RESEARCH ARTICLE

Clinicopathological Features of Indonesian Breast Cancers with Different Molecular Subtypes

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Abstract

<u>Background</u>: Breast cancer is a heterogeneous disease with molecular subtypes that have biological distinctness and different behavior. They are classified into luminal A, luminal B, Her-2 and triple negative/basal-like molecular subtypes. Most of breast cancers reported in Indonesia are already large size, with high grade or late stage but the clinicopathological features of different molecular subtypes are still unclear. They need to be better clarified to determine proper treatment and prognosis. <u>Aim</u>: To elaborate the clinicopathological features of molecular subtypes of breast cancers in Indonesian women. <u>Materials and Methods</u>: A retrospective cross-sectional study of 84 paraffin-embedded tissues of breast cancer samples from Dr. Sardjito General Hospital in Central Java, Indonesia was performed. Expression of ER, PR, Her-2 and Ki-67 was analyzed to classify molecular subtypes of breast cancer by immunohistochemistry. The relation of clinicopathological features of breast cancers with molecular subtypes of luminal A, luminal B, Her-2 and triple negative/basal-like were analyzed using Pearson`s Chi-Square test. A p-value of <0.05 was considered statistically significant. <u>Results</u>: Case frequency of luminal A, Luminal B, Her-2+ and triple negative/basal-like subtypes were 38.1%, 16.7%, 20.2% and 25%, respectively. Significant difference was found in breast cancer molecular subtypes in regard to age, histological grade, lymph node status and staging. However it showed insignificant result in regard to tumor size. Luminal A subtype of breast cancer was commonly found in >50 years old women (p:0.028), low grade cancer (p:0.09), negative lymph node metastasis (p:0.034) and stage III (p:0.017). Eventhough the difference was insignificant, luminal A subtype breast cancer was mostly found in small size breast cancer (p:0.129). Her-2+ subtype breast cancer was more commonly diagnosed with large size, positive lymph node metastasis and poor grade. Triple negative/basal-like cancer was mostly diagnosed among <50 years old women. <u>Conclusions</u>: This study suggests that immunohistochemistry-based subtyping is essential to classify breast carcinoma into subtypes that vary in clinicopathological features, implying different therapeutic options and prognosis for each subtype.

Keywords: Breast cancer - molecular subtypes - clinicopathological features

Introduction

Breast cancer is a heterogeneous disease, consists of several molecular subtypes with different biological behavior, epidemiological risk factor, natural histories, response against local and systemic treatment and also prognosis (Goldhirsch et al., 2011). Based on the molecular expression of ER, PR, Her-2 and Ki-67, breast cancer is classified into luminal A, luminal B, Her-2+ and triple negative subtypes/basaL-like (Perou et al., 2000; Onitilo et al., 2008; Kao et al., 2009; Blows et al., 2010). Molecular characteristics of the luminal A subtype are ER + and/or PR+, Her-2– and low proliferation rate, while the luminal B subtype is characterized by ER + and/or PR+, Her-2+ and high proliferation rate. The Her-2+ subtype characteristics are ER/PR and Her-2+

expression, meanwhile the triple negative/basal-like subtype is characterized by negative expression of ER/PR and Her-2-.

Sixty percent of breast cancers are luminal subtype cancers arising from luminal epithelial cell that lined the duct of mammary gland. The luminal subtype of breast cancer tend to have a better prognosis compared with the non-luminal subtype because the luminal subtype is a hormone receptor-positive. Therefore, it is more sensitive to hormone therapy approach. The Her-2+ and triple negative/basal-like molecular subtypes arising from the basal cell of the mammary gland. These subtypes of breast cancer have a fairly poor prognosis and more prone to early and frequent recurrence and metastasize. Prognosis of Her-2+ subtype is better compared with triple negative/basal-like subtype since it can be treated with the drug

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trastuzumab /Herceptin (Sorlie et al., 2003; Chanrion et al., 2007; Campbell et al., 2011).

Breast cancer study among Chinese women showed that frequency of luminal A, luminal B, Her-2+, triple negative/basal-like subtypes were: 48.6%, 16.7%, 13.7% and 12.9%, respectively. The luminal A subtype was significantly higher among 70 years old women, early stage, small size and low grade tumors, and it is reported to have longer survival rate compared to the non luminal one. The triple negative/basal-like subtype was significantly correllated with familial breast cancers (Su et al., 2011). Another research in China found that luminal A cancers have the best prognosis, whereas Her-2+ cancers have the poorest (Jia et al., 2014). Breast cancer study in Egypt found the highest frequency was luminal A subtype, followed by triple negative, Her-2+, and luminal B cancers. The luminal subtype is significantly associated with low grade cancers and low lymph node metastasis (El-Hawary et al., 2012). A study of early stage breast cancers in Iran suggested that Luminal A cancers have the best disease-free survival and Her-2+ cancers have the worst (Najati et al., 2013). A study done by Chuthapisith et al., suggested that percentages of Her-2+ and basal-like cancers in Thai women were higher, as compared with a study from the USA (Chuthapisith et al., 2012).

The frequency of Indonesian breast cancers based on molecular subtypes are still unknown. Previous study reported that 65% of Indonesian breast cancers were found already in late stage, poor grade and with lymph nodes metastasis (Widodo et al., 2013). Therefore, study of clinicopathological features of Indonesian breast cancers with different molecular subtypes need to be done, in order to determine proper cancer management and prognosis.

Materials and Methods

This study is a retrospective cross-sectional study, analyzing 84 embedded paraffin blocks of breast carcinoma taken from Dr. Sarjito General Hospital Indonesia in the year of 2008-2009. Samples were chosen using a consecutive sampling method. Samples containing small specimen were excluded from this research.

Samples were stained histologically with Hematoxyllin Eosin to determine histological grade and lymph node status. Histological grade of cancer was grouped into low, moderate and poor grade based on the Elston and Ellis criteria (Tavassoli and Devilee, 2003). Lymph node status was classified into negative lymph node metastasis, tumor metastasis to ≤ 3 lymph nodes and tumor metastasis to >3 lymph nodes. Cancer Staging was classified into stage I, II and III. Tumor size was categorized into <2cm, 2-5cm and >5 cm. Patient age was grouped into ≤ 50 and >50 years old.

Immunohistochemical (IHC) staining using MoAb anti ER (M 7046 Dako, dilution 1:50), PR (PgR 636 Dako, dilution 1:50), Her-2 (CB 11 Dako, dilution 1:100) and Ki-67 (ab 16667 abcam, dilution 1:100), DAB chromogen and counter stain Hematoxyllin Mayer, was performed to classify molecular subtype of breast cancers. Normal breast tissue was used as positive control, meanwhile negative control was obtained by omitting the primary antibodies. Interpretation of IHC expression was determined using Photoshop-based image analysis.

ER/PR expression is considered positive if it is stained in >1% of tumor nuclei of the total tumor cells (Hammond et al., 2010). Her-2 positive cancers if they were scored 3+ (Wolff et al., 2007). Cancers with Her-2 scored 2+ (indeterminate) were considered negative for Her-2 in the absence of fluorescent in situ hybridization (FISH) or CISH data. High ki-67 rate is positive if >14% of cancer cells show positive nuclear staining (Gnant et al., 2011). Characteristics of breast cancer of luminal A subtype are ER and or PR+, Her-2 -, and low Ki-67 proliferation rate. Luminal B cancer subtype characteristics are: ER and or PR+, Her-2+, and high ki-67 rate. Her-2+ molecular subtype will show ER and PR negative, but positive Her-2 expression. Triple negative/basal-like cancer subtype is characterized by ER/PR and Her-2 negative staining. The differences of molecular subtypes of Indonesian breast cancers in regard to several clinicopathological features were analyzed using Pearson`s Chi-Square test. A p-value of <0.05 was considered statistically significant.

Results

This study found, mean of patients` age at diagnosis was 53.15±10.89 years old (range from 31-81 years old). Patients were divided into >50 years old (54.8%) and <50 years old (45.2%). Clinicopathological characteristics of breast cancer in Indonesia are described in Table 1. Based on histological grade in breast cancer, moderate differentiation is the most common grade (44%), compared to well and poor differentiation grade. Breast cancer cases are mostly reported by patients when the tumor is already >5cm in size (46.5%) and with positive lymph nodes metastasis (60.8%). Breast cancer patients in Indonesia usually identify the tumor when it is already in late stage (54.8%). Based on immunohistochemistry result (Figure 1), luminal A molecular subtype of breast cancer showed the highest percentage (38.1%), followed

Table 1. Characteristic and Frequency of Indonesian Breast Cancer Based on Histological Grade, Tumor Size, Lymph Node Status, Stage and Molecular Subtypes

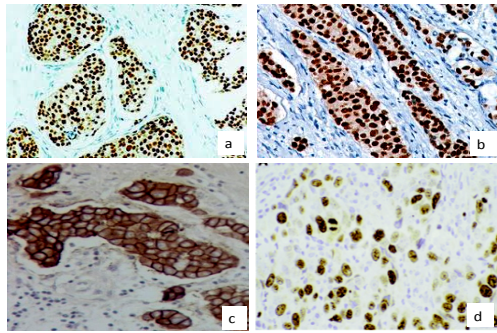| Characteristic		Number (%)
Histological grade	well	13 (15.5%)
	moderate	37 (44%)
	poor	34 (40.5%)
Tumor size	<2 cm	19 (22.6%)
	2– 5 cm	26 (30.9%)
	>5 cm	39 (46.5%)
Lymph node status	negative	33 (39.2%)
	Metastasis to ≤ 3 lnn	26 (31%)
	Metastasis to >3 lnn	25 (29.8%)
Stage	I	11 (13.1%)
	II	27 (32.1%)
	III	46 (54.8%)
Molecular subtype	Luminal A	32 (38.1%)
	Luminal B	14 (16.7%)
	Her-2 positive	17 (20.2%)
	Triple negative/basal-like	21 (25%)

Figure 1. Immunohistochemistry result. A) Nuclear ER expression. **B)** Nuclear PR expression. **C)** Membranous Her-2 expression. **D)** Nuclear ki-67 expression
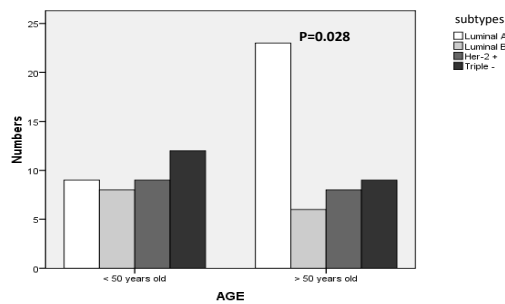

Figure 2. Association between Molecular Subtype of Breast Cancer and Age. Pearson`s Chi-Square test showed that there is significant difference of molecular subtype of breast cancer between patient more than 50 year old and patient less than 50 year old (p =0.028)
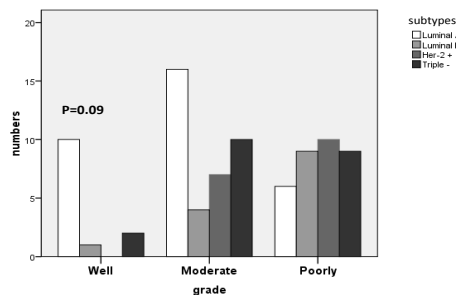

Figure 3. Association between Molecular Subtype of Breast Cancer and Grade. Statistical analysis showed that there is significant difference of molecular subtype of breast cancer among well, moderate and poorly differentiated grade cancer (p=0.09)
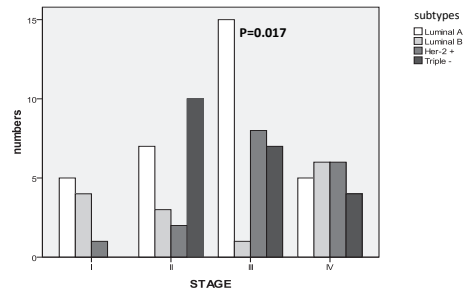

Figure 4. Association between Molecular Subtype of Breast Cancer and Stage. Statistical analysis showed that there is significant difference of molecular subtype of breast cancer among different stages of breast cancer (p=0.01)
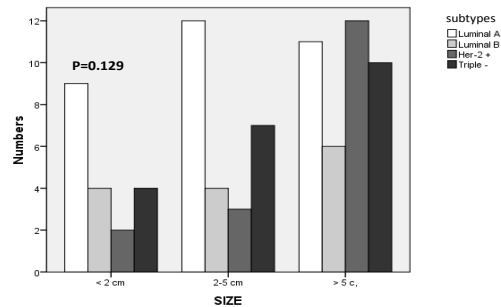

Figure 5. Association between Molecular Subtype of Breast Cancer and Tumor Size. Statistical analysis showed that there is no significant difference of molecular subtype of breast cancer among different sizes of the tumor (p=0.129)

by triple negative/basal-like (25%), her-2 positif (20.2%) and luminal B molecular subtype (16.7%).

Statistical analysis showed highly significant differences between the breast cancer subtypes in regard to most tested variables, as shown in Figure 2, 3, 4, and 5. Significant difference was found in breast cancer molecular subtypes in regard to age, histological grade, lymph node status and staging. However, molecular subtype in regard to tumor size was not significantly different (p:0.129). Luminal A subtype of breast cancer was commonly found in >50 years old patient (p:0.028), low grade cancer (p:0.09), negative lymph node metastasis (p:0.034) and stage III (p:0.017). Even though luminal A subtype was more commonly found in small size cancer, the difference was not significant (p:0.129). Her-2 positive

subtype was mostly diagnosed in large size tumor, positive lymph node metastasis and poor grade variable. Luminal B subtype number is more prone to increase in regard to poor grade, late stage and tumor size, as they showed high expression of cell proliferation markers, Ki-67. Triple negative/basal-like cancer was mostly found in <50 years old women and the number tend to increase in regard to bigger tumor size.

Discussion

Mean of patient`s age in this study was 53.15 years old, similar to Thai study in which the average age was 52 yers old (Chuthapisith et al., 2012). Studies in Iran got lower results in which the mean age at diagnosis was 50±12 years old and 47.9 years old (Kadivar et al., 2012; Najafi et al., 2013), meanwhile higher mean of patient's age (62.7%) was found in Marshfiled Clinic/St Joseph Hospital Wisconsin study (Onitilo et al., 2009). In this study the number of breast cancer patients of >50 years old was higher (54.8%) compared to <50 years old patients (45.2%). Diffferent results were got from the study in Iran in which the number of patients <50 years old was higher than patients >50 years old (Kadivar et al., 2012; Najafi et al., 2013). Different etnic and genetic may highlight in those different results.

Percentage of luminal subtypes in this study was higher (54.8%) than non luminal subtypes. The luminal A subtype was the most common cancers. This results were similar with others (Perou et al. 2000; Sorlie et al, 2003; Milikan et al., 2008; Jia et al., 2010; Su et al., 2011; Najafi et a., 2012, Chuthapisith et al., 2012). Different result was reported

in breast cancer study of African-American women and African women, in which the highest frequency was triple negative subtype (Carey et al., 2006; Huo et al., 2009) and a study among North African breast cancer women that luminal B subtype was in the first rank (El-Fatemi et al., 2012). In Pakistan, percentages of non luminal cancers were higher than the luminal. Among luminal subtypes, luminal B cancer was more frequent than luminal A cancer (Khokher et al., 2013). In this study luminal B subtype was the rarest cancer similar to previous studies in Iran, Egypt and Thai (Chuthapisith et al., 2012; El-Hawary et al., 2012; Kadivar et al., 2012), but differ from another study in Iran in which the rarest cancer was Her-2+ subtype (Najafi et al., 2013). Among non luminal subtypes in this study, triple negative/basal-like subtype was more frequent (25%) than Her-2+ subtype (20.2%), similar to Iran and Thai studies (Kadivar et al., 2012; Chuthapisith et al., 2012; Najafi et al., 2013). This various distribution of molecular subtypes among world-wide population suggested the important role of human race or etnicity in molecular subtyping of breast cancers.

This study found significant differences of molecular subtypes of breast cancers in regard to age, histological grade, lymph node status and stage, but no significant difference in regard to tumor size. The luminal A subtype in this study mostly diagnosed in >50 years old, low histological grade, negative lymph node metastasis, similar to Marshfield Clinic/St Joseph's, China and Iran studies (Onitilo et al., 2009; Su et al., 2011, Najafi et al., 2013). Even though not significantly correlation, in this study small size tumor mostly found in luminal A sybtype, similar to Iran study (Kadivar et al., 2012). The Luminal B subtype in this study is more prone to increase in regard to poor grade, late stage and tumor size. This is related to its characteristic as hormonal positive cancers with poor prognosis due to the high expression of cell proliferation markers such as Ki-67 and cyclin B1 (Fan et al., 2006; Loe et al., 2007). Study in china found luminal B cancer had smaller tumor size than luminal A cancer. However, luminal B and luminal A subtypes showed similar rates of lymph node metastasis and age (Jia et al., 2014). Her-2+ subtype in this study was mostly occured in large size tumor, positive lymph node metastasis and poor grade variable. Meanwhile, triple negative subtype commonly found in <50 years old, similar to studies in Thai and Iran (Chuthapisith et al., 2012; Kadivar et al., 2012; Najafi et al., 2013). These results suggested that luminal A subtype cancers were associated with favorable clinicopathological factors while Her-2 positive and triple negative subtypes were associated with poor outcomes (Carey et al., 2006; Bosch et al., 2010; Su et al., 2011; Jia et al., 2014).

The favorable clinicopathological factors of luminal A subtype cancers in compare to non luminal cancers were supported by many studies showed low frequency (12-15%) of p-53 mutation and cell proliferation rate in luminal A subtypes, while non luminal cancers have high results (40%) (Carey et al., 2006; Bosch et al., 2010). P-53 mutation and cell proliferation rate in breast cancer are important markers for poor prognostic prediction. Luminal A subtype cancers also had lower recurency rate (27.8%) and higher survival rate (median survival rate: 2.2 years)

in compare to non luminal cancers (Eroles et al., 2012; Guarneri et al., 2009). A study in Chinese cancer women found that luminal A subtype had the highest locoregional relaps -free survival (93.2%), distant metastasis-free survival (91.5%) and disease free survival rates (87.5%) at 5 years, while Her-2+ subtypes showed the highest rate reccurence (27.5%) and locoregional recurrence (11.4%) (Jia et al., 2014).

This study found that among group of stage I-III, luminal A subtype was the most common cancers. This surprising result mainly due to imbalance proportion between number of stage I, II and III samples. Frequency of stage III samples was high (58.4%) while frequency of stage I samples was only 13.1%. Therefore, study of breast cancers with equal number of samples in each stage is very important to be done in order to know the role of cancer stage in molecular subtypes.

In conclusion, this study suggests that immunohistochemistry-based subtyping is extremely important to classify breast carcinoma into molecular subtypes that vary in clinicopathological features. Different molecular subtypes will lead to different prognosis and therapeutic option. Thus, molecular subtyping is essential for breast carcinoma management.

Acknowledgements

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