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CHANGES OF mRNA CASPASE-3 AFTER FIRST CYCLE OF CHEMOTHERAPY AS BIOMARKER ASSOCIATE TO CHEMOTHERAPY NEGATIVE RESPONSE IN LOCALLY ADVANCED BREAST CANCER

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Background: Problems caused by behaviour of biology molecular in locally advanced breast cancer still unpredictable. This study aims to identify the mRNA caspase-3 as a predictive biomarker associated to chemotherapy sensitivity following neoadjuvant chemotherapy (NAC) in patients with locally advanced breast cancer. **Methods:** Open biopsy before and core biopsy after first cycle neoadjuvant chemotherapy of 62 locally advanced breast cancer patients was analysed for mRNA caspase-3 by Reverse Transcription - Polymerase Chain Reaction (RT-PCR) and this was correlate with response to NAC using American Joint Committee on Cancer (AJCC) creteria. **Results:** The total mean of mRNA caspase-3 expression before chemotherapy was 12.51±3.03 and after chemotherapy was 11.64±3.13. Negative response to chemotherapy was 44 (70.96%) and positive response was 18 (29.03%). The result of data with Phi and Cramer's V analysed showed that decrease of mRNA caspase-3 after chemotherapy first cycle as a risk factor to chemotherapy negative response in patients with locally advanced breast cancer was significantly (p = 0.007). **Conclusion:** Decrease of mRNA caspase-3 after chemotherapy first cycle correlated with chemotherapy negative response in patients with lacally advanced breast cancer.

Keywords: mRNA; caspase-3; neoadjuvant; chemotherapy; locally advanced breast cancer.

INTRODUCTION

Breast cancer is the most common cause of cancer in women and is expected to account for 29% all new cancer cases and 14% of all cancer death among women in 2013 Worldwide. Approximately 6% of breast cancer is diagnosed as a de novo metastatic disease and 30% of women with early stage develop recurrent advanced or metastatic disease. Although several therapeutic options are available, there are few validated biomarkers to predict the benefit of specific treatment.[1]

In United State, locally advanced breast cancer continues to be a significant problem, with multiple modality therapies for five years survival rate is approximately 50% for stage III and compared with 87% for stage I. All of the upon problems caused by behaviour of biology molecular in locally advanced breast cancer still unpredictable.[2,3]

Combined treatment with radiation plus surgery was also attempted in this era , but yielded no significant improvement in disease control, although combine therapy before surgery with chemotherapy (*neoadjuvant chemotherapy/NAC*) for overallsurvival and disease free survival was improvement, especially in pathological complate response (pCR).[2] Many studies have analysed a series of tumour, related characteristics such as clinicopathological status (age, menstrual status, tumour size, node status and type of tumours) and certain biology markers status (such as ER, PR, HER-2, Ki67, p53, Bcl-2. BAX, p21, TopoIIα, NF-kB, apoptotic index, tumour cellularity, mitosis) for predicting response to NAC with inconsistent result.[4]

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Other biologic markers corralate to cell death after induction of chemotherapy is mRNA caspase-3. This gene is the effector caspase necessary for efficient cell killing especially in programed cell death or apoptosis. The mRNA caspase-3 is a active gene to produce protein (protease) and it is call caspase (cystein aspartyl-specific protease). This caspase plays an important a role in cytoplasm when the cells induced chemotherapy or others cytotoxic. Caspase-3 especially, is a protease enzyme produced or translated by mRNA caspase-3, as a effector or executioner caspase works at downstream in apoptosis procedure and play a critical role in apoptosis cascade.[5,6]

MATERIALS AND METHODS

This study was designed as a longitudinal prospective study (cohort study) for 62 patients with locally advanced breast cancer (LABC) as a subject research. At first study, all of the LABC included criteria research as an intended sample. Afterwards, mRNA Caspase-3 as a independent variable was analysed by Reverse Transcription - PCR assay and chemotherapy response as a dependent variable using American Joint committee on Cancer (AJCC) creteria. Twenty four hours after first cycle NAC, mRNA caspae-3 reexamination and neoadjuvant chemotrherapy continued untill thirth cycles and the end of study, responce chemotherapy evaluated. Breast cancer tissue was obtained prechemotherapy open biopsy and postchemotherapy with core biopsy, fixated with L6 liquid. Reverse Transcription - PCR assay performed with primers and the TaqMan probe were designed using the primer design software Primer Express (Perkin-Elmer Applied biosystems). Primers and TaqMan probe for glycerol-dehyde-3-phosphate dehydrogenase (GAPDH; TaqMan GAPDH control kit) also purchaced from Perkin-Elmer Applied biosystems.[7]

RESULTS

Total 62 locally advanced breast cancer patients were included in this study, with an age range of 24-70 years (mean, 46.16 ± 10.42) and with a range of tumour size 3-20 cm (mean, 8.66 ± 4.26). In this study, it was observed that 43 (69.35%) patients were premenopausal and 19 (30.64%) patients were posmenopausal status. Histology type of tumor was observed 61 patients (98.38%) infiltrating ductal carcinoma /IDC and one patient (1.61%) infiltrating lobular carcinoma / ILC. Histology grading was 6 (9.67%) patients grade I, 18 (29.03%) patients grade II and 38 (61.29%) patients grade III. The total mean of mRNA caspase-3 expression before neoadjuvant chemotherapy was 12.51±3.03 and after neoadjuvant chemotherapy was 11.64±3.13. Negative response to neoadjuvant chemotherapy was 44 (70.96%) and positive response was 18 (29.03%) included 16 (88.88%) partial response / PR and 2 (11.11%) pathological complate response / pCR. The result of data with *Phi and Cramer's V* analysed, showed that decrease of mRNA caspase-3 after neoadjuvant chemotherapy first cycle as a risk factor to chemotherapy negative respone in patients with locally advanced breast cancer was significantly ($p = 0.007$) and (r = 0.343).

DISCUSSION

This study report data from a cohort of 62 LABC patients were included in this study, with an age range of 24-70 years (mean, 46.16 ± 10.42) and with a range of tumour size 3-20 cm (mean, 8.66 ± 4.26). In this study, it was observed that 43 (69.35%) patients were premenopausal and 19 (30.64%) patients were posmenopausal status. Histology type of tumor was observed 61 (98.38%) patients infiltrating ductal carcinoma /IDC and one (1.61%) patient infiltrating lobular carcinoma / ILC. Histology grading was 6 (9.67%) patients grade I, 18 (29.03%) patients grade II and 38 (61.29%) patients grade III. Afterwards, the efficacy of biomarker, mRNA caspase-3 in predicting response to NAC was examined. The mRNA caspase-3 is an active gene to produce protein (protease) and this caspase plays an important role in cytoplasm when the cells induced chemotherapy or others cytotoxic. Caspase-3 especially, is an effector or executioner caspase works at downstream in apoptosis procedure and play a critical role in apoptosis cascade. In the result of analysis of significance for this data with *Phi and Cramer's V* test showed that decrease of mRNA caspase-3 after neoadjuvant chemotherapy first cycle was correlated to chemotherapy negative response ($p = 0.007$). Other study was report that of all parameters examined, only the apoptosis - related genes (Bcl-2 and BAX) seemed to exert some influence on the response to NAC.[4] Report in defference research was about the cyclin-dependent kinase inhibitor roscovitine and the nucleoside analog sangivamycin induce apoptosis in caspase-3 deficient in MCF7 human breast cancer cells independent of caspase mediated P-glycoprotein cleavage. Result of this study showed, that P-gp cleavage was not detected in vivo in MCF7 cells induced to undergoing apoptosis by either roscovitine or sangivamycin, despite activation of both caspase-6 and -7. P-gp overexpressing MCF7 cells were more sensitive to either roscovitine or sangivamycin than wild-type and suggesting that result support the concept that caspase-3 is the only caspase responsible for in vivo cleavage of P-gp and also highlight small molecules which could be effective in treating P-gp overexpressing cancer.[8] Other study was report about role of caspase-3 in effect of an isolated active compound (BVIO3) of Boehmeria virgata (Forst) Guill leaves on anti-proliferation in human cancer cervix HeLa cells. This result showed that

p53 and caspase-3 are involved in the process of BVIO3-induced antiproliferative in HeLa cell.[9] Caspase-3 is an effector or executioner caspase works at downstream in apoptosis procedure and play a critical role in apoptosis cascade. Resent study performed that caspase-3-mediated cleavage of Mammalian protein kinase C-interacting cousin of thioredoxin (PICOT) in apoptosis, that data raise the possibility that the pro-apoptotic role of PICOT is actively regulated via caspase-3-mediated cleavage.[10] Cadmiun (Cd) is a well-known metal carcinogen associated with tumor formation and carcinogenasis. The research about cadmium modulates H-ras expression and caspase-3 apoptotic cell death in breast cancer epithelial MCF7 cells performed and this data showed the work presented suggests that cadmium acts as an inducer of caspase-3 apoptosis, while promoting variable H-ras protein expression.[11]

CONCLUSION

Based on research results and the above discussion it can be concluded that mRNA caspase-3 as a predictor factor to chemotherapy response in patients with locally advanced breast cancer / LABC.

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