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RESEARCH ARTICLE

MDR1 mRNA EXPRESSION BY REAL TIME - PCR AND RELATIONSHIP WITH CLINICAL RESPONSE BEFORE AND AFTER GIVING NEOADJUVANT CHEMOTHERAPY REGIMEN CYCLOPHOSPHAMID-DOXORUBICIN-5FU CDF) IN LOCAL ADVANCED BREAST CANCER LABC)

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ABSTRACT

Background: Chemoresistanceinvolving P-gp / MDR1 as uncertain factors affect the clinical response after the administration of neoadjuvant chemotherapy NAC) regimen Cyclophosphamid-Doxorubicin-5FU CDF). Response to chemotherapy can be assessed by changes in mRNA expression of MDR1 gene due to DNA damage in the cancer cell DNA synthesis by examination of quantitative Real Time PCR qRT-PCR). Objective: Knowing the direction of change in MDR1 mRNA expression before and after administration of NAC regimen CDF on Local advanced breast cancer LABC) based clinical response.

Method: Longitudinal observation with cohort prospective design of 20 samples before and after NAC regimen CDF with assessing the expression of MDR1 mRNA in LABC by qRT-PCR examination. Expression MDR1 mRNA analysis by T test independent, paired T-test, Chi-Square Test.

Results: qRT-PCRExamination of 20 samples pre and post NAC regimen CDF showed that a decline of MDR1 mRNA expression $p < 0.05$. In the clinical response responsive decreased of MDR1 mRNA expression $p<0.05$ while nonresponsive decrease MDR1 mRNA $p> 0.05$. Increased of MDR1 mRNA expression in nonresponsive clinical response was 38.5%

Conclusion: There was a decrease significantly of MDR1 mRNA expression both before and after NAC regimen CDF as well as on clinical response responsive MDR1 mRNA expression before NAC regimen CDF as a predictor of response to chemotherapy.

INTRODUCTION

The number of new cases in the USA in 2013 an estimated 232 340 newly diagnosed invasive breast cancer (Desantis *et al*., 2013). The incidence of local advanced breast cancer LABC) in Indonesia gained 40-80% (Murray *et al*., 2012) whereas in developed countries obtain 20-30% of breast cancer have metastatic or locally advanced disease, and another 30% have recurrences or metastases (Chia, 2008). LABC is a breast cancer that is inoperable and who poorly survivalwith monotherapy (Vishnukumar, 2013). Management of LABC there is a tendency to change from radical surgery into a multimodality approach involving surgery, radiotherapy and chemotherapy (Taheri, 2013). The main problem in the treatment of breast cancer is the development of resistance to chemotherapeutic agents (Luqmani, 2008) that the mechanism involves changes in the expression of P-gp/MDR1 (Gonzalez-Angulo *et al*., 2007).

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In general, systemic therapies responded initially to 90% in primary breast cancer and 50% in metastases, but for a time period occurs progressive (Yardley *et al*., 2013). Although the role of Pgp/MDR1 in inducing drug resistance in cancer has been studied clinically, gene expression MDR1 in breast cancer is unclear, and the data is still controversial (Luqmani, 2008). Resistance is caused by tumor cells are resistant or Multidrug-Resistant MDR) to various chemotherapy drugs that can cause treatment failure more than 90% in breast cancer who had metastasis and disease increased progressively over a period of less than one year (Martin *et al*., 2014). Resistance can occur intrinsic or acquired through multiple mechanisms, complex and not exclusive (Iwao, 2001). Response to chemotherapy can be assessed by changes in expression of gene mRNA its caused by the mRNA has the genetic code and can be assessed in real gene expression changes with quantitative Real Time PCR qRT-PCR) examination with high accuracy (Hammond, 2010) compared to the Immunochemistry IHC). qRT-PCR one of the methods that are sensitive, efficient, fast and reproducible to measure gene expression and can be used to measure the quantity of mRNA levels (Taheri, 2013).

Rate biomarker changes after therapy NAC as an evaluation of the efficiency and also used for guiding the handling of the breast cancer (Dede, 2013). Neoadjuvant chemotherapy regimen changes biological tumor marker or causing tumor fraction in which the selection of biologically different from chemotherapy-naïve tumor (Boom, 1990). Examination of the changes in biomarker expression levels of genes mRNA by qRT-PCR cause the reason and the focus of this research in the genomics era so that changes in biomarkers before and after NAC regimen CDF can be used to guide clinicians in planning treatment as predictive and prognostic factors.

METHODS AND SAMPLES

This research was conducted longitudinal observation with cohort prospective design, the sample collection with a purposive sampling a number of 20 samples before and after NAC regimen CDF in Wahidin Sudirohusudo Hospital, Makassar South Sulawesi, Indonesia. NAC regimen CDF is given before surgery for 3 cycles Cyclophosphamide 500 mg/m2, Doxorubicin 50mg/m2, 5-fluorouracil 500 mg/m2 at intervals of every three weeks. The tissue sample was taken from an incisional biopsy before NAC regimen CDF and a mastectomy in patients LABC responsive while the nonresponsive LABC samples were taken by core biopsy. Chemotherapy response was measured with calipers in cm by RECIST Response Evaluation Criteria in Solid Tumors). The response to chemotherapy is classified into two parts: a) Non Responsive, RECIST: stable disease and progressive disease. If the reduced tumor size <30%, a fixed size, the size increases or discovered a new tumor. b) Responsive, RECIST: Partial response and Complete response: Tumor massdisappeared, or at least the reduction occurred tumor size by up to 30% as measured by bi-dimensional and not found a new tumor. Etichal Clearance obtained from research Committee, Faculty of medicine, Hasanuddin University, Makassar, Indonesia.

Quantitative real-time PCR Assay

Quantitative real-time PCR system Applied Biosytems) using Power SYBR®. Green PCR Mix Applied Biosytems). Detecting gene MDR1 mRNA using a specific premier forward and reverse PCR protocol: Performed with the DNA replication cycle of 94°C for 3 minutes, the cycle is repeated 38 times at 54°C 30 seconds). Detecting GAPDH gene using the forward / sense primer MDR1 mRNA: a) Forward: 5'-TGACATTTATTCAAAGTT AAAAGCA-3'; PCR protocol: 94°C 10 min); 32 cycles of 54°C 30 seconds) and reverse/antisense primer: MDR1-reversed: 5'-TAGACACTTTATGCAAACATTTCAA-3'. in accordance with the protocol Tomomi Yajima.

Data analysis

Data were analyzed with SPSS Statistics Packed for Social Science) version 22. Bivariable analysis was used to test the significance of variables mean difference before and after chemotherapy regimen CDF. If the distribution of the normal distribution of data used parametric independent t-test, paired T test and Chi Quare test.

RESULTS

MDR1 mRNA expression before chemotherapy regimen CDF with mean is 11.53±0.69 and after chemotherapy mean CDF is 10.32±2,32. There was a decrease MDR1 mRNA expression before and after chemotherapy regimen CDF showed a significant difference with p <0.05. The table 2 shows that the clinical response nonresponsive mean MDR1 mRNA expression decreased after chemotherapy CDF of 11.79±0.87 pg/ml to 10.51±2.76 pg/ml. While the decline responsive tumor response after chemotherapy CDF of 11.39±0.55 pg/ml to 10.23±2.16 pg/ml. It was found also that the MDR1 mRNA expression was higher in tumor nonresponsive. T Test paired result that tumor nonresponsive decrease

Table 1. ER mRNA expression of before and after chemotherapy CDF on LABC

Variable	Mean	Difference	95%CI	p-value
mRNA MDR1				
Before chemotherapy n=20	11,53±0,69	1,20±1,80	0,36-2,05	0,01*
After chemotherapy n=20	10,32±2,32			
Clinical response				
Before chemotherapy n=20	10.20±3.70	4.65±3.01	3.24-6,06	0.00*
After chemotherapy n=20	5.55±2.85			

Note: p* = significant p< 0.05

Table 2. Expression of MDR1 RNA based Clinical Response

Variable	Mean	Difference	95%CI	p-value
Nonresponsive				
mRNA MDR1 Before chemotherapy n=7	11.79±0.87			
After chemotherapy n=7	10.51± 2.76	1.28±1.99	0.56-3.12	0.139
Responsive				
mRNA MDR1				
Before chemotherapy n=13	11.39±0.55	1.16±1.78	0.09-2.23	0.036
After chemotherapy n=13	10.23±2.16			
p= T pair test				

Table 3. The MDR1 mRNA expression before chemotherapy CDF against tumor response after the administration of neoadjuvant chemotherapy CDF on LABC

Variable		Expression mRNA MDR1				Value p (1-side)
		Risk (≥11.38750)		No risk (<11.38749)		
		n (%)	Risk Estimates	n (%)	Risk Estimates	
Clinical response	N					
	Nonresponse	5 (38,5)	1,161	2 (28.6)	0,743	0,526
	RResponse	8 (61.5)		5 (71.4)		
Total		13 (100)		7 (100)		

MDR1 mRNA expression before and after NAC regimen CDF is not significant p = 0.14 whereas in tumors responsive to significant p = 0:04, so it can be stated that the effect of chemotherapy regimen CDF to clinical response decreased mean mRNA ficantly in responsive tumors. Table 3 shows that higher MDR1 mRNA expression the proportion of nonresponse tumor incidence amounted to 38.5% 5 of 13 LABC), while the responsive tumor incidence the proportion of 61.5%. MDR1 mRNA probability of high risk for tumor response by 1,161 times. Furthermore, mRNA MDR1 expression lower incidence of proportion tumor nonresponse was 28.6% 2 of 7 LABC) and tumor responsiveness was 71.4% with risk estimates for 0,743 times.

DISCUSSION

This study showed that decreased of MDR1 mRNA expression allegedly caused cancer cells are sensitive have apoptosis and left resistant cell clones. Biomarker changes after giving NAC regimen CDF allegedly due to changes in the characteristics of epigenetic gene MDR1. Examination of DNA methylation profile on MDR1 gene therapy after the administration of Doxorubicin on LABC obtained absence of methylation in the promoter regions of genes MDR1 (Burger, 2003). Abnormal methylation can cause MDR1 gene transcription process in forming the lower MDR1 mRNA. Furthermore, this study showed a decrease in mRNA MDR1 expression in nonresponsive group 11.79±0.87) to 10.51±2.76) and MDR1 mRNA expression was higher in tumor nonresponsive. Similarly, the results of another study showed a higher MDR1 in tumor nonresponsive (Luqmani, 2008); (Korn, 2002). Research on P-gp / MDR1, Zhang *et al*, 2014 reported that 46.4% breast cancer showed increased expression of P-gp in tumors after NAC nonresponsive disease progression). This means, first, that anthracycline regimen is substrate of P-gp, so that the tumor cells that P-gp negative more sensitive and killed by NAC therapy while the expression of P-gp high tumor will survive.

Second, acquired drug resistance after exposure to the NAC therapy. Regardless of whether the expression of P-gp primary or acquired, expressionP-gp is high both in the pre and post NAC NAC therapy as a predictor of poor treatment outcome. Examination of the DNA methylation biomarker profile after the administration of Doxorubicin therapy in treatment Doxorubicin response LABC obtained absence of methylation in the promoter regions of genes MDR1. Hipomethylation promoter MDR1 gene associated with TP53 mutation, a tumor with a CpG island region hipomethylation MDR1 associated with mutations in the loop domains L2 / L3 is an area that is associated with resistance to anthracycline therapy base, causing the expression of positive MDR1 impact on the clinical response nonresponsive. The absence of methylation in the promoter on MDR1 associated with disease progression during therapy Doxorubicin reverse DNA methylation status of the promoter MDR1 associated with survival (Burger, 2003). NAC regimen CDF in responsive tumors showed that a decline in MDR1 mRNA expression decreased significantly in clinical response responsive. Clinical response responsive occur due to various factors that contribute beyond this study. Research Korn *et al*., 2002 showed significant increase in gene expression before and after chemotherapy regimen of anthracycline base, in this case the gene cyclin-kinase inhibitor dependents p21) (Modlich, 2004; Vousden, 2000). The increase genes transcription that describe the response of the p53 gene to Doxorubicin induces DNA damage that causes cell cycle break (Zhang, 2014). Furthermore, it also obtained Thymidylate synthase gene expression changes immediately after neoadjuvant chemotherapy Doxorubicin and cyclophosphamide) (Vousden, 2000). Thymidylate synthase TS) plays an important role in the early stages of DNA biosynthesis. In this study, the chemotherapy regimen given CDF, where 5 Fu as inhibitors of the enzyme thymidylate synthase thus antimetabolite that irreversibly inhibit TS causing DNA damage.

The results showed that administration of NAC regimen CDF on the high expression of MDR1 mRNA proportion of incidence tumor nonresponsivewas 38.5%. Status of P-gp is an important factor influencing the pathological complete response manner pCR). Changes in the expression of P-gp, from negative to positive after the NAC indicate drug resistance as a result of chemotherapy. The expression of P-gp were low before NAC associated with improvement in pCR figures. This suggests that P-gp is a predictive tool for the benefit of NAC. Furthermore, the increase in P-gp significantly after NAC there are trends tumor nonresponsive pNR). P-gp expression status provides information prognostic for NAC therapy (Tanei, 2011). The study showed that 72 %) of the 103 non-pCR patients LABC P-gp expression before and after NAC is useful as a prognostic factor in LABC.

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