RESEARCH ARTICLE

Expression of Nuclear Factor Kappa B (NF-κB) as a Predictor of Poor Pathologic Response to Chemotherapy in Patients with Locally Advanced Breast Cancer

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Abstract

Background: NF-κB inhibits apoptosis through induction of antiapoptotic proteins and suppression of proapoptotic genes. Various chemotherapy agents induce NF-κB translocation and target gene activation. We conducted the present study to assess the predictive value of NF-κB regarding pathologic responses after receiving neoadjuvant chemotherapy. Materials and Methods: We enrolled 131 patients with locally advanced invasive ductal breast carcinoma. Immunohistochemistry (IHC) was used to detect NF-κB expression. Evaluation of pathologic response was elaborated with the Ribero classification. Results: Expression of NF-κB was significantly associated with poor pathological response (p=0.02). From the multivariate analysis, it was found that the positive expression of NF-κB yielded RR=1.74 (95%CI 0.77 to 3.94). Conclusions: NF-κB can be used as a predictor of poor pathological response after neoadjuvant chemotherapy.

Keywords: Locally advanced breast cancer - NF-κB - pathological response - neoadjuvant chemotherapy

Asian Pac J Cancer Prev, **15 (2)**, 595-598

Introduction

Breast cancer is the most common malignancy found in women worldwide, with a relatively high incidence of 20% of all malignancies. GLOBOCAN Data 2008 issued by the WHO International Agency for Research on Cancer, stated that the incidence of new breast cancer cases in Indonesia was 39,831 (25.5 %) and was the most common type of malignancy in women.

Neoadjuvant chemotherapy role in the management of advanced breast cancer has been properly defined in terms, providing benefits in downstaging tumor loco regionally, reducing the micro metastatic burden, and improving overall survival (Gonzalez-Angulo et al., 2005; Schott and Hayes, 2012). However there were still approximately 70% patients who did not achieve complete pathology response. Whereas it posed marker which constitutes the long-term survival (Smith et al., 2002; Von Minckwitz et al., 2012). No optimal responses, worse indeed for neoadjuvant chemotherapy due to chemoresistance mechanism (Chuthapisith et al., 2006; Chen and Tiwari, 2011).

Malfunctions in apoptosis process could lead to the development of resistance in cells towards chemotherapeutic agents, because the induction of apoptosis is a key role of drug induced cancer cell death (Basseres and Baldwin, 2006; Ghavami et al., 2009). Those are many evidence suggesting the regulation of NF-κB on oncogenetic and tumor progression. NF-κB inhibits apoptosis through induction of anti-apoptotic protein and suppression of pro-apoptotic gene, so that tumor cell reinforced with NF-κB activation could avoid apoptosis. Activated NF-κB, that inhibits p53 function, is considered to contribute in chemotherapy resistance (Fan et al., 2008; Barre et al., 2010; Jones et al., 2011).

Materials and Methods

Patients and specimens

Demographic and clinical data from 131 patients with locally advanced invasive ductal breast carcinoma receiving neoadjuvant chemotherapy from January 2008 to December 2011, were collected from Kariadi Hospital Semarang, Margono Hospital Purwokerto, and Hasan Sadikin Hospital Bandung. Major pathological parameters were taken from pre-chemotherapy initial status, including tumor size, lymph node status, histological grade as assessed by modified Bloom-Richardson classification, and lymphovascular invasion. Immunohistochemistry staining for estrogen receptor, progesteron receptor, HER2, Ki-67, and NF-κB were performed using formalin-fixed, paraffin-embedded biopsy specimens (Chang and Hilsenbeck, 2004). All patients received neoadjuvant chemotherapy containing doxorubicin 50 mg/m^2; fluorouracil 500 mg/m^2; and cyclo-phosphamide 500 mg/m^2 given on day 1. After three or four cycles of chemotherapy, all patients underwent mastectomy or breast conserving surgery. Evaluation of pathological

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response from paraffin-embeddded mastectomy or breast conserving surgery (BCS) specimen were performed using Ribero classification.

The primary end point was pathological response which was categorized into poor response, including no response and minor response, and good response, including major response and complete response.

Immunohistochemistry (IHC)

Concisely, 5-micron sections were cut, deparaffinized in xylene, rehydrated in a series of graded alcohols and placed in a tris buffer bath. Endogenous peroxidase activity was complied using 0,6% hydrogen peroxide. Preimmune goat serum was used to block nonspecific staining, and sections were stained with primary antibodies, respectively. After the slides were stained with ER1D5 for estrogene receptor (ER), immunoreactivity was detected using the labeled Streptavidin-biotin (Daco LSAB2 kit). Similar method was used for progesterone receptor (PR) using Monoclonal Mouse Anti-Human Progesterone Receptor Clone PgR 636 (Dako Laboratories, Glostrup, Denmark. Expression of ER/PR was graded as positive if >1% the cells are stained.

HER2 was evaluated using Concentrated and Prediluted Monoclonal Antibody c-erbB-2 CB11 (Biocare Medical, Concord, USA), streptavidin-biotin-peroxide-complex was added and reaction was detected by diaminobenzidine (DAB). Tissue was sustained with Hematoxylin and Eosin (HE). Scoring HER2 was used according to FDA Scoring System for HER2; 2008. HER2 expression was considered positive if the score was 3+.

Immunohistochemistry method of avidin–biotin–peroxidase complex was performed on paraffine block with Concentrated and Predilute Monoclonal Antibody Ki67 M (Biocare Medical, Concord, USA) with 1:200 dilution as the antibody to determine Ki67 status. Cut-off point for positive Ki67 was made if more than 13% cells stained.

The primary antibody used for NF-κB was Rabbit polyconal to NF-κB p65-ChIP Grade ab7970 (Abcam, Cambridge, UK) with 1:600 dilution. 4 μm slides from tumor specimen were deparaffinized in xylene and alcohol, endogen peroxidase was blocked by 0.03% hydrogen peroxide for 5 minutes. After washed within Tris-buffered saline, the slides were incubated with primary antibody. The slides were contacted toward 3,3'-diaminobenzidine as chromogen for 5 minutes and counterstained using hematoxyline. NF-κB/p65 was assesed independently and blindly by patologist. We used semiquntitative scoring to determine the slide's intensity and distribution to evaluate expression of citoplasm. Distribution criteria was defined as focal (≤10%), regional (11-50%) or diffuse (>50%). Intensity criteria was defined as weak, moderate and intense subjectively. NF-κB/p65 cytoplasm was considered positive when the samples presented intense-diffuse, moderate-diffuse, or intense-regional.

Statistical analysis

Chi square was used to examine the corelation of predictive factor with pathologic response. Binary Logistic Regression was used to examine the corelation of each factor independently with pathologic response, by controlling confounding variables. p value <0.05 was considered as significant.

Results

One hundred and thirty one (131) women with locally advanced breast cancer were included in this study. Among 131 samples, 29.8% showed negative NF-κB expression and 70.2% samples considered to be positive. Majority of the samples (57.3%) showed minor response, 28.2% samples showed major response, and, complete response was achieved in 14.5% samples. Bivarian analisis was

Table 1. Frequency of *Nuclear Factor Kappa* B (NF-κB) Expression

Expession of NF-κB	Frequency	%
Negative	39	29.8
<50% weak	19	14.5
<50% moderate	8	6.1
<50% strong	7	5.3
>50% weak	15	11.5
>50% moderate	15	11.5
>50% strong	28	21.4
Total	131	100

Table 2. Frequency of Pathologic Response after Neoadjuvant Chemotherapy

Pathologic response	Frequency	%
No Response (residue 100%)	0	0
Minor Response (residue ≥50%)	75	57.3
Major response (residue <50%)	37	28.2
Complete response (residue 0%)	19	14.5
Total	131	100

Table 3. Correlation between Predictive Factors with Pathologic Response

Variables		Pathological Response NR & mR* (n=75)	MR & CR** (n=56)	RR (95%CI)	p value
Age (years)	≤ 45	31 (63.3)	18 (36.7)	1.49 (0.72-3.07)	0.28
	> 45	44 (53.7)	38 (46.3)		
Menopausal status	Pre (<50)	45 (62.5)	27 (37.5)	1.61 (0.80-3.24)	0.18
	Post (≥50)	30 (50.8)	29 (49.2)		
Histological grade	3	53 (57.0)	40 (43.0)	0.96(0.45-2.07)	0.92
	1 & 2	22 (57.9)	16 (42.1)		
Lymph vascular invasion					
	Yes	61 (62.2)	37 (37.8)	2.24 (1.00-4.99)	0.047
	No	14 (42.4)	19 (57.6)		
Molecular subtype	Triple ve-	25 (50.0)	25 (50.0)	-	0.28
	HER2	12 (66.7)	6 (33.3)		
	Luminal B	22 (68.8)	10 (31.2)		
	Luminal A	16 (51.6)	15 (48.4)		
ER expression	ve-	48 (55.2)	39 (44.8)	0.78 (0.37-1.62)	0.5
	ve+	27 (61.4)	17 (38.6)		
PR expression	ve-	47 (57.3)	35 (42.7)	1.01 (0.49-2.06)	0.98
	ve+	28 (57.1)	21 (42.9)		
HER2 expression	ve+	17 (65.4)	9 (34.6)	1.53 (0.63-3.75)	0.35
	ve-	58 (55.2)	47 (44.8)		
Ki67 expression	>13%	37 (68.5)	17 (31.5)	2.23 (1.08-4.62)	0.03
	ve- & <13%	38 (49.4)	39 (50.6)		
Pgp expression	ve+	29 (65.9)	15 (34.1)	1.72 (0.81-3.66)	0.15
	ve-	46 (52.9)	41 (47.1)		
NF-κB expression	ve+	58 (63.7)	33 (36.3)	2.38 (1.11-5.08)	0.02
	ve-	17 (42.5)	23 (57.5)		
ALDH1 expression	ve+	18 (69.2)	8 (30.8)	1.90 (0.76-4.74)	0.17
	ve-	57 (54.3)	48 (45.7)		

*NR=no response; mR=minor response; **MR=major response; CR=complete response

Table 4. Predictive Factors for Poor Pathologic Response (no response and minor response)

Variables		Unadjusted RR (95%CI)	Adjusted RR (95%CI)
Menopausal status			
Post menopause		1	1
Premenopausal		1.61 (0.80-3.24)	1.87 (0.87-3.99)
Lymph vascular invasion	ve-	1	1
	ve+	2.24 (1.00-4.99)	2.14 (0.90-5.11)
Ki67 expression	ve-	1	1
	ve+	2.23 (1.08-4.62)	2.12 (0.99-4.55)
Pgp expression	ve-	1	1
	ve+	1.72 (0.81-3.66)	1.67 (0.75-3.72)
NF-κB expression	ve-	1	1
	ve+	2.38 (1.11-5.08)	1.74 (0.77-3.94)
ALDH1 expression	ve-	1	1
	ve+	1.90 (0.76-4.74)	1.76 (0.65-4.77)

used to examine the corelation between each predictive factor with pathologic response using chi square analisis. From table 3, it can be concluded that vascular invasion (p=0.008), expression of Ki67 (p=0.03), and NF-κB (p=0.02) significantly correlates with pathologic response.

Multivariate analysis using Binary Logistic Regression showed strong predictive factors for poor pathologic factor (no response and minor response) which included lymph vascular invasion (RR 2.14; 95%CI 0.90-5.11) and expression of positive Ki67 (RR 2.12; 95%CI 0.99-4.55). Although expression of P-glycoprotein RR=1.67 (95%CI 0.75-3.72); nuclear factor kappa B RR=1.74 (IK95% 0.77-3.94); and ALDH1 RR=1.76 (95%CI 0.65-4.77) were not strong predictive factor for poor pathologic response, but it offered high relative risk for poor pathologic response (no response and minor response).

Discussion

NF-kB expression showed significant effect on the pathologic response after neoadjuvant chemotherapy with FAC regiment on locally advanced invasive ductal breast carcinoma. Malfunction in apoptotic process may result in cell resistance toward chemotherapy agents, because apoptotic induction was a key element causing cancer cell death induced by drugs (O'Gorman and Cotter, 2001; Vobořilová et al., 2011). There was evidence suggested the regulation of NF-κB toward oncogenetic and tumor progression. NF-κB inhibited apoptosis through induction of anti-apoptotic protein and suppression of pro-apoptotic gene. Various chemotherapeutic agents (taxanes, vinca alkaloids, irinotecan, doxorubicin) induce NF-κB translocation and activation of target genes (Bourgarel et al., 2001; Godwin et al., 2013). Thus, NF-κB can be used as a predictor of poor pathological response after neoadjuvant chemotherapy.

Evaluation of response after neoadjuvant chemotherapy pathologically can be performed routinely, as it can objectively assess cellular response, so that further intervention can be done to anticipate worse outcome.

Routine histological examination such as histological grade, lymphovascular invasion, and routine immunohistochemical examination such as estrogene and progesterone receptor, HER2, Ki67 and NF-κB expression should be conducted in the clinical practice of breast cancer management, especially in locally advanced stage, as shown to provide clinical benefit.

In conclusion, NF-κB expression offered good results in estimating neoadjuvant chemotherapy response pathologically, so it could be used in clinical practice. But it might necessary to do further research in larger and multicenter researches, especially in dealing with locally advanced breast carcinoma.

Acknowledgements

We want to thank Kariadi Hospital, Roemani Hospital, Sultan Agung Hospital, Margono Soekardjo Hospital, and Hasan Sadikin Hospital for giving us opportunity to do this research.

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