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Cohort Study

The relationship between NFKB, HER2, ER expression and anthracycline -based neoadjuvan chemotherapy response in local advanced stadium breast cancer: A cohort study in Eastern Indonesia

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ABSTRACT

Introduction: Neoadjuvant chemotherapy has become the standard form of treatment for locally advanced breast cancer. Chemoresistence is a problem that limits the effectiveness of chemotherapy. Therefore, predictive biomarkers are needed to choose the appropriate chemotherapy to the right patient. The role of NF-κb expression as a predictive biomarker of neoadjuvant chemotherapy response needs to be investigated in patients with locally advanced breast cancer who are treated with a regimen of cyclophosphamide-doxorubicin-5FU (CAF).

Methods: This observational study used the prospective cohort method to examine 62 samples. CAF was administered at 3-week intervals for 3 cycles of chemotherapy. The data utilized in this study include the positive and negative expression of NF-κB, ER, and HER2 overexpression. The cases were divided into groups that were responsive and non-responsive to the neoadjuvant chemotherapy.

Results: The average age in the youngest group was 26 years, and that in the oldest was 66 years. The highest age group was subjects in their 50s, which had 26 cases (41.9%). The majority of the cases were moderate grade with 38 cases (61.3%). The percentage of responsive subjects was higher in the groups with negative NF-κB expression (82.5%), positive HER2 status (85.7%), and negative ER status (71.9%). It was found that 37 cases (59.7%) were responsive to CAF, while 25 cases (40.3%) were non-responsive. There was a significant relationship between NF-κB expression and chemotherapy response ($p < 0.05$), and the percentage of responsive subjects was higher among those with negative NF-κB expression (82.5%) than positive NF-κB expression (18.2%).

Conclusion: NF-κB expression, ER status, and HER2 have a significant relationship with the response to anthracycline-based neoadjuvant chemotherapy for local advanced breast cancer, and NF-κB expression has the most significant relationship with the chemotherapy response. Therefore, NF-κB expression should be considered as a predictive biomarker for the response to CAF regimens.

1. Introduction

Breast cancer is the most frequently diagnosed cancer and causes the most deaths among women worldwide [1,2]. Data from the Jakarta Cancer Registry show that breast cancer is the most prevalent form of cancer in Indonesia with an incidence of 18.6 cases per 100,000 population every year [3,4]. Currently, chemotherapy is a very important component in the treatment of breast cancer [5]. Furthermore,

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neoadjuvant chemotherapy has become the standard form of treatment for local advanced breast cancer and is the therapy of choice in the operable early stages of cancer [6]. Anthracycline-based chemotherapy is a very important regimen and is widely used in both neoadjuvant and breast cancer adjuvant chemotherapy [7]. However, it has a limited effect on breast cancer due to drug resistance [8].

Cancer cells have the ability to protect themselves against apoptosis by activating NF-κb [9]. The ability of NF-κb to induce resistance to chemotherapy is mediated by its role in the regulation of various anti-apoptotic genes. These genes include 1) pro-survival genes, such as Bcl2, Bcl-xl, and Bfl-1/A1; 2) the apoptosis cellular-inhibitor genes c-IAP1, c-IAP2, TRAF1, TRAF2, Zinc-finger protein A20, and c-FLIP; and 3) the antiapoptosis gene x-IAP [10].

Predictive biomarkers are generally used to predict a patient's responsiveness or resistance to a certain chemotherapy. This is important in cancer management because patients with the same histology can respond differently to the same drug, with a variation of 10–90% [11]. Therefore, the clinical end point of predictive biomarkers is the improvement in overall survival (OS) after certain drugs have been administered. Estrogen receptor (ER) is the first predictive biomarker recommended for routine use in breast cancer by the Tumor Marker Panel of the American Society of Clinical Oncology. It is used to predict a patient's responsiveness to hormonal therapy. In addition, Human Epidermal Growth Factor Receptor 2 (HER2) is expressed in about 25% of breast cancer patients (immunohistochemical identification) and is also a suitable predictive biomarker for a favorable response to trastuzumab [12].

Chemoresistence is a problem that limits the effectiveness of chemotherapy [13]. Furthermore, predictive biomarkers are needed to give the right chemotherapy to the right patients [14]. An ideal biomarker needs to be able to predict a tumor's response to certain chemotherapy agents (whether sensitive or resistant) before the procedure is carried out. The aim of this study is to investigate the role of NF-κb expression as a predictive biomarker of the response to neoadjuvant chemotherapy among patients with locally advanced breast cancer and chemotherapy responses to cyclophosphamide-d-oxorubicin-5FU (CAF).

2. Methods

This observational study was conducted using the prospective cohort method. The study was approved by the Ethics Commission (registration number: 1296/H4.8.4.5.31/PP36-KOMETIK/2016) and has been registered with the Research Registry (no. 6503). Neoadjuvant chemotherapy is a treatment method that entails the administration of cytostatic drugs in combination with a CAF regimen. It is given to breast cancer patients at 3-week intervals for 3 cycles of chemotherapy. NF-κb expression in breast cancer tissue was examined at the Anatomical Pathology Laboratory of the Medical Faculty of Hasanuddin University, Makassar, Indonesia. This study has been reported in line with the "Strengthening the Reporting of Cohort Studies in Surgery" (STROCSS) guidelines [15].

2.1. Population and sample

The study was conducted at Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia, and the number of subjects was selected as 62 based on an unpaired categorical comparative analytical relationship test. The inclusion criteria were women with locally advanced breast cancer, histopathologically invasive ductal mammary carcinoma, and willingness to undergo neoadjuvant chemotherapy with a CAF regimen. The exclusion criteria were bilateral breast cancer, negative tumor tissue samples for IHC examination, and patients not continuing neoadjuvant chemotherapy until cycle III.

2.2. Clinical response

The clinical response was evaluated using calipers by measuring the size of the tumor before the first chemotherapy cycle and at 3 weeks after the third cycle. The result was calculated by deducting the final tumor size from the initial size and then dividing the result by the initial tumor size multiplied by 100. The RECIST criteria were then applied [16].

2.3. Sample preparations

The tissue samples taken at the time of biopsy were fixed with 10% formalin buffer. They were then placed in a cassette and incubated in neutral formalin buffer for 2 h, 70% ethanol for 30 min, 80% ethanol for 30 min, 90% ethanol for 30 min, 96% ethanol for 30 min, 100% ethanol and xylene (1:1) for 15 min, and paraffin for 30 min (histoplast). The sample was then embedded in liquid paraffin and cooled until it solidified, after which it was cut using a 4-μm-thick microtome. Next, it was placed on a glass slide coated with L-polysine. The slide preparation was immersed in warm water 400 °C and then dried on a hot plate at 600 °C for 1 h. Finally, the sample was stored at room temperature.

2.4. Immunohistochemistry (IHC)

The immunohistochemical staining process was performed according to standard protocols using 4–6-μm-thick sections from the original 4–6-mikron blocks. The staining was performed using the avidin-biotin method. Next, the slides were placed in 1.5% blocking serum and incubated for 5–10 min. The slide was then incubated for 60 min in primary antibodies at 1:200 (human monoclonal NFκB p65 antibody, ScyTek Laboratories, Logan, UT, USA) according to the manufacturer's instructions [17]. Then, it was dripped with TrekAvidin universal link secondary antibody (Biocare Medical) according to the manufacturer's instructions.

2.5. Interpretation of the IHC results

The results of NF-κB staining are interpreted in a semiquantitative process that is carried out by examining the distribution of the percentage of expression of colored cell groups and the intensity of the staining. The percentage is obtained by high-resolution fields in each specimen and used for quantitative analyses via the image analysis software ImageJ version 1.48 (National Institutes of Health, Bethesda, MD, USA). The immunohistochemical distribution of NF-κB/p65 was assessed as focal (<10%), regional (11–50%), and diffuse (>50%), while the staining intensity was assessed as "weak," "moderate," and "strong." The results of NF-κB immunohistochemistry in the nucleus and cytoplasm are positive when the intensity and wide distribution of staining give strong-diffuse, strong-regional, or medium-diffuse results [18,19].

The ER expression is the level of ER protein detected in breast cancer tissue, and this is positive in cases where >1% of the cell nucleus is stained with weak, moderate, or strong intensity [20], while, HER2 overexpression (c-erbB-2, HER2/Neu) refers to HER2 levels detected in breast cancer tissue, and this is positive in cases where HER2 +3. However, a result of HER2 +2 requires further examination in the form of in situ hybridization (FISH) [21].

2.6. Statistical analysis

The data in this study consisted of positive/negative NF-κB, ER and HER2 expression and the responsiveness to neoadjuvant chemotherapy. Therefore, data analysis was performed using the chi-squared test, while multivariate analysis was performed using the Logistic Regression test with the Statistical Package for Social Science (SPSS) 20.0 for Windows (IBM Corp. NY, USA). The statistical test results were determined to be significant if the p value was <0.05.

3. Results

This study used a prospective cohort design to analyze the relationship between NF-κB expression and anthracycline-based neoadjuvant chemotherapy response (CAF) in patients with locally advanced breast cancer. Among the 62 breast cancer patients examined in this study, the youngest was 26 years old, and the oldest was 66 years old. The highest age group was subjects in their 50s, which comprised 26 cases (41.9%).

Based on tumor grading, the majority of the cases were moderate grade with 38 cases (61.3%), followed by high grade with 20 cases (32.3%), and low grade with 4 cases (6.4%). After examining the expression of NF-κB, 22 (35.5%) had negative expressions, and 40 (64.5%) cases were found to have positive expressions. It was found that the 37 cases (59.7%) were responsive to neoadjuvant chemotherapy, while 25 cases (40.3%) were non-responsive. The characteristics of the patient sample are presented in.

3.1. Expression profile of NF-κB in chemotherapy response

The expression of NF-κB has a tendency to influence the chemotherapy response in breast cancer. Consequently, shows that there is a significant relationship between NF-κB expression and chemotherapy response ($p < 0.05$), and the percentage of responsive subjects was higher in those with negative NF-κB expression (82.5%) than those with positive NF-κB expression (18.2%). This further indicates a relationship between NF-κB expression and chemotherapy response.

3.2. The relationship between NF-κB, HER2, and ER with chemotherapy response

HER2 status and NF-κB expression were discovered to have a significant relationship with the chemotherapy response ($p < 0.05$ each), while the ER status did not ($p > 0.05$). Based on the Wald score, the NF-κB expression was more correlated with the chemotherapy response (8.91) than HER2 status (7.70). According to the odds ratio (OR), subjects with negative NF-κB expression had a 10-times greater chance of being responsive to chemotherapy than those with positive NF-κB expression. Subjects with positive HER2 status had an 8-times higher chance of responsiveness to chemotherapy than those with negative HER2 status. Based on the R^2 value, the HER2 status and NF-κB expression had a 56.3% influence on the chemotherapy response, while other factors outside this study had a 43.7% influence.

4. Discussion

The ages of subjects in this study varied with a range of 26–66 years and most being aged 50–60 years (41.9%). A study by the American Cancer Society found 231,840 new cases of invasive breast cancer in women within the United States in 2015, and the most vulnerable group (59,990 cases) was those aged 60–69 years old [2,22,23]. Most breast cancer patients in Indonesia that come for treatment are usually in the advanced stages, and 63% are in stages III and IV at diagnosis [24,25]. This behavior is related to socio-economic problems, breast cancer stigma, and education levels [24–26].

Breast cancer in Indonesia may be considered a non-priority by the health care system in comparison to infectious diseases, which could cause the system to be less responsive to breast cancer care in terms of breast health education, awareness, and early detection. From the patient's point of view, Indonesian women do not seek help earlier due to financial problems, rural residence, health expenditure, healthcare access, and lower education level. In addition, other recognized barriers to early detection of breast cancer in Indonesia include belief in traditional medicine and a lack of autonomy in decision making [25–28].

Some studies state that age is a prognostic factor for breast cancer, but several other studies found no relationship. A retrospective cancer registry study found that those within the age range of 50–59 years were more susceptible, while those ≥60 years and ≤49 years were less susceptible [29]. In contrast, a retrospective study of age's effect as a prognostic factor found no relationship between age and prognostic indicators such as tumor stage, diameter, axillary node, and hormonal receptor status [30].

Based on the morphological characteristics of tumor grading in this study, it was found that low, moderate, and high grade had rates of 6.4%, 61.3%, and 32.3% respectively. Recent studies also confirm the importance of tumor grading as a predictive and prognostic factor in breast cancer. According to Engstrom et al., patients with breast cancer with grades 2 and 3 during the first 5 years have a worse prognosis than those with grade 1 [31]. Ogston et al. studied breast cancer patients in locally advanced stage who received chemotherapy. The results revealed a significant relationship between histopathological grading, pathological response, DFS, and OS. Therefore, histopathological grading could be a predictor of chemotherapy response [32].

In this study, the rate of positive ER status was found to be 48.4%. Estrogen has a significant role in the growth and development of target tissues, including the breast glands, where it interacts with other hormones, including growth factors and cytokines, to regulate proliferation and differentiation [33]. Shapochka et al. examined the ER status of KPD patients and obtained a rate of positive ER of 76% [34], while Zhang et al. found high plasma estradiol levels to be significantly associated with the risk of postmenopausal breast cancer, especially ER/PR(+) tumors, but not with ER(+)/PR (−) intermediates and ER/PR(−) [35].

In this study, positive HER2 status was discovered to have rate of 56.5%. The HER2 gene consists of 4 transmembrane protein kinase receptors that are significant for mediating cell growth, differentiation, and survival. The amplification or overexpression of this gene is present in about 18–20% of breast cancer cases associated with tumor aggressiveness and high rates of recurrence and mortality [36].

Pathmanathan et al. carried out a study on several countries in the Asia-Pacific region and obtained a HER2 expression range of 19.7–44.2% in the patient population in Asia, with an average of 23.5% [37]. This study concluded that the breast cancer population in Asia tends to comprise mostly younger ages with high histopathological grading and greater overexpression of HER2 compared to western countries.

Based on the molecular characteristics of NF-κB, its expression in breast cancer was found to be 35.5%. NF-κB is a transcription factor that acts as a transcription catalyst for target genes that cause all aspects of tumorigenesis. These aspects include growth without the need for stimulation of exogenous growth (self-sufficiency in growth signals), insensitivity to anti-growth signals, decreased apoptosis ability, unlimited proliferative ability, angiogenesis ability, invasion, and metastasis, which are hallmarks of cancer [38]. Epidermal growth factor (EGF) and HER2 increase tumor growth by activating NF-κB [10,39,40].

Invasion and metastasis of tissues are two important events in the development of tumors that are regulated by NF-κB-mediated genes [10,39,41–43]. These include matrix metalloproteinase (MMP) genes, urokinase plasminogen activator (uPA), IL-8, adhesion molecules VCAM-1, ICAM-1, and ELAM-1, and chemokine receptors such as CXCR4 [10,44,45]. NF-κB is also involved in the regulation of angiogenesis, the process by which tumor cells promote neo-vascularization, which is an important step in cancer growth and invasion [10,42].

Vascular endothelial growth factor (VEGF) is also a major member of the angiogenic family of factors under the control of NF-κB transcription. Furthermore, the alteration of gene expression is involved in apoptotic regulation and a feature of neoplastic cells. It is caused by the activity of NF-κB, which mediates the transcription of antiapoptotic genes. These genes are the tumor necrosis factor receptor-associated factors 1 and 2 (TRAF 1 and 2), inhibitors of apoptosis proteins (xIAP, cIAP 1, and 2), members of the Bcl-2 family, c-FLIP, GADD45β, and ferritin heavy chain (FHC). By promoting these genes, NF-κB facilitates chemotherapy resistance [10,46].

Several studies were conducted to determine the relationship

between NF-κB and breast cancer. Sovak et al. discovered an increase in the NF-κB expression of breast cancer cell cultures [47]. Furthermore, Montagut et al. discovered a 45.9% increase in NF-κB expression in locally advanced breast cancer tissues (17/37). It was also discovered that NF-κB activation is a predictor of chemotherapy resistance in breast cancer [48]. Another study conducted on a population of Asian female breast cancer patients in India found an association between NF-κB expression, clinical stage, tumor size, high grade, high Nottingham prognostic index, negative ER status, and HER2 overexpression [18].

The results of this study showed 59.7% clinical responsiveness to anthracycline-based neoadjuvant chemotherapy, and anthracycline-based regimens were found to be frequently used in neoadjuvant chemotherapy for breast cancer. However, only a small proportion of patients can achieve a pathological complete response (pCR), and some indicated resistance [49]. Drug resistance is a major factor limiting the effectiveness of chemotherapy. The tumors are either intrinsically resistant prior to chemotherapy or initially sensitive initially and become resistant during treatment [50].

In a study by Kim et al., administering regimen of anthracycline-based neoadjuvant chemotherapy in locally advanced breast cancer caused a clinical response of 60%. This provides an advantage when it is followed by adjuvant chemotherapy in the form of decreased local recurrence and metastasis [46]. Yao et al. studied HER2 predictive factors for anthracycline-based neoadjuvant chemotherapy and reported that breast cancer patients with positive HER2 status had better responsiveness than those with negative HER2 [51].

A bivariate analysis of NF-κB expression on the response of anthracycline-based neoadjuvant chemotherapy in locally advanced breast cancer was performed using the chi-squared test, and a significant relationship was obtained ($p < 0.05$). The percentage of responsive subjects was found to be higher in those with negative NF-κB expression (82.5%). This means that there is a relationship between NF-κB expression and chemotherapy response. The aim of chemotherapy is to kill cancer cells through apoptosis. However, the ability of cancer cells to undergo apoptosis is often altered, which causes chemotherapy resistance [46]. Various studies have revealed the mechanism by which cancer cells protect themselves against apoptosis, which is the activation of NF-κB transcription factor [10,43,46].

Inactive NF-κB in the cytoplasm becomes active when there is a certain signal response. Then, it is released and translocated to the nucleus, where it activates a number of target genes that inhibit apoptosis. These genes include the Bcl-2, Bcl-xL, and Bfl-1/A1 gene families, which inhibit the pro-apoptotic release of cytochrome-c and Smac/Diablo from mitochondria, thereby inhibiting chemotherapy agents that perform cell death programs. They also include cellular apoptosis inhibitor genes, namely c-IAP, c-IAP2, TRAF1, TRAF2, zinc-finger protein A20, and c-FLIP, which inhibit TNF-α-induced apoptosis, death receptor, and chemotherapy agents. Lastly, the x-IAP antiapoptotic gene inhibits the activity of procaspase-9, caspase-7, and caspase-3, while GADD45β activates NF-κB and inhibits the apoptotic signaling pathway through JNK due to the TNF-α response. The same mechanism is used by FHC to control ROS production and mediate apoptosis through the JNK pathway caused by chemotherapeutic responses [10,43,46,52].

Bottero et al. conducted an in vitro study on the activation of NF-κ by the toxin topoisomerase SN38 and a doxorubicin enzyme in a HeLa cell culture. It was found that SN38 and doxorubicin activate NF-κB through several processes, such as stimulating the IKK complex functional pathway, IKK activation, Ijb-α phosphorylation, and NF-κB degradation. The activation of NF-κB protects cancerous cells from the apoptotic effects of chemotherapy drugs [53].

Thomas et al. carried out a study on 82 breast cancer patients who received a CAF regimen of neoadjuvant chemotherapy (cyclophosphamide, doxorubicin, 5-fluorouracil). It was discovered that NF-κB, Bcl2-bax protein expression, and the activation of the NF-κB/Bcl2 pathway were closely related to chemotherapy resistance [54]. Montagut et al. conducted a study on 51 patients with locally advanced breast cancer that received doxorubicin and taxan-based chemotherapy. The results showed that tissue samples that expressed NF-κB according to immunohistochemical staining had a clinical response of 20%, while the rest had a higher response of 91%. Therefore, there was a significant relationship between NF-κB expression and chemotherapy response [48].

The percentage of responsive subjects was discovered to be higher for those with positive HER2 status (85.7%). This indicates there is a significant relationship ($p < 0.05$) between HER2 expression and chemotherapy response, which is in accordance with the study by Zhang et al. on the relationship between HER2 and the response to adjuvant chemotherapy in 1625 patients. They stated that HER2 overexpression was associated with resistance to a cyclophosphamide-metrotrexata-5FU (CMF) regimen and sensitivity to anthracycline or taxane-based regimens [55].

A greater percentage of responsive subjects were discovered to have negative ER status (71.9%), which shows there is a significant relationship ($p < 0.05$) between ER expression and chemotherapy response. This is in line with the study by Osako et al. involving 103 locally advanced KPD patients. The patients were given anthracycline and taxane-based neoadjuvant chemotherapy. Negative ER and PR expression results had a significant relationship with the pCR chemotherapy results [56].

Based on these results, the expression of NF-κB, HER2, and ER status have a significant relationship with the chemotherapy response. Therefore, a multivariate analysis was performed to determine the distinct relationship between the expression of NF-κB and the chemotherapy response. The results showed NF-κB expression and HER2 status were associated with the chemotherapy response ($p < 0.05$ each), while the ER status did not have any significant relationship with it ($p > 0.05$). According to the Wald scores, the expression of NF-κB was more significantly associated with the chemotherapy response (8.91) compared to the HER2 status (7.70).

From the OR, the subjects with negative NF-κB expression had a 10-times greater chance of being responsive to chemotherapy compared to the subjects with positive NF-κB expression. The participants with positive HER2 status had an 8-times greater chance of being responsive to chemotherapy compared to the negative HER2 counterparts. Furthermore, based on the R2 value, HER2 status and NF-κB expression were discovered to have a 56.3% influence on chemotherapy response, while other factors outside this study had a 43.7% influence.

Crosstalk occurs between NF-κB and other pathways in breast cancer, which has been observed in relation to resistance to chemotherapy. Numerous studies have examined the role of HER2-expressed expression in breast cancer due to NF-κB activation. Biswas et al. discovered that HER2 overexpression activates NF-κB, and this in turn influences the apoptotic response by TNFα through the NF-κB pathway [57]. Grandage et al. demonstrated NF-κB activation via the PI3K pathway [58]. Merkhofer et al. studied KPD tissue cultures and discovered that IKKα has a significant role in controlling HER2's ability to activate NF-κB through canonical pathways (IkBα and RelA/p65 phosphorylation, IKK activation, and the regulation of target gene expression). They study also discovered that IKKα controls cell invasion through HER2, and the PI3K pathway contributes to NF-κB activation [41].

According to Crowley et al., numerous Growth Factor (HER2) receptors are able activate NF-κB through the P13K/AKT pathway [59]. AKT is a signaling molecule that acts as a cell-growth and survival pathway hub and mediates NF-κB activation through the mTOR effector. This in turn forms the catalytic subunit of the two mTOR signal molecular complexes, mTORC1 and mTORC2. The mTORC1 promotes the regulation of NF-κB mRNAs, including Bcl-2, Bcl-xL, and cyclin D, through the 4E-binding protein 1 (4E-BP1) effector [60]. The addition of herceptin inhibits NF-κB activity by activating NEMO and consequently blocking cell cycle progression, proliferation, and the induction of apoptosis [57].

In addition, several studies have shown a correlation between NF-κB activity and estrogen independence. In breast cell culture, increased

levels of NF-κB DNA activity were observed in negative-ER cells compared to positive-ER cells. Also, a study by Nakshatri et al. reported that mouse mammary adenocarcinoma cells with the hormone-independent phenotype had a two-fold increase in NF-κB expression within primary breast tumors [61]. This is in line with other evidence showing that ER status is inversely proportional to NF-κB activity in tumor cells [62]. Laere et al. reported that the expression of the target gene NF-κB was most prominent in breast cancer samples with low ER expression [63], and Biswas et al. discovered that breast-cancer NF-κB activity is increased in negative-ER cases, particularly with HER2 amplification [57].

NF-κB is able to inhibit ER transcription through AKT activation, and this in turn inhibits the activity of Forkhead (FOXO3A). This protein plays a role in ER synthesis, thus inhibiting FOXO3A activity and consequently reducing ER transcription. Conversely, NF-κB stimulates Zeste Homolog 2 (EZH2) enhancer activity, and this in turn inhibits ER. This is also done by upregulating Blimp-1.

In addition to the suppression of ER by NF-κB, ER is conversely able to suppress NF-κB. This is done by increasing the transcription of the NF-κB p105 subunit in the cytoplasm until partial degradation occurs. The activation of the PI3K signaling pathway also leads to NF-κB accumulation within the cytoplasm. Another mechanism is the increase of interaction with co-repressors and competition for co-activators with ER [64].

A limitation of this study was the small sample sizes, and the measurement of NF-κB levels was only done before neoadjuvant chemotherapy. Neoadjuvant chemotherapy can only be carried out within 3 cycles with the aim of downsizing the tumor, but no pCR was obtained. Also, the variables were measured manually with calipers, which is only an approximate measurement method. Ideally, measurements should be made with the aid of USG, CT scans, MRI, or a more sensitive method using proliferation biomarkers, such as Ki-67 or the apoptosis index.

5. Conclusion

Based on the findings of this study, there is a significant relationship between NF-κB expression, ER status, HER2, and the response to CAF regimen for local advanced breast cancer. NF-κB expression had the most significant relationship with the chemotherapy response. Particularly, breast cancer with negative NF-κB expression had a 10-times greater chance of being responsive to chemotherapy compared to the positive counterparts. However, further research is required to support this study with a larger sample size, measurement of NFKB levels before and after neoadjuvant chemotherapy, and the measurement of chemotherapy response using CT scans or MRI.

Provenance and peer review

Not commissioned, externally peer reviewed.

Declaration of competing interest

The authors declare that they have no conflict of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2021.02.010.

Ethical approval

The study was conducted after obtaining approval from the Ethics Commission of Hasanuddin University, number: 1296/H4.8.4.5.31/PP36-KOMETIK/2016.

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Author contribution

IND, CM, WH, AGB, SAS, PRI, NS, MF, AAI, and DS: authorship implies a substantial contribution to the study concept and design, analysis and/or interpretation of data, critical writing or revising of intellectual content, and final approval of the version to be published. IND, CM, WH, AGB, PRI, and AAI: authorship implies a substantial contribution to the study concept and design and analysis and/or interpretation of data. IND and CM: analysis and/or interpretation of data.

Registration of research studies

This cohort study has been registered with the Research Registry (no. 6503).

https://www.researchregistry.com/browse-the-registry#home/registrationdetails/60140b40b04958001cacd9f8/

Guarantor

Indra.

Consent

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The patients have given their written informed consent on admission to use their prospective data base and files for research work.

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