Role of Hormonal Receptor in Predicting Sentinel Lymph Node Metastasis in Early Breast Cancer

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ABSTRACT
Background: Sentinel lymph node biopsy is one of the minimally invasive techniques that can confirm the presence of metastasis of regional lymph nodes in cancer. Sentinel lymph node biopsy can be done with a lymph mapping technique using blue-dye, radiotracer, or a combination of both. In developing countries, sentinel lymph node biopsy is often done with a single agent, which is the blue dye. The limitation of conducting SLNB in Indonesia is the availability of patent blue dye and radioisotope tracer. To overcome that, a hormonal receptor is expected to aid in predicting sentinel lymph node metastasis. Objective: The aim of this study was to investigate the hormonal receptor as a prognostic factor of sentinel lymph node metastasis in breast cancer. Methods: This study was conducted in Universitas Sumatera Utara Teaching Hospital with the acknowledgment from the Ethics Committee of the respected hospital by the number of 116/KEP/USU/2020. Total of 51 patients participated in this research. Results: Statistically, the p-value in each immunohistochemistry group is > 0.05 in all ER (+) / PR (+); ER (+) / PR (-); ER (-) / PR (+) groups. This shows that there is no significant relationship between hormonal receptors on sentinel lymph node metastases. Conclusion: The statistical evaluation showed that there is no significant correlation between the hormonal receptor and sentinel lymph node metastasis (p>0.05), but is found clinically significant. Therefore, hormonal receptors should be considered as a predicting factor for sentinel lymph node metastasis. Keywords: Hormonal Receptor, Sentinel Lymph Node Biopsy, Early Breast Cancer, Breast Cancer

1. BACKGROUND

Even though breast cancer incidence is higher in developed countries, the mortality rate is higher in developing and underdeveloped countries according to STATS. Due to the high incidence of advanced breast cancer, screening and early diagnosis are the key points to treat cancer in developing countries like Indonesia (1). Axillary lymph nodes have been established as one of the most substantial prognostic factors in women with early breast cancer (2). The five-year survival rate in breast cancer with axillary metastasis is 40% lower than those without axillary metastasis and axillary lymph nodes status is directly proportional to overall survival despite the size of the tumor. Lymph node dissection in ALND is controversial due to its tremendous morbidity in patients without axillary lymph nodes (3). Eighty percent of women who underwent axillary dissection experienced complications like a decrease of mobility, seroma, paresthesia or lymphedema, ipsilateral pain where these complications are difficult to manage and may affect the quality of life significantly (4). Brar et al reported that 60% of patients complained about one or more complications, therefore a newer and non-invasive method has been used such as sentinel lymph node biopsy (SLNB) that has lower morbidity and mortality rate compare to ALND (5).

Sentinel lymph node biopsy is one of the minimally invasive techniques that can confirm the presence of metastasis of regional lymph nodes in cancer (6, 7). Sentinel lymph node biopsy can be done with a lymph mapping technique using blue-dye, radiotracer, or a combination of both. In developing countries, sentinel lymph node biopsy is often done with a single agent, which is the blue dye. The limitation of conducting SLNB in Indonesia is the availability of patent blue dye and radioisotope tracer. To overcome that, a hormonal receptor is expected to aid in predicting sentinel lymph node metastasis.
Lymph node biopsy, minimally invasive surgery, is expected to support a more accurate prediction of lymph node metastasis in breast cancer patients without altering the standard therapy of breast cancer and minimizing the morbidity and mortality rate (4).

2. OBJECTIVE
The aim of this study was to investigate the hormonal receptor as a prognostic factor of sentinel lymph node metastasis in breast cancer.

3. MATERIALS AND METHODS

Study Design
This study was conducted in Universitas Sumatera Utara Teaching Hospital with the acknowledgment from the Ethics Committee of the respected hospital by the number of 116/KEP/USU/2020. 51 patients participated in this research. All patients were suspected of malignant breast tumors in the early stage who visited Universitas Sumatera Utara teaching hospital and fitted the inclusion criteria. Histopathology data is obtained from the medical record. Evaluation of lymph node metastasis is done by the histopathology sample that has been paraffin-embedded and immunohistochemistry stained.

4. RESULTS
In this study, we collected several samples that fit the inclusion and exclusion criteria which add up to 51 patients, where all the samples were female. The age range is 31 years to 70 years, with an average age of 49.39 years, a median of 49 years, and a standard deviation of 8.511 years with the domination of female gender as many as 51 person (100%).

Based on the characteristics of breast cancer patients in this study, the most common type of breast cancer is nonspecific type in 49 patients (96.1%), followed by invasive lobular carcinoma 2 patients (3.9%), respectively. T2 breast tumor size was 44 patients (86.3%) and T1 was seven patients (13.7%). The highest grade was grade 3, as many as 40 patients (78.4%), followed by grade 2, as many as eight patients (15.7%), and finally grade 1, three patients (5.9%). Positive lymphovascular invasion (LVI) was detected in 25 patients (49%), and negative in as many as 26 patients (51%). For tumor-infiltrating lymphocyte (TIL) was divided into 2 groups, mild and severe. Severe TIL were 34 patients (66.7%) and mild TIL were 17 patients (33.3%). Immunohistochemical examination in 51 of these patients showed that ER (+) / PR (+) results were found in 22 patients (43.1%); ER (+) / PR (-) in 1 patient (2%), and ER (-) / PR (+) in 2 patients (3.9%), and HER2 (+) / ER (-) / PR (-) was present in 10 patients (19.6%), with a summary of the patient’s characteristics in the table 1 below.

In the Table 2 showing distribution of samples based on lymph node sentinel examination, sentinel lymph gland biopsy was positive in 21 patients (41.2%) and negative in 30 patients (58.8) as showed below:

Relationship of Hormonal Receptors to Sentinel Lymph node Metastasis
The result showed in table 3 stated that there are 8 (36.4%) out of 22 patients with ER (+) / PR (+) who had sentinel lymph node metastases and 14 patients with ER(+)/PR(+) and negative sentinel lymph node (63.6%) with the PR of 0.812 and p value of 0.543. Moreover, 1
patient (50%) with ER (-)/PR (+) having sentinel lymph node metastases. While one patient of ER (+)/PR (-) but there was no sentinel lymph node metastasis in this patient with the PR of 1.724 and p value of 0.398. With the category of ER (-)/PR (+), one patient (50%) had sentinel lymph nodes metastasis and one other patient (50%) had negative sentinel lymph nodes metastasis with PR value of 1.225 and p value of 0.796. In positive hormonal receptor, 9 out of 25 patients (36%) had positive sentinel lymph nodes metastasis and 16 out of 25 patients (64%) had negative sentinel lymph nodes with PR value of 0.78 and p value of 0.461.

Statistically, the p-value in each immunohistochemistry group is > 0.05 in all ER (+)/PR (+); ER (+)/PR (-); ER (-)/PR (+) groups. This shows that there is no significant relationship between hormonal receptors on sentinel lymph node metastases. The PR value in the ER (+)/PR (+) group is 0.812 means the risk of having sentinel lymph node metastasis is 0.812 times in the positive ER (+)/PR (+) group. The PR value in the ER (-)/PR (+) group is 1.225 means there is 1.225 times the risk of having a lymph node sentinel metastasis. The PR value in the ER (+)/PR (-) group is 1.724, where the risk of not having sentinel lymph node metastasis is 1.724 times higher. Below is the statistical calculation:

5. DISCUSSION

Sentinel lymph node biopsy (SLNB), an insignificant-ly intrusive technique, has been displayed to precisely organize the axilla and elevate lesser dismalness contrasted with axillary lymph node dissection (ALND). The planning strategy has been among the main components influencing the distinguishing proof rate and bogus negative pace of SLNB in breast malignancy. A mix of lymphoscintigraphy, blue color, and intraoperative gamma test for SLNB has as of now been viewed as the best quality level for recognizing sentinel nodes (SNs) in beginning phase breast malignancy (8).

The current gold-standard (best quality level) for SLNB is a double technique strategy (radioisotope and blue staining). The method consolidates organization of a technetium-named nano colloid and blue coloring interstitially into the breast on all sides of the tumor or periareolar area. In the wake of restricting itself in the lymphatic organization, a glimmer counter is used to decide SLN which acknowledges the central waste from the tumor through a lymphatic circulation (vessel). The blue staining will help with confinement after entry point and nodes that are blue, radioactive, or both are perceived as SLN, subsequently taken out and inspected (5).

In Indonesia, notwithstanding, SLNB for breast malignant growth presently can’t seem to become mainstream thinking about a few variables, including patient qualities (most as of now had the high level stage illness), hazy proposals, and restricted offices for radioisotope use because of significant expenses and inaccessibility in most disease communities (9). The previously mentioned factors are compounded by the way that Indonesia comprises of numerous islands, making admittance to malignancy focuses considerably more troublesome. All such factors have added to the obstacles in the use of SLNB. Therefore the usage of single method SLNB with methylene blue is the main procedure to be done due to the fact that limitation that developing countries have faced (8).

As of late, the role of SLNB is quite limited in axillary metastasis breast cancer, therefore the needs to explore the predictive factor is urgent in this case. Theoretically the predictive factor for axillary metastasis lymph node is hormone receptors. The function of estrogen in estrogen receptor (ER) positive breast cancer is primarily mediated by ER. Estrogen receptor a (ERa) activation promotes tumorigenesis in different types of cancer, including axillary metastasis breast cancer. The estrogen receptor is a member of the nuclear receptor superfamily and is involved in various developmental and physiological processes (5, 9).

The two main mechanisms of ER-dependent gene transcription are estrogen/ligand-dependent and estrogen/ligand-independent. In ligand-dependent signaling mechanisms, the binding of estrogen with ER causes a conformational change, which allows various coregulators to stimulate transcription of ER-target genes. The direct genomic or classical pathway regulates the expression of ER target genes by the direct binding of estrogen-activated ERαs to DNA binding at EREs. During estrogen binding with ER, and the heat shock proteins (HSP70 and HSP90) dissociate ER from this binding in the cytosol, and change their conformation, then migrate as dimers into the nucleus to bind with EREs. This conformational change also allows helix 12 (H12) to accept coactivators and activate gene transcription (7, 10).

In indirect genomic/non-classical pathways, estrogen receptors regulate the transcription of genes that do not contain EREs through indirect binding to DNA. The indirect ER binding is mediated by different co-factors (like SP-1, AP-1, and NF-kB) that stimulate gene transcription through interaction with DNA. Specificity protein 1 (Sp-1) is the main transcriptional factor that binds with ER and contributes to coactivator recruitment which leads to progression to metastasis in breast cancer (11).

The binding of membrane-localized ER and estrogen interact directly with RTK, the p85 regulatory subunit of PI3K, Src, and Shc to activate RAS/RAF/MEK1/2 and ERK1/2, PI3K/Akt/mTOR signaling pathway. These kinase pathways not only induce cell survival and cell proliferation but also phosphorylate ER and its coregulators, which result in the activation of nuclear genomic transcription (11). Estrogen activates growth factor signaling via non-genomic actions of ER and the growth factor signaling, in turn, activates ER, hence forming a vicious cycle. Coregulatory proteins such as proline-glutamic acid, leucine-rich protein 1, and metastasis-associated proteins are important to activate nongenomic activity (12).

Growth factors interact with activated receptor tyrosine kinases (RTK) like human epidermal growth factor receptors, insulin-like growth factor-1 receptor (IGF-1R), and the fibroblast growth factor receptor (FGFR),
which leads to activation of the phosphatidylinositol 3 kinases (PI3K) signaling pathway. Phosphatidylinositol 3 kinase contains a catalytic domain (p110) and a regulatory domain (p85), and it phosphorylates phosphatidylinositol diphosphate (PIP2) to phosphatidylinositol triphosphate (PIP3), which in turn facilitates the phosphorylation of the Akt. Then, Akt activates mTOR via the inhibition of tuberous sclerosis 1/2 (TSC1/2) (13).

Tuberous sclerosis 1/2 is a tumor suppressor and heterodimer of tuberin and hamartin, which acts as a guanosine triphosphatase activating protein and negatively regulates Rheb-GTP by converting it into its inactive guanosine diphosphate-bound state. The tumor suppressor gene phosphatase and tensin homolog deleted on chromosome ten (PTEN) has an inhibitory effect on PI3K by dephosphorylating PIP3 to PIP2, and inositol polyphosphate 4-phosphatase type II (INPP4B) is also dephosphorylated PIP3 to PIP26. Activation of the ER target gene in the PI3K/Akt/mTOR pathway is mediated by phosphorylation of ER on S167. Taken together, activation of the PI3K/Akt/mTOR pathway plays a central role in breast cancer, and blocking of this pathway is an attractive treatment target, especially in endocrine-resistant ER-positive breast cancer (14).

Growth factors binding with the RTK receptors also lead to activation of the Ras/Raf/MEK/ERK signaling pathway. The binding of growth factor with RTK activates RAS. Activated Ras can then bind with Raf and activate the downstream signaling pathway. When Raf is activated, its C-terminal catalytic domain can interact with MEK, and its catalytic VIII subregion is phosphorylated at the Ser218 and Ser222 activation loop, which activates MEK1/2. MEK1/2 is further activating ERK1/2 by phosphorylating the Tyr and Thr regulatory sites. Activated ERK1/2 are then translocated to the nucleus and promote phosphorylation of Ser 118 in the AF-1 domain of ER and activates its ER-target gene transcriptional activity. These procedures are quite essential in the progression of metastasis of breast cancer through axillary lymph node (5, 9).

The discordance rates between metastatic lesions and primary tumors for ER, PR, and HER2 receptor were 20.70%, 37.78%, and 11.48%, respectively. The conversions were mainly losses in ER and PR expression, while the majority of HER2 conversions were gains. Overall, the discordance rates (and discordance-rate orders of the three receptors) observed in this study were similar to those in previous reports; as frequently reported, loss of receptor expression exceeded the gains. According to most studies, PR has the highest discordance rate, followed by ER and HER2. Few reports have suggested that HER2 ranks second and ER third (5, 8).

In terms of receptor discordance, our major findings suggested that adjuvant endocrine therapy positively correlates with PR discordance, and ER discordance is associated with chemotherapy. Additionally, persistent ER negativity conferred poorer survival. The conversion of HR appeared to have a significant impact on survival. Although we conclude that HER2 conversion has no effect on survival, most of our patients did not receive targeted therapy; thus, we could not conclude that HER2 conversion had an impact on survival. This demonstrated that patients with tumors that were ER-/PR- and HER2- had the worst prognosis. Multivariate analysis showed that HR loss was a significant and independent predictor of poorer clinical outcomes. Changes in receptor profile between primary and metastatic lesions have been widely studied. In general, ER or PR losses represent a therapeutic challenge in breast cancer. The development and progress of breast cancer are regulated by many hormones. ER is a key HR, and its positivity indicates a highly differentiated tumor with relatively low malignant behavior; these patients may respond to endocrine treatment and have a good prognosis. PR is a downstream effector of ER signals; with the ER status (7).

In the present study, we found that, increasing tumor size and higher tumor grade are significantly associated with ALN involvement, and this result is explained through the tumor size and grade could have been the main reason for the higher probability of ALN involvement. The tumor size in our study is significantly associated with the increased number of involved lymph nodes (p < 0.01). The three predictive markers, estrogen receptor, progesterone receptor (ER, PR) and HER2/neu have an independent prognostic value. ER expression was demonstrated in 80-90% of breast-cancer cases, while PR expression was demonstrated in 70-80% of cases. HER2/neu is over-expressed in about 15-20% of breast cancer cases (7).

In breast cancer, tumor-infiltrating lymphocytes (TIL) have been reported to vary by subtype. In particular, HER2-enriched breast cancer and TNBC have been reported to show significantly higher TIL density than HR+/HER2− breast cancer, and TILs have been proven to predict the therapeutic effect of chemotherapy. For a few SLNB strategies have been accounted for with great adequacy and wellbeing information, including methylene blue dye, nanocarbon, near infrared (NIR) fluorescence imaging, 99 m-Technetium (99-Tc), and double tracing modality. Mapping sentinel lymph node areas with methylene blue staining alone outcomes in a worthy identification rate yet an extreme false negative rate, as per the American Society of Breast Surgeons’ guidelines, and has been adapted in developing countries especially Indonesia (7).

As of now, helpful dynamic in BRCA depends on the articulation status of three receptors: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). In spite of the fact that ER, PR, and HER2 status is generally dictated by immunohistochemistry (IHC), with the coming of high-throughput advancements for quality articulation examination, new atomic subtypes of BRCA have been depicted. These incorporate luminal A, luminal B, HER2-enhanced, basal-like, and typical like breast tumors. The clinical meaning of these inherent BRCA subtypes has been featured by their capacity to anticipate treatment reaction and visualization; henceforth...
their utilization in clinical practice has expanded over ongoing years (10).

Based on the characteristics of breast cancer patients in this study, the most common type of breast cancer is nonspecific type in 49 patients (96.1%), while the most common breast tumor size was T2 in 44 patients (86.3%) and the highest grade was grade 3, as many as 40 patients (78.4%). Positive lymph vascular invasion (LVI) was detected in 25 patients (49%), and negative in as many as 26 patients (51%). For tumor-infiltrating lymphocyte (TIL) was divided into 2 groups, mild and severe. Severe TIL were 34 patients (66.7%).

Immunohistochemistry examination reveals that ER(+)/PR(+) found in 22 patients (43.1%); ER(+)/PR(-) found in 1 patient (2%), ER(-)/PR(+) found in 2 patients (3.9%), and HER2(+)/ER(-)/PR(+) found in 10 patients (19.6%). Further analysis resulted in a p-value above 0.05 for all groups. This finding indicates that there is no significant relationship between hormonal receptor and sentinel lymph node metastasis. This result also aligns well with the findings of the study conducted by Onder Karahalt, et al. (2016), their study included 89 subjects which they found no significant relationship between hormonal receptor and the sentinel lymph node metastasis with a p-value of 0.507. Other studies showed that there is a correlation between hormonal receptor and sentinel lymph node metastasis. Nathason, et al. 2006 showed that there is a relationship between overexpression of estrogen receptor and or progesterone receptor and sentinel lymph node metastasis.

A study by Hikmet, et al. 2018 also showed that there is a correlation between the expression of estrogen receptor and/or progesterone receptor and sentinel lymph node metastasis, especially in invasive lobular breast cancer. In this study, 98.1% of the study sample showed that there is a positive correlation between ER(+) with sentinel lymph node metastasis. A similar correlation was also expressed in PR(+), about 87% of the study sample showed a positive correlation. There are several differences in results and opinions regarding the association between hormonal receptor and lymph node metastases. These differences may exist because the samples in this study are relatively smaller compared to other studies, therefore further research is needed for early detection of lymph node metastases from breast cancer.

6. CONCLUSION

The statistical evaluation showed that there is no significant correlation between the hormonal receptor and sentinel lymph node metastasis (p>0.05), but is found clinically significant. Therefore, hormonal receptors should be considered as a predicting factor for sentinel lymph node metastasis.

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