



Published by DiscoverSys

A Cohort Study: Caspase-3 As Prognostic Factor of Radiation Therapy in The Advanced Stage of Cervical Cancer (IIB-IIIIB) Patients



CrossMark

Hermawan Udiyanto,^{1*} Ambar Mudigdo,² Sri Sulistyowati,¹
Bambang Purwanto,³ Vitri Widyaningsih,⁴ Brian Wasita²

ABSTRACT

Background: The treatment of the advanced stage of cervical cancer (stage IIB, IIIIB) using internal (brachithery) and external radiation therapy raises question in how to determine the prognosis of the disease. The role of caspase 3 in predicting radiation response in the advanced stage of the cervical cancer (stage IIB-IIIIB) is still unknown. This study aim to analyze the role of Caspase-3 expression as a prognostic factor of radiotherapy in patients with the advanced stage cervical cancer.

Methods: This study is an analytical cohort prospective study from June 2019 to February 2020 in the Department of Obstetrics and Gynecology in association with the Department of Anatomic Pathology of Dr. Moewardi General Hospital, Surakarta, Indonesia involving 40 patients diagnosed with cervical cancer. The staging of cervical cancer

was determined using the FIGO 2019 staging system. The Caspase-3 expression level were determined using immunohistochemical examination. The association between the caspase 3 expression and changes of tumor volume is analyzed using chi square and fisher exact test for parametric and non-parametric test, respectively. A p-value of less than 0.05 is considered to be statistically significant.

Results: The analysis shows that there is a significant association between the Caspase -3 expression and the changes of tumor volume ($p = 0.04$). The odds of the changes of tumor volume in the score 3 of the caspase -3 expression is 22.67 times higher than the score 2 of the caspase-3 expression (95%CI, 1.56 - 328.95, $p=0.04$).

Conclusion: Caspase-3 expression has a significant association with the changes in the tumor volume of the advanced stage cervical cancer.

Keywords: Caspase-3, Radiation Therapy, Cervical Cancer

Cite This Article: Udiyanto, H., Mudigdo, A., Sulistyowati, S., Purwanto, B., Widyaningsih, V., Wasita, B. 2020. A Cohort Study: Caspase-3 As Prognostic Factor of Radiation Therapy in The Advanced Stage of Cervical Cancer (IIB-IIIIB) Patients. *Bali Medical Journal* 9(2): 501-503. DOI:10.15562/bmj.v9i2.1783

¹Department of Obstetrics and Gynecology, Dr. Moewardi General Hospital/Faculty of Medicine UNS, Surakarta, Indonesia;

²Department of Anatomic Pathology, Dr. Moewardi General Hospital/Faculty of Medicine UNS, Surakarta, Indonesia;

³Department of Internal Medicine, Dr. Moewardi General Hospital/Faculty of Medicine UNS, Surakarta, Indonesia;

⁴Department of Public Health, Faculty of Medicine UNS, Surakarta, Indonesia;

INTRODUCTION

The incidence and mortality rate of the cervical cancer for approximately 55% remained to be a major health problem.¹ In Indonesia, the mortality rates is tremendously high. It is approximated that there is one death in every hour caused by the cervical cancer.² Moreover, the 5-year survival rates for cervical cancer are lower in the advanced stage of FIGO staging.³ The treatment of the advanced stage of cervical cancer (stage IIB, IIIIB) using internal (brachithery) and external radiation therapy raises question in how to determine the prognosis of the disease.⁴

The current method for evaluating the cancer response to therapy is using Response Evaluation Criteria in Solid Tumours (RECIST) based on the clinical examination, Ultrasonography (USG), CT-Scan, and MRI.⁵ However, the advancement of the molecular biology of cancer provide a potential prognostic factor for assessing the response of cervical cancer to radiotherapy. The DNA damage resulted from the radiotherapy induces apoptosis via the activity of tumor suppressor gene p53 which are influenced by caspase proteins. The

activation of initiator caspases trigger the activation of the caspase cascade from the extrinsic pathway of apoptosis, in which caspase-3 plays a dominant role.⁶ However, the role of caspase 3 in predicting radiation response in advanced stage of the cervical cancer (stage IIB-IIIIB) is still unknown. Therefore, this study aims to analyze the role of Caspase-3 expression as a prognostic factor of radiotherapy in patients with the advanced stage cervical cancer.

MATERIALS AND METHODS

This study is a cohort prospective study conducted from June 2019 to February 2020 in the Department of Obstetrics and Gynecology in association with the Department of Anatomic Pathology of Dr. Moewardi General Hospital, Surakarta, Indonesia. This study was approved by The Research Ethics Committee of The Dr. Moewardi General Hospital Surakarta (Number 814/VII/HREC/2019). The participants were taken from the patients diagnosed with the cervical cancer within June 2019 – February 2020 period. The patients' medical record was reviewed and those

*Correspondence to:
Hermawan Udiyanto, Department of Obstetrics and Gynecology, Dr. Moewardi General Hospital/ Faculty of Medicine UNS, Surakarta, Indonesia
herry.udyanto@yahoo.com

who are diagnosed with stage IIB and IIIB based on the FIGO 2019 staging system are considered eligible for the study. A total of 40 participants were included in the study. The demographic data such as age, parity, body mass index (BMI), occupation, education status are collected from the medical record. The transabdominal USG examination is conducted before and after radiation therapy to evaluate the tumor volume changes as a response to radiotherapy. A tumor volume reduction of more than 70% relative to tumor volume is considered responsive to therapy.

A formalin fixed, paraffin-embedded tissue blocks of epithelial cervical cancer patients contained biopsy of patients cervix before radiation therapy were taken for Caspase-3 immunohistochemical analysis from Department of Pathology Anatomy for immunohistochemical analysis. The specimen was stained with Polyclonal Antibody of Caspase-3 Precursor (procaspase-3, bs-2593R) obtained from Bioss Biotech, and the tissues were incubated 18 hours inside of a refrigerator. The caspase 3 level was determined using Olympus CX22 Microscope under 400x magnification, if the stained (immunoreactive) cells make up less than (<) 10%, 10% to 50%, and more than 50% of the total field of view are scored 1, 2 and 3, respectively.⁷

The Statistical analysis was performed with the use of SPSS 22.0 software (SPSS Inc., Chicago, Illinois, USA). The association between the caspase 3 expression and changes of tumor volume is analyzed using Chi square and Fisher's exact test for parametric and non-parametric test. A p-value of less than 0.05 is considered to be statistically significant.

RESULTS

A total of 40 patients included in our research study with the mean age of 55.40 ± 9.82 years old and the mean BMI of 23.26 ± 5.50 kg/m². Most of the patients have history of number of parity of more than one (80%). The squamous cell carcinoma is accounted for 90% of the cervical cancer cases. Further details can be seen in [Table 1](#) and [Table 2](#).

The analysis shows that there is a significant association between the Caspase-3 expression and the changes of tumor volume with a p-value of 0.04. The odds of the changes of tumor volume in the score 3 of the caspase -3 expression is 22.67 times higher than the score 2 of the caspase-3 expression (95% CI, 1.56 - 328.95, p=0.04).

DISCUSSION

This study showed a significant association between the Caspase-3 expression and the tumor volume

changes with a p-value of 0.04. Our finding support that the low expression of caspase-3 often increases the number of cancer cells and makes cells more resistant to stress.⁸ This is similar with the previous research findings conducted on oral cancer which shows that the positive or strong expression of caspase-3 has oncogenic suppressor effects in patients on the differentiation stage.⁹ Although this study shows a significant association which shows the potential of caspase 3 as a prognostic factor, we acknowledge that the odds ratio can be ranged from 1.56 to 328.95 in the population. This might be affected from the small sample size in this study. Therefore, it is important to interpret this finding with caution. Furthermore, there is a need for further research with larger sample size to confirm with our findings.

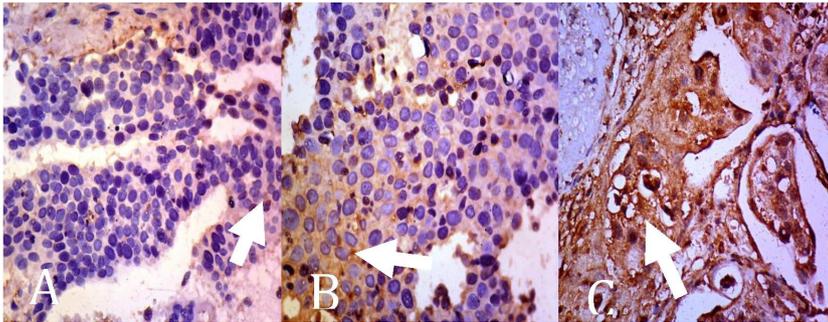
Table 1 The characteristics of subjects

Variables	n = 40 f (%)
Age in years (mean ± SD)	55.40 ± 9.82
BMI in kg/m ² (mean ± SD)	23.26 ± 5.50
Number of Parity	
Primiparity	7 (17.5%)
Multiparity	32 (80.0%)
Grandmultiparity	1 (2.5%)
Education	
Elementary School	24 (60.0)
Junior High School	7 (17.5)
High School	6 (15.0)
University	3 (7.5)
Occupation	
Housewife	22 (55.0)
Retired	1 (2.5)
PNS	1 (2.5)
Private	2 (5.0)
Farmer	5 (12.5)
Entrepreneur	9 (22.5)
Cancer Stage	
IIB	7 (17.5)
IIIB	33 (82.5)
Histopathology	
Adenocarcinoma	3 (7.5)
Adenosquamous carcinoma	1 (2.5)
Squamous cell carcinoma	36 (90.0)
Grading	
Bad	13 (32.5)
Moderate	8 (20.0)
Good	19 (47.5)

SD: Standard Deviation

Table 2 The proportion of subjects based on Caspase 3 expression and tumor volume changes

Caspase 3 expression	f (%)	Tumor Volume Changes	
		Response (>70%/cm ³) f (%)	No response (<70%/cm ³) f (%)
Score 2 (10%-50% of total field view)	3 (7.5)	1 (5.0)	2 (2.5)
Score 3 (>50% of total field view)	37 (92.5)	34 (85.0)	3 (7.5)



Picture 1 Immunohistochemical staining of Caspase-3 expression
 A. positive with 5% tumor cell; B. positive with 50% tumor cell;
 C. positive with 100% tumor cell (signification of 100x). The arrow shows caspase-3 in cytoplasmic cell marked with brown colours

CONCLUSION

Caspase-3 has a significant association with the changes in the tumor volume of cervical cancer.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The author reports no conflicts of interest in this study. The authors are responsible for the study funding without the involvement of grant or any other resource of funding.

ACKNOWLEDGEMENT

This study is fully funded by the first author. The authors would like to thank the Department of Pathology Anatomy, Faculty of Medicine, Sebelas Maret University; the Department of Obstetric and Gynecology of Dr. Moewardi General Hospital

Surakarta, and the Department of Radiology and Radiotherapy of Dr. Moewardi General Hospital Surakarta, who have been supporting in this study.

REFERENCES

- Campion MJ, Canfell K. Berek & Hacker's gynecologic oncology. 2015.
- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394–424.
- Ayhan A, Gultekin M, Dursun P. Advanced Cervical Cancer. In: Marnitz S, Kohle C, editors. Textbook of gynaecological oncology. Günes Publishing; 2012.
- Edianto D. Kanker Serviks, Buku Acuan Nasional Onkologi Ginekologi, Yayasan Bina Pustaka Sarwono Prawiroharjo. Edisi Pertama, Cetakan Pertama, Jakarta; 2006.
- Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer.* 2009;45(2):228–47.
- Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol.* 2007;35(4):495–516.
- Yamashita H, Nishio M, Toyama T, Sugira H, Zhang Z, Kobayashi S, et al. Coexistensi of HER2 Over-Expressing and p53 Protein Accumulation is a Strong Prognostic Molecular Marker in Breast Cancer. *Am J Pathol.* 2004;164(3):1031–8.
- Jakubowska K, Guzińska-Ustymowicz K, Famulski W, Cepowicz D, Jagodzińska D, Pryczynicz A. Reduced expression of caspase-8 and cleaved caspase-3 in pancreatic ductal adenocarcinoma cells. *Oncol Lett.* 2016;11(3):1879–84.
- Liu P-F, Hu Y-C, Kang B-H, Tseng Y-K, Wu P-C, Liang C-C, et al. Expression levels of cleaved caspase-3 and caspase-3 in tumorigenesis and prognosis of oral tongue squamous cell carcinoma. *PLoS One.* 2017;12(7).



This work is licensed under a Creative Commons Attribution