Research Article

Caspase-3 can not be Used to Predict the Response to Neoadjuvant Chemotherapy Regiment PVB in Cervical Cancer Stage IB-IIA

Kaspase-3 tidak dapat Digunakan sebagai Prediktor Keberhasilan Pemberian Kemoterapi Neoajuvan Regimen PVB pada Kanker Serviks Stadium IB-IIA

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Abstract

Objectives: To determine the factors that may be used as the prognostic parameter for the therapeutic efficacy of neoadjuvant chemotherapy, which can be used to revising the management of early stage cervical cancer patients with large lesions.

Methods: This was a retrospective cohort study. The study was conducted in the Dr. Cipto Mangunkusumo Hospital, Faculty of Medicine, University of Indonesia. The subjects were 15 cervical cancer stage IB_2 and IIA patients with lesions' size of > 4 cm, who would be treated with neoadjuvant chemotherapy, consisted of cisplatin 50 mg/m^2, vincristine 2 mg/m^2 and bleomycin 15 mg regiment. The patients' response would be evaluated after completing 3 series of chemotherapy. Data was retrieved from medical records and cervical biopsy paraffin blocks and examined histopathologically using IHC staining to see expression of caspase-3 with histoscore assessment score. Data was analyzed by univariate, bivariate analysis.

Results: Response to PVB neoadjuvant chemotherapy was found in 5 out of 15 patients. None of the clinicopathology variables can be used to predict response to therapy. Expression of caspase-3 as a marker of apoptosis, can not predict the response of the therapy before administrating neoadjuvant chemotherapy either. There is a significant difference between the levels of caspase-3 in epidermoid carcinoma with adenocarcinoma, with p value of 0.02 (RR 6;95% CI 1.69-21.26).

Conclusion: Clinicopathologic factors and the expression of caspase-3 before getting chemotherapy neoadjuvant can not predict the succeed of the therapy.

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Keywords: caspase -3, clinicopathologic, early-stage cervical cancer lession in large, neoadjuvant chemotherapy response to therapy

Abstrak

***Tujuan**: Untuk mengetahui faktor-faktor yang dapat menjadi parameter prognostik keberhasilan terapi kemoterapi neoajuvan sehingga dapat memperbaiki tata laksana penanganan penderita kanker serviks stadium awal lesi besar.*

***Metode**: Penelitian kohort retrospektif yang dilakukan pada Rumah Sakit Dr. Cipto Mangunkusomo Fakultas Kedokteran Universitas Indonesia terhadap 15 sampel kanker serviks stadium IB_2 dan IIA dengan lesi > 4 cm yang akan dilakukan pemberian kemoterapi neoajuvan dengan regimen cisplatin 50 mg/m^2, vincristine 2 mg/m^2 dan bleomycin 15 mg dan dievaluasi responsnya setelah selesai pemberian kemoterapi 3 seri. Data diambil dari rekam medis dan blok parafin biopsi serviks diperiksa histopatologinya dan dilakukan pengecetan IHK untuk melihat ekspresi kaspase-3 dengan penilaian skor histoskor. Data dianalisis dengan analisa univariat, bivariat.*

***Hasil**: Respons terhadap kemoterapi neoajuvan PVB ditemukan pada 5 dari 15 subjek. Tidak satupun variabel klinikopatologi yang dapat di gunakan untuk memprediksi respons terapi. Ekspresi kaspase-3 sebagai penanda eksekutor apoptosis sebelum diberikan kemoterapi neoajuvan tidak dapat memprediksi respons terapi. Terdapat perbedaan bermakna kadar kaspase-3 antara karsinoma epidermoid dengan adenokarsinoma dengan p 0,02 (RR 6;95% CI 1,69-21,26).*

***Kesimpulan**: Faktor klinikopatologik dan ekspresi kaspase-3 sebelum mendapatkan kemoterapi neoajuvan tidak dapat memprediksi keberhasilan terapi.*

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***Kata kunci**: ekspresi kaspase-3, kanker serviks stadium awal lesi besar, klinikopatologik, neoajuvan kemoterapi, respons terapi*

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INTRODUCTION

Cervical cancer is the second most common cancer in women and the third leading cause of death in women worldwide. In 2007, there were approximately 550,000 new cases and 310,000 deaths per year. More than 83% of new cases and deaths occured in developing countries.[1,2] In Indonesia, cancer is the fifth leading cause of death, and its increasing number associated with a higher female life expectancy. In Indonesia, cervical cancer is the most common cancer from based on pathology reports in 2002.[3]

Patients in the early stages of cervical cancer, that are stage IB_2 and IIA, with the size of the lesions > 4 cm, have worse prognosis, and the therapeutic modalities of choice still remains controversial. Based on the Figo 2002, there are 3 alternative of therapeutic modalities, namely primary chemoradiation, primary radical hysterectomy and bilateral pelvic lymphadenectomy chemoradiation followed by adjuvant chemoradiation, and neoadjuvant chemotherapy followed by radical hysterectomy and bilateral pelvic lymphadenectomy followed by adjuvant radiation or chemoradiation.[4]

Not all patients give a good response to neoadjuvant chemotherapy, causing a need of a good predictor to predict the respond of therapy prior choosing the primary therapy type.[5]

Several predicting factors of dynamic cellular activity were autophagy, necrosis, mitotic disturbance and apoptotic. Some indicators such as Bcl_2, Ki-67, p_{21}, p_{53}, PRB, PCNA (proliferating cell nuclear antigen) give varying results. It was stated that p_{53}, Bcl_2, Ki-67 were not good predictor, while the PRB, PCNA, MDR_1, p_{21} were good predictor.[6]

The research aims to find out whether clinicopathologic factors and examination of the expression of caspase-3 before administration of neoadjuvant chemotherapy could be used as a biologic markers that can predict the response to therapy, therefore the selection of therapeutic modalities can be considered in early stage cervical cancer patients with large lesions.

METHODS

Patients and Methods

A retrospective cohort study conducted at the Dr. Cipto Mangunkusumo Hospital - Medicine Faculty University of Indonesia from July 2009 to July 2010 to 15 patients of cervical cancer stage IB2 and IIA with lesions > 4 cm who would be treated using neoadjuvant chemotherapy followed by radical hysterectomy surgery.

Treatment plan: Patients were given neoadjuvant chemotherapy regiment of PVB (cisplatin, vincristine, Bleomicyn), with a dose of cisplatin 50 mg/m^2 administered intravenously, Vincristine 2 mg/m^2 administered intravenously, and 15 mg Bleomycin administered intramuscularly. This regiment were given for 3 cycles with a 1-week interval.[7]

Evaluation of treatment response would be performed using RECIST criteria. Complete response was defined as the the loss of all target lesions, which was confirmed after > 4 weeks of chemotherapy completed. Partial response was a decrease of at least 30% of the target lesion, which refers to the diameter of the initial lesion. Progressive disease was characterized by the increment of at least 20% from the the diameter of the target lesion or new lesions encountered. Stable disease (SD) was defined as the lack of sufficient tumor shrinkage as to be classified as partial responses.[8]

Immunohistochemical Assay

Paraffin blocks from the the biopsy was reexamined with HE to confirm the diagnosis. Then the staining of caspase-3 expression by immunohistochemical assay was done. Primary antibodies used were mouse monoclonal antibody Santa Cruz Biotechnology, Inc, which was diluted 1,600 times. The reading of the IHC performed by Immunoreactive score (IRS)/histoscore. Further, data were analyzed using univariate and bivariate analysis. Since all the p value was more than 0.025, multivariate analysis was not performed.[9]

RESULTS

Patient Characteristics

Characteristics of 15 patients who received chemotherapy are listed in Table 2. Out of 5 subjects, the mean age, the youngest age and the oldest age were 45.5 ± 7.50 years, 27 years old 54 years old; respectively. The highest frequency occurred in the age group 40-49 years (7/15 or 46.7%) followed by age group over 50 years (5/15 or 33.3%). Mean while the mean age of first intercourse was 17.1 ± 2.46 years, and the youngest and the oldest age of first intercourse were 13 years old and 21 years old. The highest frequency of marital age was below the age of 20 years (13/15 or 86.7%). The most common educational level of the subjects was primary school 6/15 (40%). Most of the subjects had married once (12), and only 3 subjects have married at least 2 times. According to the number of parity, the subject are equally divided into 1-3 parity group (7/15), and 4-6 parity group (7/15). Distribution normality test of the data of age groups using Shapiro-Wilk was abnormal with p = 0.08.

Evaluation of Treatment

Complete response is defined as loss of all tumor mass. Partial response is defined as 30% or more reduction in tumor mass, whereas the tumor mass of less than 30% and progressive is defined as the onset of new lesions or tumors grow more than 20%. Patients were categorized as respond to therapy in case of complete response and partial response, and were considered as unrespond when there's are no progressive changes in result. The success rate of neoadjuvant chemotherapy with regiment PVB was found in 5 out of 15 subjects. Clinicopathologic factors showed no significant results for treatment response, as found in the following Table 1.

Table 1. Response NAC

	Therapy Response n(%)	
	Yes	No
Progresive		7 (46.7%)
Stable		3 (20%)
Partial	3 (20%)	
Complete	2 (13.3%)	

Table 2. Relations between Clinicopathologic Factors and Response Theraphy

Independent variable	Response to Theraphy		p	RR (95%CI)
	Negative	Positive		
Age				
< 50 years	6 (60%)	4 (80%)	1.00	1.33(0.68-2.60)
> 50 years	4 (40%)	1 (20%)		
First sexual intercourse				
< 20 years	9 (90%)	3 (60%)	0.24	2.25(0.44-11.52)
> 20 years	1 (10%)	2 (40%)		
Education				
No education	1 (10%)	1 (20%)		
Elementary	4 (40%)	2 (40%)	0.64	—
Junior High	3 (30%)	0 (0%)		
Senior High	2 (20%)	2 (40%)		
Married				
once	7 (70%)	5 (100%)	1.00	1.71(1.06-2.76)
twice	3 (30%)	0 (0%)		
Parity				
1 - 3	5 (33%)	2 (13%)	1.00	0.88(0.43-1.78)
> 3	5 (33%)	3 (20%)		
Histopathology assay result				
Squamous cell Ca (n=12)	9 (90%)	3 (60%)	0.24	0.44(0.09-2.28)
Adenocarcinoma (n=3)	1 (10%)	2 (40%)		
Differentiation				
poor (n=4)	2 (20%)	2 (40%)		
intermediate (n=8)	7 (70%)	1 (20%)	0.17	—
good (n=3)	1 (10%)	2 (40%)		
Lymphovascular stroma invation				
positive	2 (20%)	1 (20%)	1.00	1.00(0.41-2.45)
negative	8 (80%)	4 (80%)		
Necrosis				
positive	5 (50%)	2 (40%)	1.00	1.14(0.56-2.33)
negative	5 (50%)	3 (60%)		
Shape				
endophitic (n=3)	2 (20%)	1 (20%)	1.00	1.00(0.41-2.45)
exophitic (n=12)	8 (80%)	4 (80%)		
Mass				
< 4.8 cm	3 (30%)	2 (40%)	1.00	1.17(0.51-2.66)
> 4.8 cm	7 (70%)	3 (60%)		
Stage				
stage 1 (n=12)	8 (80%)	4 (80%)	1.00	1.00(0.41-2.45)
stage 2 (n=3)	2 (20%)	1 (20%)		
Histoscore category				
negative (n=10)	7 (70%)	3 (60%)	1.00	1.17(0.51-2.66)
positive (n=5)	3 (30%)	2 (40%)		

Table 3 shown that the type of histopathology provides a significant difference on the expression of caspase-3 with p 0.02. Other clinicopathologic factors showed that there was no significant difference on the expression of caspase-3.

Table 3. Relation between Clinicopathologic and Expression Caspase-3

Independent variable	Histoscore/Expression Caspase-3		p	RR (95%CI)
	Positive	Negative		
Age				
< 50 years	3 (60%)	7 (70%)	1.00	1.33(0.31-5.58)
> 50 years	2 (40%)	3 (30%)		
First sexual intercourse				
< 20 years	3 (60%)	9 (90%)	1.00	1.00(0.17-5.98)
> 20 years	2 (40%)	1 (10%)		
Married				
one	4 (80%)	8 (80%)	1.00	1.00(0.17-5.98)
twice	1 (20%)	2 (20%)		
Parity				
1 - 3	1 (20%)	8 (80%)	0.28	3.5(0.50-24.41)
> 3	4 (80%)	2 (20%)		
Histopathology assay result				
Squamous cell Ca (n=12)	2 (40%)	10 (100%)	0.24	0.44(0.09-2.28)
Adenocarcinoma (n=3)	3 (60%)	0 (0%)		
Differentiation				
poor (n=4)	1 (20%)	3 (30%)		
intermediate (n=8)	2 (40%)	6 (60%)	0.61	—
good (n=3)	2 (40%)	1 (10%)		
Lymphovascular stroma invation				
positive	1 (20%)	2 (20%)	1.00	1.00(0.17-5.98)
negative	4 (80%)	8 (80%)		
Necrosis				
positive	2 (40%)	5 (50%)	1.00	0.76(0.17-3.32)
negative	3 (60%)	5 (50%)		
Shape				
endophitic (n=3)	2 (40%)	1 (10%)	0.24	1.00(0.41-2.45)
exophitic (n=12)	3 (60%)	9 (90%)		
Mass				
< 4.8 cm	1 (20%)	4 (40%)	1.00	1.17(0.51-2.66)
> 4.8 cm	4 (80%)	6 (60%)		
Stage				
stage 1 (n=12)	4 (80%)	8 (80%)	1.00	1.00(0.41-2.45)
stage 2 (n=3)	1 (20%)	2 (20%)		

DISCUSSION

Primary standard treatment of cervical cancer stage IB-IIA is surgery, neoadjuvant followed by surgery or by radiation immediately. Application of two modalities of treatment will increase morbidity.[4]

In recent decade, the incidence of cervical cancer in younger age is increasing (<35 years). Based on research reports cancer statistics by SEER from the NCI in 1999-2006, the average age of patients with cancer was 48 years.[10,11] In this research, the mean age was 45.5 years (SEM [standard error of mean] 1.93) with a span of age was 27-54. The highest incidence occurred in the age group 40-49 years. It showed the decline in age of onset of cervical cancer, possibly due to the implementation of early detection program that were conducted by government. Other studies in Indonesia in 2002 showed the highest incidence was in the age group of 55-64 years.[3] In this study 80% of patients had first sexual intercourse when they were under 20 years. There were no differences in therapeutic response in patients with the age group below 50 years than over 50 years, with p = 0.6.

The relationship between clinicopathologic factors and biological markers have been studied as a predictor of efficacy in cancer treatment, including gynecological.[12] Several categories of dynamic cellular activities that lead to cell death such as apoptosis, autophagy, necrosis, mitotic destruction can be used for this purpose. Several indicators of predictor factors in the administration of chemotherapy has been investigated as gene Bcl-$_2$, Ki-67, p_{21}, p_{53}, PRB, VGEF, MDR_1 (Multidrug Resistance), PCNA (proliferating cell nuclear antigen) with varied results. Bcl-$_2$ and Ki-67 is not a good predictor with p> 0.05, while the PRB, PCNA, MDR_1, and p_{21} are a good predictor with p <0.05.[6,13,14]

In this study, clinicopathologic factors of stage, histological type, differentiation, size of the mass can not be used as a predictor factor for efficacy of neoadjuvant chemotherapy treatment, same as the result that proposed by Panicci, de Jounge et all.[8,15-18]

In this study, Clinicopathologic factors did not affect the expression of caspase-3 as an apoptotic protein that plays a role as an executor of apoptosis. All patients who had adenocarcinoma histology types showed strong expression of caspase-3 in IHC staining. There were significant differences compared to other types of squamous cell carcinoma.

There were five patients with positive appearance of caspase-3, two patients responded to neoadjuvant chemotherapy and the three others did not, and it was not statistically different, with p = 1. Similar to previous studies conducted by de Jounge and Tsuyoshi[19] Ghavami et al found a mutation in the gene caspase-3 thereby making it uncolored by caspase-3 monoclonal antigen which were not mutants. Caspase-3 mutations found in gastric adenocarcinoma, squamous cell carcinoma and adenocarcinoma of the lung, colon adenocarcinoma. The mutation occurs in Exon 6, Exon 5, Exon 4, is silent and missense mutations.[20] Previous study described that p_{53} might regulate cell proliferation and apoptosis induced by chemotherapy or radiation, but the cancer cells might lose p_{53} or p_{53} mutations.

Disruption or mutation in p_{53} would allow for a mutation in caspase-3 that play a role as an effector of apoptosis.[17,21-23] Other condition that contributes to cell death in apoptosis is the variety of substrates to be degraded, so it takes the role of non-caspase to degrade it. Like Calpain enzymes, Lysosomes, Protease and the others.[22,24] The cell death that occurs due to chemotherapy is not only a result of apoptosis pathway, but also result from other processes such as necrosis, autophagi and the destruction of mitotic.

Caspase-3, as the effectors of apoptosis, plays an important role in the process of apoptosis, and some chemotherapy agents can improve this process. In this study, caspase-3 expression does not affect the effect of neoadjuvant chemotherapy administration.

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