Original Article

HISTOLOGIC PROFILE AND CD44 EXPRESSION IN BREAST CANCER TISSUE OF BALUR NANO DIVINE KRETEK VOLUNTEERS

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ABSTRACT

Breast cancer is the most common cancer in woman. Accumulation of free radical in the body is one of cancer risk factors. Since 2009, Research Institute for Free Radicals Shedding Malang has been developing balur nano divine kretek method, combination of balur and divine tobacco smoke to decay free radicals. It has been tested on volunteers with cancer, including breast cancer. This study aimed to assess the effect of balur nano divine kretek method to breast cancer tissue and CD44 expression. The results showed that 66.67% tumor samples suffered extensive damage (grade 3) and high CD44 expression. The high expression of CD44 in most of the samples may indicate better prognosis. The augmentation for the effectiveness consistency of balur nano divine kretek should be continuously conducted with greater samples and more complete molecular markers such as CD24 and ALDH.

Keywords: *breast cancer, balur nano divine kretek, CD44*

INTRODUCTION

Breast cancer is a cancer type that commonly strikes women. Breast cancer may occur as a result of uncontrollable cells growth in breast tissues. There are a number of risk factors for breast cancer, among others; age, alteration of inherited gene, hormone therapy, alcohol consumption, free radicals and obesity (Suzuki et al., 2009, McTiernan, 2003). Free radicals are an atom with free electrons in their outermost shell (Halliwell and Gutteridge, 1984). Free radicals play a pivotal role in carcinogenesis of breast cancer (Pelicano et al., 2009). Free radicals are highly reactive and unstable, thus it can react with surrounding molecules that produce other free radicals and non-radical compounds. The free radicals yielded during cell metabolism are mainly through mitochondrial respiration and several other causes oxidative stress in cells. Oxidative stress is a redox imbalance condition caused by increasing free radicals and decreased antioxidant capacity (Halliwell and Gutteridge, 1984). In several cases of cancer, an oxidative stress happens along with the increase in free radicals in cells. The increase in free radicals in cancer cells may affect certain molecules and result in some impacts, such as stimulation of cell prolif-

eration, cell differentiation, sensitivity alteration towards anti-cancer agent, mutations and genetic instability (Pelicano et al., 2004). The accumulation of mutations in certain gen allows the cells to divide excessively, thus the emerging tumors will eventually metastasize (Hanahan and Weinberg, 2000).

Balur is a Javanese traditional treatment used to improve the quality of one's life. This balur method is adopted from the term kerikan or pilisan (Javanese) using coconut vinegar and onions. The method of balur nano divine kretek is aimed to attract free radicals and heavy metals, such as mercury in human body. Free radicals and heavy metals coming out of the body via balur can be found in balur waste (Zahar and Sumitro, 2011). In such balur method, several antioxidant agents are used, acting as a provider of electrons, originating from natural ingredients and amino acids. The materials used are amino acids, sabrang onion (*Eleuthrine palmifolia* L. Merr), balur coffee (*Coffea arabica* L., mixed with aspirin), coconut (*Cocos nucifera* L.), secang (*Caesalpinia sappan* L.) and cigar smoke from a mixture of cloves and tobacco (*Nicotiana tobacum* L.). Fluid and extracts of the materials used are massaged throughout the body (Zahar and Sumitro, 2011).

The balur treatment method has been conducted by Research Institute for Free Radicals Shedding, Malang, East Java, Indonesia since 2009. The volunteers receiving the therapy were cancer patients in general. Clinically, most of cancer patients undergoing this therapy showed life quality improvement. During 2009-2012, there were

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6 volunteers with breast cancer who underwent mastectomy procedure after complete balur treatment. This research was aimed to examine the cellular and molecular alteration occurs in breast cancer tissue from the balur volunteers by assessing histologic profile and CD 44 expression, a stem cell marker that is widely used as a prognostic tool in breast cancer.

METHODS

This is an observational research to assess histological profile and CD44 expression of breast cancer tissue from 6 volunteers after balur procedure. The samples were paraffin blocks of breast cancer tissue from volunteers that received a mastectomy after undergoing a complete balur procedure. The research was conducted in the Anatomical Pathology Laboratory of Medical Faculty of Jenderal Soedirman University Purwokerto. Hematoxylin-Eosin staining was performed to assess histological profiles. Avidin biotin complex method was performed for immunohistochemical analysis. FFPE tissues were cut into 4-μm thick sections and transferred to positively charged slides. Then, sections were subjected to dewaxing, rehydration, blocking with hydrogen peroxide, and antigen retrieval (Dako target retrieval solution, citrate buffer pH 6.0) with microwave. The slides were then incubated overnight at 2-8^{0}C with primary CD44 monoclonal antibody from Biocare Medical (Cat Number: PM 380 AA). Incubation with secondary antibody and product visualization (Dako) was performed with diaminobenzidine substrate as the chromogen. The slides were finally counterstained with Mayer's hematoxylin and washed once each with distilled water and PBS.

The assessment of histologic profile and CD44 expression had been performed by two anatomical pathologists. The histologic profile was assessed using Nottingham Histologic Score ("Elston grade"). CD44 expression was detected in cell membrane with the following assessment: negative when 0% cell was smeared positive (score 1); weak expression when 1% - 50% cells were positively smeared (score 2); and strong expression when 51% - 100% cells were positively smeared (score 3). The descriptive analysis was in the form of percentage.

RESULTS

The results showed all samples were invasive ductal carcinoma with Nottingham score range between 7 to 9. There were 2 samples (33.33%) with histological grade 2 and 4 samples (66.67%) with histological grade 3. CD44 expression showed 4 samples (66.67%) with high expression, 1 sample (16.67%) with weak expression and 1 sample (16.67%) with negative expression (Table 1). Spearman correlation test showed no significant correlation between tumor grade with CD44 expression (p = 0.817).

DISCUSSION

Based on Jayanti and Saraswati (2016), during the 2009-2012 period, there were 65 female volunteers with cancer who undergo balur therapy, 27.69% of them did not show improved quality of life including 6 volunteers who underwent mastectomy and became the subject of this study. The results of this study showed no significant correlation between the degree of breast tumor histology with CD44 expression. In this study, we found evidence that 1 high grade tumor with negative CD44 expression (Figure 1) and 1 moderate grade tumor with low expression of CD44 (Figure 2). Most of tumor samples are high grade degree with high expression of CD44 (Figure 3).

CD44 with CD24 and ALDH have been identified as a marker of breast cancer stem cells (BCSCs), a subpopulation of tumor cells having high growth capacity in vivo (Velasco-Velazquez et al., 2012). CD44 is believed to be responsible for mediating communication and adhesion between cells, as well as between cells and extra cellular matrix, in order to maintain tissues integrity. CD44 can directly affect growth signals and intracellular motility, so as to play a role in the growth of many types of cancer. CD44 is capable of promoting tumorigenic signals via main signal network, including the activation of Rho GTPases,that encourage cytoskeletal remodeling and invasion, in addition to PI3K/ AKT and MAPK-Ras pathways, promoting cell growth, survival and invasion. Complex of CD44 and key oncogenes increase the activity of tumorigenesis and angiogenesis. This complex is even capable of modifying microenvironment of the tumor by promoting hyaluronan division to support tumor growth (Louderbough and Schroeder, 2011, Horiguchi et al., 2010).

In another research CD44 role indicate to have two antagonistic roles (dual nature) as tumor promoter and tumor suppressor in breast cancer. From several existing research, it is concluded that CD 44 expression is increasing in invasive breast tumors, but does not correlate positively with clinical outcome (Louderbough and Schroeder, 2011). Other research suggests that high CD 44 expression has a strong association with Relapse Free Survival (RFS) of breast cancer patients (Horiguchi et al., 2010). Diaz et al (2005) in a study of 230 breast cancer patient's paraffin blocks stained with anti-CD44 prove that CD44 is positive as favorable prognostic factor for invasive breast cancer patients without lymph node metastases. The research by Ahmed et al (2012) in 1036 samples of primary invasive breast cancer proved that CD44 is associated with beneficial prognostic criteria, including lower Nottingham prognostic index, ER +, HER 2 – and luminal phenotype. CD44 may also function as an independent predictor for good prognosis. The combination of phenotype CD44+ / CD24- has the best prognostic significance with 10 years breast cancer survival and metastases free survival at 84% and 80%, conversely, CD44- / CD24+ are only 62% and 60%.

**Table 1.** Nottingham Histologic Assesment and CD 44 Expression

Samples	Nottingham Histologic		CD 44 Expression	
	Score	Grade	Proportion	Score
1	7	2	35	2
2	8	3	85	3
3	7	2	80	3
4	9	3	80	3
5	9	3	70	3
6	9	3	0	1
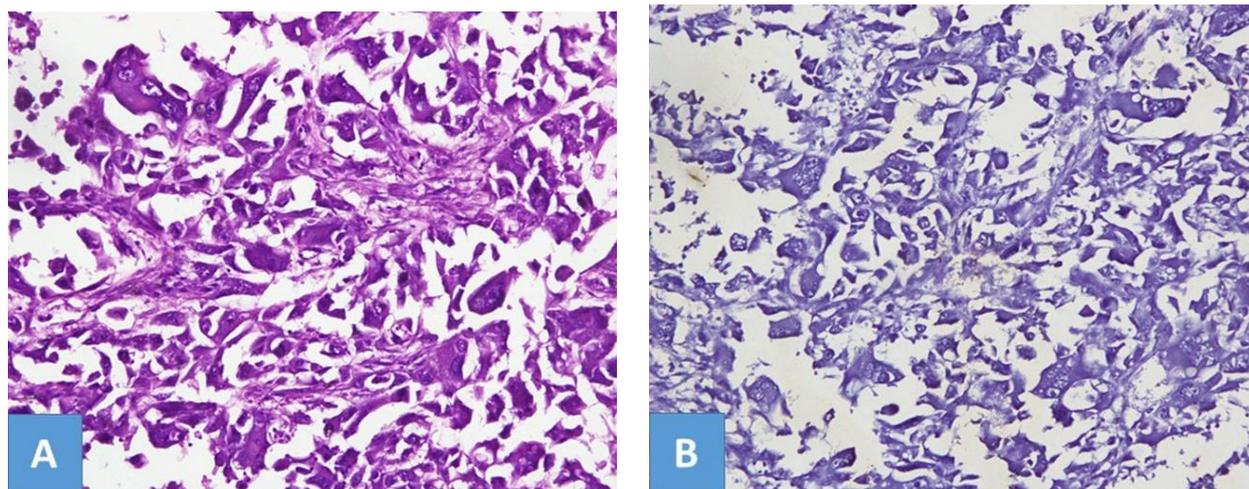
Figure 1. Sample diagnosed as invasive ductal carcinoma grade 3 (A) with negative expression of CD 44 (B). (HE and IHC, magnification x 200).
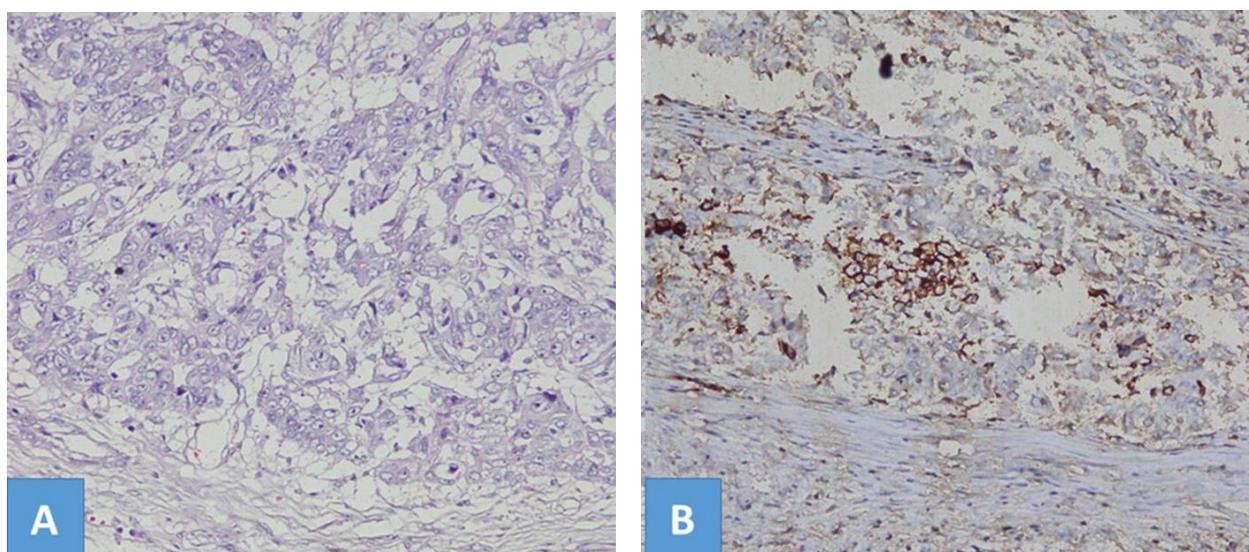
Figure 2. Sample diagnosed as invasive ductal carcinoma grade 2 (A) with low expression of CD 44 (B). (HE and IHC, magnification x 200).
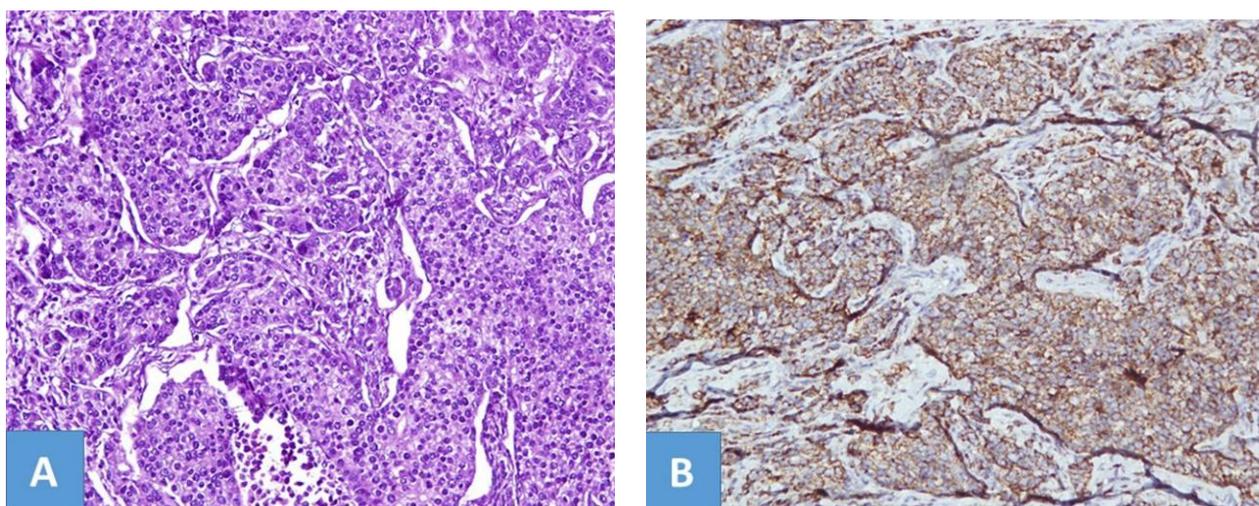
Figure 3. Sample diagnosed as invasive ductal carcinoma grade 3 (A) with high expression of CD 44 (B). (HE and IHC, magnification x 200).

The present research explain the failure of balur in improving quality of life of some volunteers with breast cancer due to the advanced degree of the tumor. Nevertheless, the high expression of CD44 in most of the samples may indicate better prognosis. The augmentation for the effectiveness consistency of balur nano divine kretek should be continuously conducted with greater samples and more complete molecular markers such as CD24 and ALDH.

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