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Overexpression of nuclear factor kappa B (NF-κB) protein as a risk factor for anthracyclin chemoresistance in luminal a subtype locally advanced breast cancer (LABC) at Sanglah General Hospital, Bali - Indonesia

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ABSTRACT

Background: Around 68.6% of women who have breast cancer seek medical treatment at a locally advanced stage (LABC), where the neoadjuvant chemotherapy (NAC) modality is the initial choice therapy. Luminal A was recorded as the subtype with the chemotherapy response being the most resistant among others. The cause of chemoresistance is multi-factorial, and several studies correlate this condition with several anti-apoptotic agents expressed in the nucleus of cancer cells, especially nuclear factor-kappa B (NF-κB). The NF-κB transcription factor as an anti-apoptotic agent in some cancers blocks the apoptotic pathway through the extrinsic and intrinsic pathways. NF-κB plays a vital role in the development of chemoresistance. Understanding the NF-κB pathway and perceiving more about the cellular characteristics of LABC is very important to develop NF-κB inhibitors as therapy. NF-κB as a transcription factor can be considered a promising research target because it can affect oncogenes' transcription and play an essential role in prognostic and predictive biomarkers. This study was conducted to determine whether NF-κB overexpression is a risk factor for anthracycline-based neoadjuvant chemoresistance in Luminal A subtype LABC patients at Sanglah General Hospital.

Methods: An unpaired case-control study was conducted at Sanglah General Hospital. The research subjects were 40 people, divided into case groups (chemoresistance) and control groups (chemosensitive). Hereafter the NF-κB H-Score was carried out by IHC examination. Data are presented in table form and analyzed by Chi-square test or Fischer exact.

Results: Samples of cases with NF-κB overexpression were obtained 13 (65%), compared with the control group 7 (35%) ($p<0.05$). Multivariate analysis has shown that NF-κB overexpression is an independent factor against anthracycline-based chemotherapy resistance in advanced LABC patients with local luminal subtypes with $p=0.013$; OR 3.45 (95% CI=2.94-12.65).

Conclusion: NF-κB expression in Luminal A subtype LABC can be used as a predictor for anthracycline-based neoadjuvant chemoresistance. NF-κB overexpression is a predictor factor for NAC chemoresistance in luminal A subtype LABC.

Keywords: NF-κB, Chemoresistance, NAC, Luminal A subtype LABC

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INTRODUCTION

Breast cancer is the most common malignancy found in female population both in developed and underdeveloped countries. Number of deaths by breast cancer was estimated to be more than half a million females around the world in 2011. Breast cancer has the highest new case rate at 43.3% and the age-adjusted mortality rate was 12.9%.[1]

According to Indonesian Ministry of Health National Data in 2013, the prevalence of cancer for all ages in Indonesia was 1.4‰ or around 347.792 people, whereas the number of breast cancer in Bali Province was recorded 8462 patients. Around 68.6% of patients with breast cancer came to health care service at the locally advanced stage (stadium IIa and IIB), and only 22.4% came in the early stage (stadium I and II). Prevalence of breast cancer in university hospital across Indonesia such as Cipto Mangunkusumo General Hospital Jakarta, Dharmais Cancer Hospital Jakarta, Pringadi Hospital Medan, and Mohammad Hoesin Hospital Palembang also showed higher prevalence of advance breast cancer (stadium III and IV) at 57.9%-76.9%.[2]

Locally advance breast cancer (LABC) is classification of breast cancer characterized by local spreading to adjacent tissue of breast organ without distant metastasis. "locally advance" term also used when the spreading of cancer cell was limited to regional lymph nodes. As the Saint Gallen Consensus 2011, LABC further divided

into 4 subtypes with distinct clinicopathology and therapeutic responses.[3]

Neoadjuvant chemotherapy was integrated in multimodal treatment strategy of LABC, and continuing studies are still conducted until recently regarding treatment regimen, especially with anthracycline, to increase long term control of LABC. Clinical management of LABC could be modified according to biological subtype and treatment approach could be adjusted individually to optimize the treatment.[4] Some studies suggest of resistance against some anti-apoptotic agent expressed by the nucleus of cancer cells, especially NF-κB.[5] Luminal A is one of LABC subtype with the best prognosis among others. Most clinical cases with high risks were given adjuvant chemotherapy, although small benefit should be expected because of low proliferation rate found in this group.[6]

Transcriptional factor NF-κB expressed by normal cells as an anti-apoptotic agent became overexpressed in some cancer types by blocking an intrinsic and extrinsic pathway.[7] Nuclear factor-kappa B plays a significant role in development of chemoresistance. Preclinical studies showed resistance against several chemotherapeutic agents such as anthracycline and taxane groups following the activation of NF-κB pathway.[8] This caused by gene that encoded anti-apoptotic factors induced by transcriptional factor NF-κB in the nucleus of cancer cells.[4]

Understanding NF-κB pathway and cellular characteristics of breast cancer are pivotal in developing NF-κB inhibitor as therapeutic agents.[5] Overexpression of NF-κB as a transcriptional factor is considered a promising research target because it can affect oncogene transcription and also can act as a biomarker for prognostic, therapeutic, and chemotherapy resistance.[9] This study aimed to determine whether NF-κB overexpression is a risk factor for anthracycline-based neoadjuvant chemoresistance in Luminal A subtype LABC patients at Sanglah General Hospital.

METHODS

This study was an unpaired case-control study conducted by Department of Surgical Oncology, Udayana University at Sanglah General Hospital Denpasar Bali in August 2018. Twenty cases of each case and control group were enrolled in this study. Inclusion criteria consist of female patients with LABC, subtype Luminal A according to IHC examination, already received three times neoadjuvant chemotherapy Anthracycline, and complete medical records. Exclusion criteria were male, pregnancy, refusal of chemotherapy, uncompleted neoadjuvant chemotherapy, neoadjuvant chemotherapy using other regimen, and paraffin block unavailable.

Patients fulfilling inclusion criteria were recorded and data collected was including age, histological grade, lymphovascular invasion (LVI), and tumor infiltrating lymphocytes (TIL). Samples were tissue collected from cancer mass with the size according to clinical stadium and subtype, and undergone NF-κB expression examination. NF-κB family measured by IHC was ReIA (p65). NF-κB expression level was divided into 2 categories, low expression if H-score < cut-off point; and over expression if ≥ cut-off point. Cut-off point was determined by H-score calculation of ROC (Receiver Operating Characteristic) and Youden Index.

Collected data were grouped according to type and purpose of the data. Statistical analysis was done using SPSS 23. Descriptive analysis was used to describe characteristics of samples. Bivariate analysis using chi-square test was used to determine relation between NF-κB and chemotherapy response. Multivariate analysis using logistic regression was used to control confounding variables. Significant value considered if $p \leq 0.05$.

RESULTS

Characteristics based on on age, the youngest subject was 32 years old and the oldest was 72 years old with mean age was 51,4 ± SD 10,12 years old (Table 1). The age group less than 50 years old was 19 subjects and more than 50 years old was 21 subjects. Based on pathology characteristics, all samples were invasive carcinoma of NST. Histology grading was varied, with 7 (17.5%) were grade I, 19 (47.5%) were grade II, and 14 (35%) were grade III. Lymphovascular invasion (LVI) was found in 14 (35%) whereas 26 (65%) others were negative. Based on TIL, 33 (82.5%) were low and 7 (17.5%) were high.

Normality test for numeric variables using Shapiro-wilk test showed all data were not normally distributed. Mann-Whitney statistical test showed no significant difference between resistant and sensitive groups based on age, menopausal state, histological grade, LVI, and TIL with p-value >0.05 (Table 2).

To find out the role of NF-κB solely on chemoresistance, numerical values were converted into categorical data using cut-off points determined by ROC curve and Youden Index (Figures 1 and 2).

ROC Curve of H-score NF-κB with under curve value of 0.724 and p=0.015 showed that H-score calculation could be a significant predictor to differentiate chemotherapy responses. Youden Index determines cut-off point from sensitivity

and specificity judgment yielding intersection of H-score NF-κB value at 185 (Figures 1 and 2).

From statistical analysis, the odd ratio (OR) of H-Ssore NF-κB with cut-off point 185 to chemoresistance was found 3.45 (95% CI 2.94-1265) with p value=0.013 (p<0.05). This result means that overexpression of NF-κB has 3.45 higher chance of resistance against chemotherapy compared to low expression of NF-κB in LABC Luminal A subtype in Sanglah General Hospital. This result also emphasized that overexpression of NF-κB as a risk factor for developing chemoresistance, in this study against anthracycline-based NAC in Luminal A subtype of LABC in Sanglah General Hospital Bali, Indonesia (Table 3).

Table 1. Subject characteristics

Group	Chemo-resistant (Case)	Chemo-sensitive (Control)	Total
Age (years)	50.9 (SD 11.5)	51.9 (SD 8.8)	-
Menopause			
Postmenopause	7	10	17
Premenopause	13	10	23
H-score NF-κB	226.75 (SD 70.69)	169.75 (SD 57.23)	-
Grade			
Low	2	1	3
High	18	19	37
LVI			
Negative	12	14	26
Positive	8	6	14
TIL			
Negative	15	17	32
Positive	5	3	8

DISCUSSION

In this study, the youngest subject was 32 years old and the oldest was 72 years old with mean age was 51.4 ± SD 10.12 years old. No significant difference was found between case and control group regarding age. The relation between age and chemotherapeutic responses were remains controversial. One study from Gajdos et al. found age was not related to either pathological nor clinical chemotherapeutic responses.[10] Other study showed a significant role of age in chemotherapeutic response where patients older than 65 years old with breast cancer would suspected to have lower pathological chemotherapeutic response (pCR) than group less than 40 years old. This especially true for HER (+)/HR (-) breast cancer.[11]

Based on menopausal states, this study found no significant difference between the postmenopausal and premenopausal group (p=0.523). This result consistent with one study in Thailand that showed distribution of menopausal state in Luminal A subtype breast cancer was not so different.[12]

This study found low and high histological grade in 15% and 85% samples respectively, and p-value of 1.00. This result was similar to study from Gadjah Mada University where high-grade histology was found in more than 50% cases of

Table 2. Subject characteristic and bivariate analysis

Group	Chemoresistant	Chemosensitive	Total	Odd Ratio	p
Age	50.9 (SD 11.5)	51.9 (SD 8.8)	-	-	0.464
Menopause					
Postmenopause	7	10	7	0.538	0.523
Premenopause	13	10	23	(95%CI 0.15-1.92)	
H-score	226.75 (70.69)	169.75 (SD 57.23)	-	-	0.015
Grade					
Low	2	1	3	2.11	1.000
High	18	19	37	(95% CI 0.17-25.35)	
LVI					
Negative	12	14	26	0.643	0.741
Positive	8	6	14	(95% CI 0,17-2.38)	
TIL					
Negative	15	17	32	0.529	0.695
Positive	5	3	8	(95% CI 0,11-2,59)	

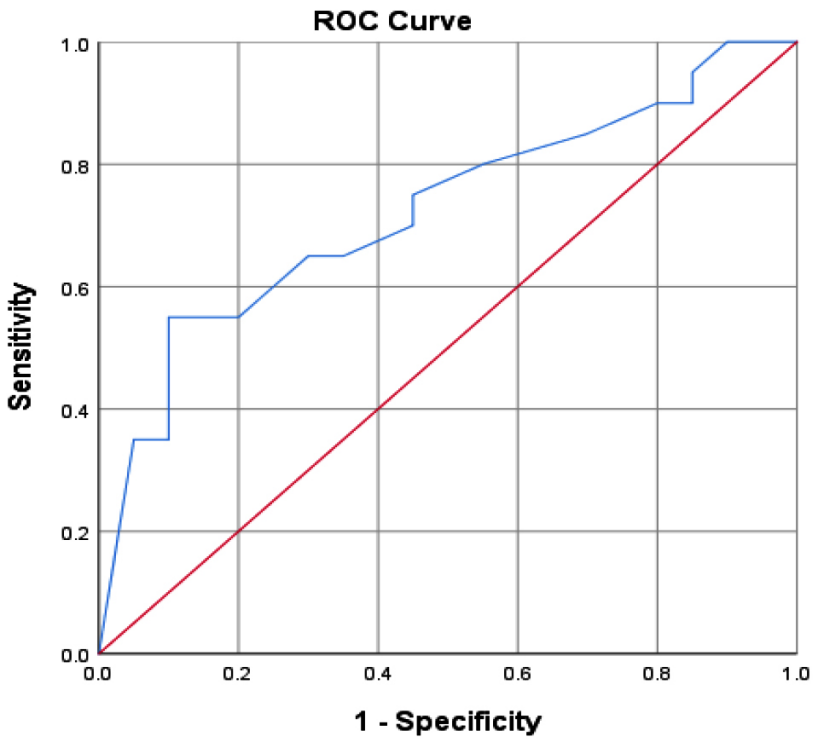

Figure 1. ROC Curve H-score NF-κB

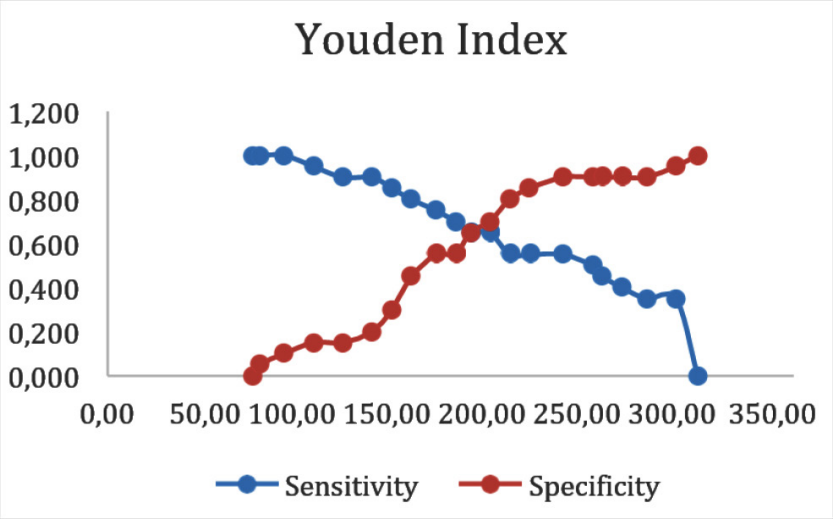

Figure 2. The cut-off point of H-Score NF-κB from intersection of Youden Index line at 185 with 65% sensitivity and 65% specificity

Tabel 3. Relation between NF-κB and Chemoresistance

H-score	Chemo-resistant	Chemo-sen-sitive	Total	*Odd Ratio*	p
H-score ≥185	13	7	20	3,45	0,013
H-score <185	7	13	20	(95%CI 2,94-12,65)	

Luminal A Subtype breast cancer. Report from Nottingham University stated that histological grade could serve as an important prognostic value in breast cancer patients, but not as predictive value of chemotherapeutic response.[14]

In this study, LVI was not different between the case and control group (p=0.741). One study at Jinan-Shandong University in China showed LVI in Luminal A Subtype was not so different with another breast cancer subtype. LVI thought to have predictive value in chemoresistance when regional lymph nodes involvement was found.[16]

Based on TIL, no difference was found in both cases and control group with p-value = 0.594. Phase III study of BIG02- 98, FinHER, ECOG2197, and ECOG1199 in BC found that, compared with other subtype, Luminal A had low TILs and could be an important predictive value only in TNBC subtype or in HER2 (+).[17]

Descriptive tables in this study showed mean H-score of NF-κB was 198.25 ± SD 69.76. Statistical analysis found significant difference between chemoresistant and chemosensitive group regarding H-score NF-κB with p-value < 0.05. Further analysis showed NF-κB as an independent risk factor for developing chemotherapy resistance against anthracycline-based NAC OR 3.45; 95% CI = 2.94-12.65 (p=0.013). According to study by C. Montagut in 2006, evaluation to activation of NF-κB/p65 by IHC could serve as predictive factor for chemotherapy resistance.[5] This result support the hypothesis that an increase in NF-κB activity would lead to decrease in Bid expression and increase the level of Bcl-xL and Bcl-2. Shift in the expression of pro- and anti-apoptosis would reflect in increased sensitivity cancer cells to chemotherapeutic agents.[18]

Other study supporting this result showed an increase of NF-κB expression by resistant cells compared with sensitive ones, and further proved implication of NF-κB as a potential mediator for resistance.[7] In vitro study in Illinois, USA using MCF-7 cell line, showed consistency in ER and NF-κB in increasing pro-survival transcription factor gene BIRC3 to protect cancer cells from apoptosis.[19] This result support as a basis for clinical research of the role NF-κB in ER modification resulting in resistance against hormonal therapy in breast cancer patients.[20] Study by Rutering et al. in 2016 regarding NF-κB and ER expression in breast cancer Luminal B subtype showed that NF-κB decreased ER expression in this subtype of breast cancer.[21]

In Indonesia, two studies have been done recently in Hassanudin University to determine the relation between NF-κB and chemoresistance in LABC patients and showed that chemoresistance

more commonly found in HR (-) and HER2 (+) patients. Research studying NF-κB expression as a risk factor for chemoresistance, especially with Anthracycline-based NAC, in LABC Luminal A subtype has not been done previously.

CONCLUSION

Overexpression of NF-κB in LABC Luminal A subtype could be used as predictor for chemotherapy response to anthracycline-based neoadjuvant regimen in Sanglah General Hospital.

CONFLICT OF INTEREST

All authors declare there is no conflict of interest regarding publication of this article.

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This study doesn't receive any specific grant from government or any private sectors.

ETHICAL CONSIDERATION

This study has been approved by Ethical Committee Faculty of Medicine, Universitas Udayana/Sanglah General Hospital, Denpasar, Bali, Indonesia. All study procedures in accordance to Helsinki Declaration of human rights.

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