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RESEARCH ARTICLE

Molecular Subtypes of Breast Cancers from Myanmar Women: A Study of 91 Cases at Two Pathology Centers

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Abstract

Background: Breast cancer is the most common cancer in Myanmar women. Revealing the hormonal receptor status, human epidermal growth factor receptor 2 (HER2) and Ki-67 expression is useful for estimating patient prognosis as well as determination of treatment strategy. However, immunohistochemical features and classification of molecular subtypes in breast cancers from Myanmar remain unknown. **Methods:** The clinicopathological features of 91 breast cancers from Myanmar women were examined. Immunohistochemistry was performed on tissue specimens with antibodies to estrogen receptor (ER), progesterone receptor (PgR), HER2, Ki-67, cytokeratin (CK)5/6 and CK14. Immunohistochemistry-based molecular subtyping was conducted. **Results:** Breast cancers in Myanmar women were relatively large, high grade with frequent metastatic lymph nodes. Of the 91 patients, tumors with ER positive, PgR positive, and HER2 positive were 57.1%, 37.4%, and 28.6%, respectively. The most prevalent subtype was luminal B (HER2-) (39.6%), followed by HER2 (22.0%), triple negative (TN)-basal-like (12.1%), luminal A (11.0%), TN-null (8.8%) and luminal B (HER2+) (6.6%). The mean Ki-67 expression of 91 cases was 33.9% (33.9% ± 19.2%) and the median was 28% (range; 4%-90%). The mean Ki-67 expression of luminal A, luminal B, HER2 and TN-basal-like/null was 7%, 30%, 40%, and 57%/43%, respectively. A higher Ki-67 expression significantly correlated with a higher grade, larger size and higher stage of malignancy. **Conclusions:** We, for the first time, investigated the histopathological features of breast cancers from Myanmar women. Myanmar breast cancers appeared to be aggressive in nature, as evidenced by high frequency of poor-prognosis subtypes with high level of Ki-67 expression.

Keywords: Breast cancer- molecular subtypes- Ki-67 expression- Myanmar

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Introduction

Breast cancer is a heterogeneous disease, composed of many morphological and molecular entities. The heterogeneity reflects different clinical outcomes (Polyak, 2007). By gene expression profiling, breast cancer is classified into 5 intrinsic subtypes (Perou et al., 2000; Sølie et al., 2001). For clinicopathological practice, 5 molecular subtypes, luminal A, luminal B (HER2-), luminal B (HER2+), HER2 and triple negative (TN), are defined based on the expression of estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), and Ki-67 (Goldhirsch et al., 2011). TN was subdivided into two subtypes, basal-like and null (Nielsen et al., 2004; Chuangsuwanich et al., 2014). It is well known that molecular subtypes are important biological markers not only for predicting prognosis but for decisions of effective treatment (Blows et al., 2010; Engstrø et al., 2013; Sung et al., 2016). Luminal types are cancers with a better prognosis in comparison to non-luminal types. Luminal breast cancer subtypes were found to predominate across racial/ethnic groups, while frequency of TN breast cancer progressively increases among white American, African-American, and Ghanaian/Africans (Amirikia et al., 2011; Huo et al., 2009; Kurian et al., 2010; Stark et al., 2010).

As with other countries, breast cancer is a leading cause of morbidity and mortality in Myanmar women (Moore, 2014). The aim of this study was to unveil the histopathological features and molecular subtypes in Myanmar breast cancer patients. We analyzed the clinicopathological characteristics and expression patterns of ER/PgR/HER2/Ki-67 using 91 female patients with breast cancer.

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Materials and Methods

Study subjects

A total of 91 female patients diagnosed with breast cancer in the year 2015 were retrieved from pathology records at Myeik General Hospital (Myeik City, Myanmar) and Sakura Specialist Hospital (Yangon City, Myanmar). Clinicopathological data such as age, tumor size, lymph node and distant metastasis status were retrieved from the clinical and pathology records. All hematoxylin and eosin-stained sections were reviewed by two independent pathologists. Criteria defined by the World Health Organization (2012) were used for the histopathological diagnosis and classification of breast carcinoma. Nottingham combined histological grading system (Elston and Ellis, 1991) was used for tumor grading. American Joint Committee on Cancer staging system 8th edition was applied for tumor staging. The experimental protocol employed in this study was approved by the Ethics Committee of Okayama University (Japan) and the Ethics Review Committee of Department of Medical Research of Yangon City (Myanmar).

Immunohistochemical analysis

Immunohistochemistry was performed by using standardized automated techniques according to the manufacturer's protocol. Tissue sections were stained with antibodies ER (SP1), PgR (1E2), HER2 (4B5), Ki-67 (MIB-1), cytokeratin (CK) 5/6 (D5/16B4) and CK14 (LL002) using a Discovery XT autostainer with iVIEW DAB detection kit (Ventana Medical Systems, Tucson, Arizona). In case of ER/PgR, a staining > 1% of tumor cell nuclei was considered positive (Hammond et al., 2010). HER2 was analyzed according to the American Society of Clinical Oncology and College of American Pathologists (Wolff et al., 2013). A score of 0 to 1+ was considered as negative and 3+ as positive. Specimens with score of 2+ were analyzed by HER2 dual-color in situ hybridization at Hyogo Clinical Laboratory Cooperation (Himeji City, Japan). Ki-67 labeling index was defined as the percentage of positive nuclei, in which over 500 tumor cells were counted in each case. CK5/6 and CK14 were scored positive if any (weak or strong) cytoplasmic and/or membranous staining was observed. Breast cancers were classified into 5 molecular subtypes according to the St. Gallen International Expert Consensus 2011 (Goldhirsch et al., 2011): luminal A; ER/PgR+/HER2-/Ki-67<14%, luminal B (HER2-); ER/PgR+/HER2-/Ki-67≥14%, luminal B (HER2+); ER/PgR+/HER2+/any Ki-67 percentage, HER2; ER/PgR-/HER2+, and TN; ER/PgR-/HER2-. TN subtype was further subdivided into basal-like type (ER/PgR-/HER2- with CK5/6+ and/or CK14+) and null type (ER/PgR-/HER2-/ CK5/6-/CK14-).

Statistical analysis

Statistical analyses were performed using SPSS software version 16.0. Fisher's exact test was used for data categorized into 2 × 2 contingency tables. The age, mitosis count and Ki-67 expression were reported using descriptive statistics and Mann Whitney U test to compare the mean of the variables. Two-sided tests were used for all analyses and p value <0.05 was considered as significant.

Results

Patient characteristics

Clinical data for the enrolled 91 female patients with breast cancer were shown in Table 1. Ages ranged from 30 to 81 years with mean age of 51.3. Tumor sizes ranged from 1.5 cm to 7.2 cm with average size of 4.0 cm. Most of the cancers (94.5%) were invasive carcinoma of no special type (NST) with high histological grade (grade II; 46.2%, grade III; 53.8%). No grade I tumor was found. There were lymph node metastases in 57.1% of patients. The numbers of patients with stage I, II, III and IV were 6 (6.6%), 50 (54.9%), 31 (34.1%) and 4 (4.4%), respectively.

Molecular subtypes and immunophenotypic analyses

ER positive, PgR positive and HER2 positive cases were 57.1%, 37.4% and 28.6%, respectively. Molecular subtypes and clinicopathological features of 91 breast cancers were shown in Table 2. The frequency of luminal A, a good prognostic subtype, was 11.0%. The most common subtype (39.6%) was luminal B (HER2-). Poor-prognosis subtypes, HER2 and TN subtypes, were

Table 1. Clinical Data for the Enrolled Breast Cancer Patients

Categories	Number of Cases (%)
Age (years)	
<35	4 (4.4)
35-50	39 (42.9)
>50	48 (52.7)
Tumor size (cm)	
≤2.0	6 (6.6)
2.1-5.0	61 (67.0)
>5.0	24 (26.4)
Histological type	
Invasive carcinoma of no special type (NST)	86 (94.5)
Mucinous carcinoma	2 (2.2)
Carcinoma with neuroendocrine differentiation	2 (2.2)
Pleomorphic lobular carcinoma	1 (1.1)
Histological grade	
Grade I	0 (0.0)
Grade II	42 (46.2)
Grade III	49 (53.8)
Metastatic lymph node	
Absent	39 (42.9)
Present	52 (57.1)
Staging	
Stage I	6 (6.6)
Stage II	50 (54.9)
Stage III	31 (34.1)
Stage IV	4 (4.4)

Table 2. Molecular Subtypes and Clinicopathological Features of Breast Cancers

	Luminal A	Luminal B		HER2	Triple Negative		Total
		HER2-	HER2+		basal-like	null	
Number (%)	10 (11.0)	36 (39.6)	6 (6.6)	20 (22.0)	11 (12.1)	8 (8.8)	91
Age (years)							
Mean (range)	58 (42-81)	52 (32-75)	47 (30-60)	51 (30-74)	49 (35-62)	50 (37-67)	
p value	reference	0.158	0.103	0.134	0.121	0.142	
Tumor Size							
≤2cm	3	2	1	0	0	0	6
>2cm	7	34	5	20	11	8	85
p value	reference	0.061	1	0.030*	0.09	0.216	
Histological grade							
Grade II	10	25	1	2	1	3	42
Grade III	0	11	5	18	10	5	49
p value	reference	0.088	0.001*	<0.001*	<0.001*	0.007*	
Nodal status							
negative	7	15	2	6	7	2	39
positive	3	21	4	14	4	6	52
p value	reference	0.159	0.302	0.056	1	0.153	
Stage							
Stage I	3	2	1	0	0	0	6
Stage II to IV	7	34	5	20	11	8	85
p value	reference	0.061	1	0.030*	0.09	0.216	
Ki-67 expression							
Mean (range)	7 (4-13)	30 (17-80)	30 (9-54)	40 (18-77)	57 (26-90)	43 (20-75)	
p value	reference	<0.001*	0.003*	<0.001*	<0.001*	<0.001*	
Mitoses/10HPF							
Mean (range)	12 (6-20)	27 (6-86)	39 (21-72)	40 (18-93)	77 (38-118)	49 (18-110)	
p value	reference	0.002*	0.001*	<0.001*	<0.001*	0.001*	

*statistically significant

22.0 and 20.9%, respectively. Among TN subtypes, 11 cases (12.1%) were basal-like type (7 cases; CK5/6+ and CK14+, 4 cases; CK5/6- and CK14+) and 8 cases (8.8%) were null type. Compared to luminal A as the reference, tumors larger than 2 cm were more in HER2 subtype. TN-basal-like also tended to be larger than 2 cm although it was not statistically significant. Histological grade III (poorly differentiated) was more frequently found in non-luminal A subtypes, particularly in TN-basal-like and HER2, with more advanced stages at diagnosis (stage II, III and IV). As compared to luminal A, HER2 subtype tended to metastasize to lymph nodes, although it was not statistically significant (Table 2). The mean Ki-67 index of all 91 cases was 33.9% (mean ± SD, 33.9 ± 19.2) and the median score was 28% (range; 4%-90%). In the subtype analyses, the mean Ki-67 expression was increased in the following order; luminal A < luminal B < HER2 < TN (null<basal-like), which corresponded well to the increased number of mitoses (Table 2).

Association between Ki-67 expression and clinicopathological features

The Ki-67 expression was divided into 3 levels (<14; 12.1%, 14-30; 40.7%, >30; 47.2%) and the associations between Ki-67 expression and clinicopathological features were investigated (Table 3). Compared to Ki-67<14 as the reference, tumors with increased Ki-67 expression were associated with unfavorable features such as more mitoses and higher grade. Furthermore, younger patients, larger size (>2 cm), and higher stage were related to tumors with >30% Ki-67 expression. Interestingly, no difference was found in nodal status. ER/PgR negative tumors showed higher Ki-67 expression, whereas Ki-67 expression was not associated with HER2 overexpression. Overall, these data indicated that Ki-67 expression was significantly accompanied by advanced and aggressive cancer.

Discussion

The present study reported the histopathological characteristics of Myanmar breast cancer patients. We analyzed 91 cases, in which 93% of the breast cancers were larger than 2 cm. There was no histological grade I and 53.8% of the cancers were grade III. Over half of the patients (57.1%) had lymph node involvement at the time of diagnosis. These findings may result from the lack of early detection system in Myanmar. It is also possible that

Table 3. Association between Ki-67 Expression and Clinicopathological Features

	Ki-67 index Categories (%)			Total
Index	<14	14-30	>30	
Number (%)	11 (12.1)	37 (40.7)	43 (47.2)	91
Age (years)				
Mean (range)	57 (42-81)	54 (32-75)	48 (30-75)	
p value	reference	0.589	0.037*	
Tumor size				
≤2cm	3	2	1	6
>2cm	8	35	42	85
p value	reference	0.072	0.023*	
Histological grade				
Grade II	10	20	12	42
Grade III	1	17	31	49
p value	reference	0.035*	<0.001*	
Nodal Status				
positive	4	22	26	52
negative	7	15	17	39
p value	reference	0.302	0.186	
Stage				
Stage I	3	2	1	6
Stage II to IV	8	35	42	85
p value	reference	0.072	0.023*	
ER				
positive	11	26	15	52
negative	0	11	28	39
p value	reference	0.048*	<0.001*	
PgR				
positive	11	15	8	34
negative	0	22	35	57
p value	reference	<0.001*	<0.001*	
HER2				
positive	1	9	16	26
negative	10	28	27	65
p value	reference	0.416	0.143	
Mitoses/10HPF				
Mean (range)	14 (6-36)	31 (6-108)	48 (6-118)	
p value	reference	0.001*	<0.001*	

Myanmar breast cancers are aggressive in nature.

Revealing the hormonal receptor status and HER2 and Ki-67 expression is useful not only for treatment strategy but for estimating the patient prognosis (malignant potential) (Goldhirsch et al., 2011). Generally, overall survival of breast cancers by subtypes from good- to poor-prognosis is: luminal A, luminal B, HER2 and TN (Hennigs et al., 2016). ER positive breast cancers are most frequent in western countries. In the United States, most (73%) breast cancers are luminal A and luminal B (HER2-) subtypes. About 10% of breast cancers are luminal B (HER2+), 4% are HER2 and 12% are TN (Howlader et al., 2014). Table 4 shows the summary of the percent composition of molecular subtypes of breast cancers in western countries, South Asia, South-east Asia and East Asia. In Asian countries except China, HER2 and

Table 4. Percent Composition of Molecular Subtypes of Breast Cancer in Different Countries

	Luminal A (%)	Luminal B (%) HER2-	Luminal B (%) HER2+	HER2 (%)	TN (%)	Number of cases	reference
United States	(72.7	)	10.3	4.6	12.2	50,571	Howlader et al., 2014
Italy	34.0	25.0	11.0	10.2	19.0	1,487	Caldarella et al., 2013
Germany	44.7	31.8	6.2	5.0	12.3	3,454	Hennigs et al., 2016
Australia	29.0	37.0	14.0	4.6	16.0	285	Mandaliya et al., 2016
India	(53.3	)	10.1	13.0	23.8	1,284	Doval et al., 2015
China	(65.3	)	19.0	6.5	9.2	3,198	Zhu et al., 2014
Japan	30.6	26.2	19.0	11.3	12.9	363	Yanagawa et al., 2012
Indonesia	38.1	(16.7	)	20.2	25.0	84	Widodo et al., 2014
Malaysia	(48.0	)	12.0	11.0	29.0	1,034	Devi et al., 2012
Thailand	39.0	8.0	10.0	18.0	25.0	100	Chuangsuwanich et al., 2014
Vietnam	10.6	33.5	23.0	19.3	13.6	237	Thang et al., 2015
Myanmar	11.0	39.6	6.6	22.0	20.9	91	Present study

TN subtypes are prevailing compared to those in western countries. In the present Myanmar study, unfavorable molecular subtypes, HER2 and TN, were responsible for 42.9% of all cancers, suggesting that most breast cancers in Myanmar are aggressive in nature. Interestingly, the composition of molecular subtypes in Myanmar (this study) is comparable to that in a Vietnamese study (Thang et al., 2015), with low luminal A and B and in contrast high HER2 and TN. These similarities may be related to some genetic or biological connections, as well as comparable environmental factors.

In conclusion, the present study for the first time highlights the clinicopathological features of breast cancers from Myanmar women, which provide valuable information for the breast cancer control. Approximately 80% of the breast cancers were positive for either hormone receptor or HER2. Hormone therapy and anti-HER2 therapy may offer significant benefits to the Myanmar breast cancer patients, when used properly. Moreover, it is essential to establish early detection of breast cancer: education to promote early diagnosis and screening.

Early detection alleviates the advanced breast cancer and increases the opportunities for successful treatment.

Conflict of Interest

Authors have no conflict of interest to declare.

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