral echo pattern (Fig. 2D) and interruption of mammary borders (Fig. 2E) of the BI-RADS 1 and control groups. There were statistically higher ratios of round mass shape ($P < 0.001$), no change of lateral echo pattern ($P = 0.028$) and no or indeterminate interruption of mammary borders ($P < 0.001$) in the BI-RADS 1 group. There were statistically lower ratios of spiculated periphery ($P = 0.021$), attenuation of lateral echo pattern ($P = 0.011$) and interruption of mammary borders ($P < 0.001$) in the BI-RADS 1 group. Figure 3 summarizes the results of the numbers and ratios of distribution of low-echoic lesions. There were statistically higher ratios of spotted distribution and lower cases of segmental distribution in the BI-RADS 1 group than in the control group ($P = 0.012$).

EVALUATION OF THE CORRESPONDING HISTOPATHOLOGICAL CHARACTERISTICS

Figure 4 summarizes the results of the numbers and ratios of results classified by histological subtypes (Fig. 4A), hormone receptor and HER2 expression of IDC (Fig. 4B), tumor size of the invasive lesion as confirmed by the histopathological examination (Fig. 4C), histological grade (Fig. 4D), mitotic counts (Fig. 4E) and lymphovascular invasion (Fig. 4F). There was statistically higher ratios of triple-negative subtype, smaller tumor size and lower case of lymphovascular invasion in the BI-RADS 1 group ($P = 0.021$, $P < 0.001$ and $P = 0.012$, respectively) compared with the control group. In addition, a higher ratio of histological grade 3 was detected in the BI-RADS 1 group but this difference did not reach the statistical significance ($P = 0.094$).

![Figure 2](image_url)

**Figure 2.** The US characteristics of BI-RADS category 1 and control groups. (A) Mass shape, (B) periphery, (C) internal echo pattern, (D) lateral echo pattern and (E) interruption of mammary borders.
DISCUSSION

Mammography has been considered a gold standard for breast cancer screening system. However, US screening combined with mammography may have the potential to become one of the useful screening systems to decrease breast cancer mortality according to the results of the J-START trial (7). Therefore, strict or rigorous conformation to high quality of interpreting the US findings is required or mandatory for the future success of an US diagnosis especially at the level of mass screening. Our present study is the first study to focus upon incremental detection of breast cancer by US in asymptomatic women with mammography-negative breasts, and focused on the US findings and the corresponding histopathological characteristics of the cases with BI-RADS category 1 mammograms.

US detected cancers are in general smaller than those identified with mammography. Results of our present study demonstrated that the BI-RADS category 1 group was associated with a statistically higher ratio of low-echoic lesions than the control group. In addition, 52.6% of low-echoic lesions demonstrated spotted distribution in the BI-RADS 1 group, whereas 25.8% of low-echoic lesions spotted...
distribution in the control group. A low-echoic lesion with spotted distribution is therefore considered one of the predicting factors of malignancy in the BI-RADS category 1 group. In addition, the tumor size of the BI-RADS 1 group was smaller, and the detected masses were characterized by a higher ratio of round shape, non-spiculated periphery, non-interruption of mammary borders and non-attenuation in the BI-RADS category 1 group. These results could be mainly affected by mammographic breast density. In addition, results of our present study also demonstrated that there was a statistically higher ratio of heterogeneously and extremely dense breast in the BI-RADS 1 group and the tumors with well-collagenized stromal reaction were also detected as architectural distortion or spiculation in dense breast mammogram. Therefore, mammographic breast density was reasonably postulated to influence characteristics of breast cancers with BI-RADS category 1. Results of previous studies demonstrated that the most breast cancer cases of BI-RADS category 1 were relatively hypoechoic within a background of hyperechoic fibroglandular tissue, which may make the lesions more conspicuous and detectable (18). However, it is also true that previous studies have not evaluated the US findings of BI-RADS category 1 cases and this is the first study demonstrating the US findings such as mass shape and periphery of BI-RADS category 1 cases. In addition, this is the first reported study to demonstrate histopathological characteristics of BI-RADS category 1 cases. The statistically higher ratio of triple-negative subtype was detected in BI-RADS category 1 cases, and histological grade 3 tended to be also higher in the BI-RADS category 1 group. Results above did indicate that the BI-RADS category 1 group was histologically characterized by a higher malignant level than those with mammographic abnormalities, but it awaits further investigations for clarification.

Previous study also demonstrated that earlier detection of breast cancer resulted in a decrement in mortality, which parallels the reduction in size distribution of cancers depicted and closely parallels the reduction in rates of node-positive breast cancer (19). Screening US also appears to detect many breast cancer cases at a smaller size and earlier stage compared with mammographic screening. In addition, in women with mammography dense breast, US was reported to be able to detect a substantially larger number of cancers with a supplemental cancer detection of 0.3–0.5% by US alone (18). Therefore, it is important to detect the US findings with the localized low-echoic lesion. In addition, among the BI-RADS category 1 group, particular attention should be paid to the US findings such as solitary differentiated masses such as oval or round shape and non-spiculated periphery because the corresponding histopathological features of the cases associated with these US findings above include a much higher ratio of triple-negative subtype and/or histological grade 3. Therefore, early detection of such solitary masses with triple-negative subtype and/or high histological grade by US may possibly contribute to the eventual reduction in breast cancer mortality.

We evaluated US findings and the corresponding histopathological characteristics for BI-RADS category 1 mammograms and noted significant differences among these findings in this study. Evaluation of these US and histopathological characteristics may provide a more accurate US screening system for Japanese women.

Acknowledgement

We thank Yayoi Takahashi, MT, for her excellent technical assistance for immunohistochemical staining.

Funding

This work was supported in part by a Grant-in-Aid from ‘Kurokawa Cancer Research Foundation’.

Conflict of interest statement

None declared.

References


