The Ratio of Ki-67/Caspase-3 Expression Determines Patient's Response to Neoadjuvant Chemotherapy in Cervical Carcinoma Stage IB2 and IIA2

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Abstract

Background: Cervical carcinoma is the second most frequent cancer that occurs in women in developing countries. The management of bulky cervical carcinoma remains controversial due to its large size. Among the choices neoadjuvant chemotherapy remains controversial as a choice of management since patient's response is unpredictable. It is necessary to study a biomarker which makes it possible to predict the patient's response to chemotherapy with bulky cervical carcinoma. This study was aimed to observe the association between Ki-67 and Caspase-3 expression and the patient's response in IB2 and IIA2 stage of cervical carcinomas.

Materials and Methods: This study was a retrospective cohort study using 41 preserved biologic specimens (PBS) of paraffin blocks taken from patients with stage IB2 and IIA2 cervical carcinoma that came to the Dr. Hasan Sadikin General Hospital from December 2012 to December 2014, and received neoadjuvant chemotherapy. The expression of Ki-67 and Caspase-3 was examined using immunohistochemistry and the correlation with tumor shrinkage was analyzed.

Results: All specimen expressed Ki-67 and Caspase-3 in the immunohistochemical staining. Pearson's correlation analysis found no correlation between the individual expression levels of Ki-67 and caspase-3 with diminution of tumor mass (p >0.05). On the other hand, the correlation between the ratio of expression of Ki-67 and caspase-3 was found to have a significant association with diminution of tumor mass (p<0.05; R = -0.3139).

Conclusion: There is no correlation between the individual Ki-67 and Caspase-3 expression and the shrinkage of tumor mass in patients with bulky cervical carcinoma. However, the ratio of Ki-67 and caspase-3 expression was associated with the downsizing of the tumor mass. Higher proliferation (Ki-67 expression) compared to apoptosis (caspase-3 expression) resulted in better tumor size reduction in stage IB2 and IIA2 cervical carcinomas.

INTRODUCTION

Cervical carcinoma is the primary tumor of both ecto- and endocervix. It still remains as the second most common cancer among women in the developing countries. As much as 527,624 new cases have been found in 2012, 86% of which were found in women in developing countries[1,2]. Indonesia's national data stated that the incidence of cervical carcinoma was the highest among all gynecological cancers (63.39%) in a one-year period. Dr. Hasan Sadikin General Hospital, the West Java Province central hospital, received about 1,000 new cases of cervical carcinoma each year[2]. Seventy percent of the patients came in with an advanced stage. It is known that the recurrence of the disease and metastasis are the main reasons of death among these patients[3-6].

The primary therapies of cervical carcinoma are surgery, radiation, chemotherapy, or a combination of those. The management of choice for early stage of cervical carcinoma (I-IIA) is surgery, whereas for the advance stage (IIB-IVA) surgery is not effective as the technique becomes more complicated with risk of ureteral injury and significant bleeding. Aside of those severe complications, the chance of metastasis to the cancer tissue margin and lymph nodes makes radiation as the treatment of choice in this

condition[1,6-8].

Bulky cervical carcinoma is defined as tumor size more than four centimeter and has a poorer prognosis than smaller cervical carcinomas. In this type of tumor, deep stromal invasion is likely to happen and a higher rate of lymph node metastasis is seen. Despite the standard treatment applied, these conditions lead to higher relapse rate as well as to local, regional, or distant metastases[6-8].

Chemotherapy as neoadjuvant is used widely in various cancer types including cervical carcinoma. Neoadjuvant chemotherapy (NACT) is given to shrink tumor size before surgery or radiation takes place. The NACT shows good response in limiting micro metastasis, increasing tumor's sensitivity to radiotherapy, and decreasing the needs of adjuvant radiation[3-5].

Various alternative NACT combinations were used for bulky cervical carcinoma. Several data from studies showed that platinum-based NACT continued by surgery gave a better outcome in comparison with radiation-only therapy. However, several failures to respond to the NACT result in problems on tumor progressivity as it delays the radiation therapy[9-14].

Various studies had been done to predict the tumor's response to chemotherapy in cervical carcinoma, but no effective method has been found yet, particularly for bulky cervical carcinoma. It is necessary to do another study to find a biomarker which is able to predict the successfulness or failure of NACT[7,13,15].

Tumor growth progresses because of imbalance in proliferation and apoptosis of cells; higher proliferation rate, lower apoptosis rate, or both. The literature proved that cell proliferation and apoptosis is association with each other. Those studies concluded that cancer growth is associated with decreased cell proliferation and increased apoptosis. Since tumor growth is associated with imbalance of proliferation and apoptosis rates, it may indicate a marker of proliferation and apoptosis that can predict the successful rate of NACT in bulky cervical carcinoma[9].

The most widely used marker of proliferation and apoptosis is expression and distribution of Ki-67 and caspase-3[16]. Ki-67 is the marker for proliferation as it has a role in rRNA synthesis. This particular protein is an excellent proliferation marker because it is detectable in G1, S, G2, and mitosis phase, yet not in G0 phase. Meanwhile, among various

markers of apoptosis, caspase-3 (cysteine-aspartic protease-3 or cysteine-dependent aspartate-directed proteases-3) is a caspase effector or an executor for both intrinsic and extrinsic pathway of apoptosis[11-14].

This study was aimed to observe the association between Ki-67 and caspase-3 expression and patient's response in IB2 and IIA2 stage of cervical carcinoma.

MATERIALS AND METHODS

Research Design

This study was a retrospective cohort study using preserved biologic specimens (PBS) in form of paraffin blocks from cervical biopsies from patients with IB2 and IIA2 stage of cervical carcinoma who came to the Dr. Hasan Sadikin General Hospital from December 2012 to December 2014 and received NACT. Histopathological examination confirmed the sample as squamous cell carcinoma, adenocarcinoma, and/or adenosquamous carcinoma of the uterine cervix. The exclusion criteria were patient with history of another treatment modalities or patients who did not receive NACT or received an incomplete NACT. The research was approved by the Research Ethic Committee of Faculty of Medicine Universitas Padjadjaran.

Outcome Measurements

The shrinkage of the tumor was defined as decrease of tumor size less than the defined size for bulky cervical carcinoma after complete NACT therapy. The size was measured by bimanual vaginal examination using three parameters: 1) laterolateral; 2) anteroposterior; and 3) and/or craniocaudal using the following formula: [(Pre-NACT size – pots-NACT size) / (Pre-NACT size) x 100%)].

To determine the expression of Ki-67, we performed immunohistochemistry and determined the expression based on the intensity and distribution of the immunostaining. The expression intensity was defined as negative (-) if there were no positive cells and positive (+) cells were defined more qualitatively: if 1-25% tumor cells expressed the Ki-67 it was defined as (+), 26-50% as (++), 51-75% as (+++), and >75% as (++++). Whereas the caspase-3 expression was considered positive based on the percentage of nucleus and/or cytoplasm of the cells with positive expression of caspase-3. Score (-) was be given if there were no positive cells. Qualitatively, if 1-25% tumor cells expressed caspase-3 it was defined as (+), 26-50% as (++), 51-75% as (+++), and >75% as (++++).

Data Analysis

Correlation coefficient (R) based on Guilford's criteria (1956) was defined as: $0.0 \leq < 0.2$ = very weak correlation and negligible relationship; $0.2 \leq < 0.4$ = weak correlation and definite but small relationship; $0.4 \leq < 0.6$ = moderate correlation and substantial relationship; $0.6 \leq < 0.8$ = high correlation and marked relationship; and $0.8 \leq 1.0$ = very high correlation and very dependable relationship. Positive correlation meant that the increase in the value of one variable will be followed by increase in another variable's value; In contrary, negative correlation meant that the increase in the value of one variable will be followed by decrease in another variable's value. The difference was declared as statistically significant if the p value was ≤ 0.05.

The data obtained was analyzed using Statistical Package of the Social Science (SPSS) version 21.0 for Windows.

RESULTS

Forty-one PBS has mean caspase-3 histoscore of 7.24 ± 2.82 and Ki-67 of 9.24 ± 2.65. The mean tumor shrinkage was 0.79 ± 0.17 in all subjects. Furthermore, the mean Ki-67 and Caspase-3 ratio was 1.47 ± 0.77. All the descriptive data are summarized in Table 1. The mean tumor size before NACT was 64.72 ± 15.93 and the mean size was 13.13 ± 15.93 after NACT (Table 2).

Table 1

Descriptive Analysis of Study Variables

Variable	Mean±SD	Median (Range)
Caspase-3 Histoscore	7.24±2.82	8.00 (2.00-12.00)
Tumor Shrinkage	0.79±0.17	0.850 (0.17-0.99)
Ki-67 Histoscore	9.24±2.65	9.00 (4.00-12.00)
Ki-67/Caspase-3 Ratio	1.47±0.77	1.33 (0.33-0.450)

Table 2

Tumor Size Before and After NACT

Time	Mean±SD
Before NACT	64.72±36.60
After NACT	13.13±15.93

We found the mean and standard deviation for Ki-67 expression was 9.2439 ± 2.65312. The Rank Spearman correlation analysis between Ki-67 and tumor shrinkage in patient with stage IB2 and IIA2 cervical carcinoma that received NACT showed no statistical significant correlation (p<0.05). The correlation coefficient (R) showed a negative correlation with very weak correlation and negligible relationship (R = -0.085). On the other hand, mean and standard deviation was 7.2439 ± 2.82649. The Rank

Spearman correlation test showed that caspase-3 had no correlation with the tumor shrinkage in patients with stage IB2 and IIA2 cervical carcinoma that received NACT (p<0.05). The correlation coefficient (R) showed a positive correlation with a very weak correlation and negligible relationship (R = 0.185). Furthermore, we also analyzed the correlation between ratio of Ki-67/Caspase-3 expression with the tumor shrinkage and found significant correlation (p<0.05) with a negative correlation and a weak correlation and definite but small relationship (R = -0.319) (Table 3).

Table 3

Ki-67 Expression of Ki-67 and Caspase-3 and Ki-67/Caspase-3 Ratio and Their Correlation with Tumor Size

	Mean±SD	Correlation Coefficient	P-value
Ki-67 Expression	9.2439±2.65312	-0.085	0.598
Caspase-3 Expression	7.2439±2.82649	0.185	0.248
Ki-67/Caspase 3 Ratio	1.4736±0.77794	-0.319	0.042*

Spearman Correlation Test. *Significant correlation if p < 0.05.

DISCUSSION

Neoadjuvant chemotherapy is one the treatment modalities for patients with bulky cervical carcinoma aside from surgery and radiotherapy. The body's response to NACT is affected by several factors, including proliferation and apoptosis rate. Actively proliferating cells give a better response in comparison with resting cells because chemotherapy has a stronger impact on actively proliferating cells and therefore an increased response rate to chemotherapy. Measuring the cells proliferation rate can be done by measuring Ki-67 expression as the biomarker. Aside from the proliferation rate, the apoptosis, cell programmed death, also holds a very important role in the chemotherapy response rate. Among all apoptosis biomarkers, caspase-3 is one of the most known markers that acts as the executor for apoptosis process[11]. In this study we found that all PBS positively expressed the Ki-67 and caspase-3. This suggested that the proliferation process, expressed as Ki-67, and apoptosis process, expressed as caspase-3, was taking place in stage IB2 and IIA2 cervical carcinomas.

In this study, the researcher found a positive response of patients to NACT in 31 (75.6%) subjects. This positive response was a sum of complete response (CR) in 5 (12.2%) subjects and partial response (PR) in 26 (63.4%) subjects. Meanwhile, 10 (24.4%) subjects expressed negative response. The response was measured according to the standard established by Response Evaluation Criteria in

Solid Tumor (RECIST) which has been used in previous studies[16].

The positive response in this study was consistent with previous studies on NACT. Better patient response was found in previous studies conducted by Hwang, 2001 (93.7%), Park, 2004 (90.7%), and poorer response in studies conducted by Friedlander, 1983 (66%), Lara, 1990 (62.5%), Buda, 2005 (48%), Bae, 2008 (69.7%), and Helena, 2010 (69.5%).[16] These data suggested that NACT in bulky cervical carcinoma remains as a good treatment modality.

The evolvement of the tumor depends on the uncontrolled proliferation activity that was determined by proliferation biomarkers, such as p53, p21, and Ki-67. The Ki-67 is known as the predictive factor for tumor growth. Several studies achieved conclusion that a high proliferation index, marked by higher Ki-67 expression, related to poorer prognosis of cancer patients, including cervical carcinoma. Rapidly proliferating cells are more sensitive to cytotoxic drugs than slowly proliferating cells. Other studies also found that accumulation of cytotoxic drug will be decreased in resting cells[16,17].

Previous studies suggested the correlation of Ki-67 expression and tumor behavior in breast cancer, lymphoma, ovarian cancer, and esophageal cancer[18,19]. Studies also found that the expression of p-16 and Ki-67 was stronger with more severe grading of CIN, yet the Ki-67 expression was not related to infection of high risk HPV[20].

In this study, we found no correlation between Ki-67 expression and the shrinkage of the tumor size (p>0.05) and the correlation coefficient was -0.085 which meant that the correlation was a negative correlation with weak correlation and negligible relationship. Based on that result, the expression of Ki-67 could not be used as prediction factor of the shrinkage of tumor size in IB2 and IIA2 stage of cervical carcinoma receiving NACT. This result might be due to: 1) Ki-67 play roles in the early phase of tumor growth rather than in later phase of tumors with large size. In the CIN lesion, the expression of Ki-67 was positively correlated with the degree of CIN. Higher expression of Ki-67 is correlated with the speed of cancer's growth into invasive cancer in comparison with lower expression of Ki-67; this also affecting poorer prognosis[17,21]; 2) Higher Ki-67 expression is related to another parameters, besides larger tumor size, such as pelvic lymph nodes infiltration, parametrium invasion, and shorter disease free survival

period[21]; 3) Different proliferation factors that can produce bulky sized tumor, p-21, p-53, and p-63, other than Ki-67 might be found in higher expression[21,22].

The expression of caspase-3 reflected cell apoptosis. This study found that all samples expressed caspase 3. In those that gave positive response to NACT, the caspase-3 expression intensity was mostly moderate (46.7%) and strong (31.3%). Weak intensity was found in 21.9% patients. In the other hand, samples that gave negative response to NACT was mostly produced a strong intensity (44.4%) and followed by weak intensity (11.1%).

Furthermore, in the correlative analysis we found no significant correlation between caspase-3 expression and shrinkage of the tumor size. The correlation coefficient (R) was a positive correlation with a very weak correlation and negligible relationship (R = 0.185).

Every apoptosis pathway, intrinsic or extrinsic, will use caspase-3 that has a role as the executor of apoptosis. Compare to other caspase families, caspase-3 has the highest level and also correlates with higher apoptosis rate and is believed to be related with better NACT response. However, this study found that caspase-3 had no significant correlation to the shrinkage of the tumor. This study suggested that caspase-3 was not able to predict the patient's response to NACT, in our study population. Several reasons might had influence these results: 1) Not all cells in our study subjects expressed caspase-3 in immunohistochemistry examination; 2) A drug resistance condition, Multi Drug Resistance (MDR 1), can cause the insensitivity of the tumor to NACT; 3) There might be different apoptosis mechanism that did not use the caspase in the process, such as the apoptosis inducing factors (AIF) pathways[6,15,23,24].

Our results showed that the ratio of Ki-67 and caspase-3 expression was significantly correlated with shrinkage of the tumor in our study population (p<0.05). Correlation coefficient (R) analysis found a negative correlation with a weak correlation but statistically significant (R= -0.319).

Further studies needs to be done by adding more independent variables which might influence the patient's response to NACT in patients with IB2 and IIA2 stage of cervical carcinoma.

In conclusion, there was no correlation between the expression of Ki-67 and Caspase-3 in the patient's response to NACT in patients with stage IB2 and IIA2 cervical

carcinoma. However, the ratio of Ki-67 and Caspase-3 expression was correlated with shrinkage of the tumor size. This result suggested that higher proliferation rate than apoptosis can predict a better patient response to NACT in patients with stage IB2 and IIA2 cervical carcinoma.

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