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Correlation of Baseline BCL-2 mRNA Expression and Clinical Response to Neoadjuvant Chemotherapy in Breast Cancer

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Abstract

Impairment of apoptosis is a hallmark of cancer. Tumor resistance to apoptosis usually caused by deregulation of the expression of BCL-2 family protein or mutation of the tumor suppressor gene p53. Over expression of Bcl-2 is commonly found in various types of cancer including breast cancer. Studies mentioned that analysis of Bcl-2 might predict response to selected endocrine and chemotherapies. This study is conducted to evaluate the correlation of BCL-2 mRNA expression and clinical response to neoadjuvant chemotherapy in breast cancer patients. Longitudinal study is used in this research, 30 subjects of breast cancer tissue samples prechemotherapy using cyclophosphamide-adriamycin-5FU regiment.

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Detection of mRNA expression of BCL-2 using qRT-PCR techniques. Evaluation of clinical response to chemotherapy is using RECIST criteria. Mean value of BCL-2 mRNA expression in breast cancer patients was 9.917± 2.568. Mean value of BCL-2 mRNA expression of responsive group was 9.887± 2.731. Mean value of BCL-2 mRNA expression of nonresponsive group was 10.017±2.122. Mean value of responsive group were lower than nonresponsive group, but there was no significant correlation between baseline mRNA expression of BCL-2 with clinical response to chemotherapy, value of r=0.378, *p=0.223 (p>0.05)*. this study shows that there was no significant correlation between baseline expression of mRNA BCL-2 with clinical response to chemotherapy.

Keywords: Breast Cancer; Chemotherapy; Clinical Response; mRNA; BCL-2; qRT-PCR.

1. Introduction

Cancer occurs due to disruption of balance of cell growth and death [1]. Tumor cells tend to interfere this balance by activating genes that promoting cell growth or activating genes that inhibit apoptosis [2, 3]. Tumor resistance to apoptosis usually caused by deregulation of the expression of BCL-2 family protein or mutation of the tumor suppressor gene p53 [4]. Over expression of Bcl-2 is commonly found in various types of cancer including breast cancer [5, 6]. BCL-2 is an important clinical prognostic marker in breast cancer, patients with BCL-2 positive tend to relapse and shorter overall survival [7, 8]. Studies revealed that analysis of Bcl-2 might predict response to selected endocrine and chemotherapies [5-7, 9]. The aim of this study was to evaluate mRNA expression of BCL-2 prechemotherapy in association with breast cancer chemotheraphy response.

2. Materials and Method

2.1. Collection of Samples

Study was conducted within a population of breast cancer patients who had been diagnosed through clinical and histopathology examination, which entered the Wahidin Sudiro Husodo Hospital in Makassar, South Sulawesi, Indonesia. All samples who fulfilled inclusion and exclusion criteria and willing to participate in the study and signing informed consent recruited as study samples. The samples consisted of 30 patients with breast cancer who have undergone combinations chemotherapy (combination of cyclophosphamide, adriamycin and 5FU).

2.2. Nucleic Acid Isolation

Nucleic acid was extracted from breast cancer tissue according to the diatom guanidinium isothiocyanate (GuSCN) method described by Boom and his colleagues 1990. Breast cancer tissue was mixed with 500µl of lysis buffer L6 (50mMTris-HCl, 5.25M GuSCN, 20mM EDTA, 0.1% Triton X100), vortexes vigorously, and centrifuged at 1,000 rpm for 5min. To obtain the nucleic acid, samples were lysed by incubation for 15 minutes at 18°C and 20µl of diatom suspension was added. The diatom containing the bound nucleic acid was centrifuged at 12,000 x g for 15 seconds to obtain diatom pellet. The diatom pellet was then washed with washing buffer L2 (5.25M GuSCN in 0.1M Tris-HCl, pH6.4), rinsed with 70% ethanol and acetone, and dried byincubation at 56°C for 10 minutes. The pellet was mixed with 60µl of 10mM Tris-HCl, pH 8.0, 1mM EDTA

buffer and the nucleic acid was eluted by incubation at 56°C for 10 minutes. After sedimentation of the diatom by centrifugation, the supernatant was collected and stored at -20°C until Real- Time PCR was performed [10].

2.3. Expression mRNA BCL-2 Genes by Real Time PCR

Detection of mRNA expression of BCL-2 was done according to Real time PCR method previously describe by Arribas, 2007. Specific primers for mRNA BCL-2 were used described as table 1 [3].

Table 1: Sequence of primers and conditions used.

Primer	Sequence 5' - 3'	Amplicon size	Annealing temperature
bcl-2a	CCCTGTGGATGACTGAGTAC		
bcl-2b	GCATGTTGACTTCACTTGTG	211 bp	54^0C
AC 1	GACCCAGATCATGTTTGAG		
AC 2	GAGTTGAAGGTAGTTTCGTG	486 bp	55^0C
Process		Time	Temperature
		RT-PCR	
Inverse transcription		30 min	50^0C
Activation prior to PCR		15 min	95^0C
		PCR	
Denaturalization		1 min	95^0C
Annealing		30 sec	55^0C
Extension		1 min	72^0C
No. of cycles		34 cycles	
Final extension		10 min	72^0C

Source [3].

2.4. Data Analysis

Data analysis using the SPSS (Statistical Package for Social Science) version 22. Normality of the samples were analyzed using shapiro wilk's test. Analysis of patient's characteristics and clinical response using chi square. Analysis of mean difference of BCL-2 mRNA expression between responsive and nonresponsive groups used wilcoxon test, to see the correlation using the pearson and spearman test.

2.5. Ethical Clearence

Ethical approval for this study was obtained from Research Ethics Committee, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.Number; 1581/H4.8.4.5.31/PP36-KOMETIK/2015 Register; UH15060492.

3. Results

We collected 30 samples of invasive breast carcinoma during July 2015 to August 2016, originated from Wahidin Sudirohusodo Hospital. Youngest age of subject was 28 years and the oldest was 64 years old, the

mean age of subjects in this research was 50.3 years. Histopathologic grading obtained Low grade 6.7%, Moderate grade 63.3% and High grade 30%. Immunohistochemistry examination panel obtained ER+ 26.7%, PR+ 36.6%, HER2 56.6%. The clinical response to neoadjuvant chemotherapy; responsive as much as 76.7% and nonresponsive 23.3%.

Table 2: Patients characteristic

Characteristic	n (%)
Age	
≤ 50	14 (46,7%)
> 50	16 (53,3%)
Grade	
Low Grade	2 (6,7%)
Moderate Grade	19(63,3%)
High Grade	9 (30 %)
Immunohistochemistry	
ER	8 (26,7%)
PR	11 (36,6%)
HER2	17 (56,6%)
Clinical response	
Responsive	23 (76,7%)
Nonresponsive	7(23,3%)

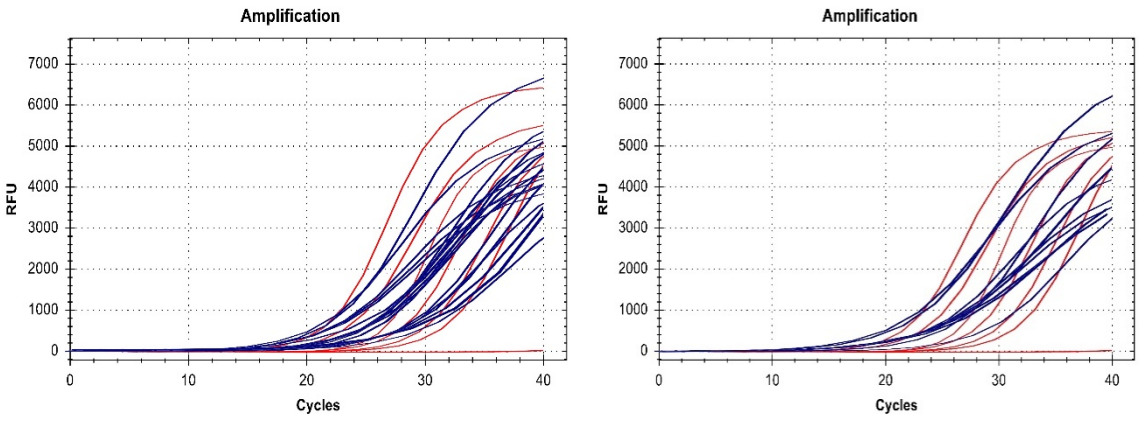

Figure 1: Amplification curve of BCL-2 mRNA expression

Table 3: Comparison expression of mRNA BCL-2 with clinical response to chemotherapy

mRNA Expression	Responsive (Mean±SD) (n=23)	Non Responsive (Mean±SD) (n=7)	*Mean difference*	*p*
BCL-2	9.887± 2.731	10.017±2.122	0.13	0.862

*p = * Wilcoxon test*

Mean value of BCL-2 mRNA expression in breast cancer patients was 9.917± 2.568. Mean value of BCL-2 mRNA expression of responsive group was 9.887± 2.731. Mean value of BCL-2 mRNA expression of nonresponsive group was 10.017±2.122. Mean value of responsive group were lower than nonresponsive group, but there was no significant in mean difference between baseline mRNA expression of BCL-2 with clinical response to chemotherapy, value of *p=0.862 (p>0.05)*.

Table 4: Correlation of expression of mRNA BCL-2 with clinical response to chemotherapy

	mRNA Expression (Mean±SD) (n=30)	Correlation with Chemotherapy response (r)	*p*
mRNA BCL-2	11.837±0.360	0.028	0.885*

*p = * pearson*

There is a positive correlation between mRNA expression of BCL-2 with clinical response to chemotherapy with value of r = 0.028, this correlation was insignificant with $p = 0.885$ ($p>0.05$).

4. Discussion

This study showed that there are no significant differences of BCL-2 mRNA expression between clinical response of responsive and nonresponsive group. Correlation test between mRNA expression of BCL-2 with clinical response was insignificant. It can be concluded from this study that the mRNA expression of BCL-2 is less influential on chemotherapy response.

Bcl-2 family proteins regulate cell death and proliferation, dysregulation of the process that occurs during oncogenic transformation. Thus, it is not surprising to find an expression of Bcl-2 in many cancers [11, 12]. Bcl-2 is able to inhibit apoptosis resulting from a variety of different signals in the intracellular pathway [13, 14]. Bcl-2 has been found to inhibit apoptosis induced by chemotherapeutic drugs, including doxorubicin, in cancer cells [7, 15].

The study on breast cancer showed beneficial effects of Bcl-2 in disease free survival (DFS) and overall survival (OS). In these studies confirm the relevance of these prognostic factors p53 decrease compared to Bcl-2 in clinical practice[16]. Five studies of 11 212 women with early stage breast cancer concluded that Bcl-2 is an independent prognostic indicator that is advantageous to all types of early stage breast cancer. This study sets the rationale for the introduction of immunohisto chemistry Bcl-2 to improve the prognostic stratification of breast cancer [8] Study of 100 samples of breast cancer, comparing examination of BCL-2 with IHC and RT-PCR techniques, found that expression of Bcl-2 in breast cancer by immunohisto chemistry or RT-PCR give very similar results, The results also suggest a link between gene expression of Bcl-2 with the favorable biological features and clinical tumor: the size of the tumor is small, nuclear low grade, hormone receptor expression, the absence of c-erb-B2 and mutant p53 expression, and the proliferation of low (an inverse correlation with the expression of Ki-67). This explains why the expression of Bcl-2 was consistently associated with a better prognosis for breast cancer in the previous report[3]. Research on breast cancer cases in 2749 concluded that Bcl-2 and Ki-67 can be effectively combined to produce an index which is an independent predictor of survival in ER-positive breast cancer, increasing their potential prognostic utility [17].

Other studies found Bcl-2 expression was not significantly associated with pCR in TNBC patients, patients belonging to the Bcl-2-negative group tended to be more chemosensitive than those belonging to the Bcl-2-positive group. This finding is in agreement with previous results showing that Bcl-2 could not predict response to neoadjuvan chemoterapy [7, 18].

5. Conclusion

BCL-2 mRNA expression in the responsive group lower than mRNA expression of BCL-2 in nonresponsive group. There is insignificant correlation between expression of mRNA BCL-2 baseline with chemotherapy response.

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