Activity of manganese superoxide dismutase (MnSOD) as a predictor of radiation therapy outcome in patients with stage IIIB squamous cell carcinoma cervical cancer

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ABSTRACT

BACKGROUND Radiation is a standard therapy for cervical cancer. Unfortunately, not all patients undergoing radiation achieve a complete response. Previous studies have shown that manganese superoxide dismutase (MnSOD) acts against free radicals generated by radiation in cancer cells thus predicting worse outcome in radiation therapy. This study was aimed to assess and evaluate whether MnSOD activity can be used as a predictor of radiation therapy responses in patients with stage IIIB squamous cell carcinoma (SCC).

METHODS A comparative cross-sectional study was conducted in the Gynecology Oncology Division, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta. The database from previous research was analyzed to identify positive and negative response samples. Measurement of MnSOD activity was done using spectrophotometry based on the McCord and Fridovich method using RanSOD® kit. The comparative data were obtained and then analyzed.

RESULTS Among 76 samples, 49 (61.8%) patients had positive responses and 27 (38.2%) had negative responses. It is shown in this study that higher MnSOD activity is related to worse radiotherapy outcome in stage IIIB cancer patients. The relative risk value of having a worse outcome with high MnSOD activity is 1.849 (1.075–3.178, 95% CI).

CONCLUSIONS Stage IIIB squamous cell carcinoma patients with high MnSOD activity are at higher risk of having a negative radiation therapy response compared with patients without high MnSOD activity.

KEYWORDS cervical cancer, MnSOD, radiotherapy, squamous cell carcinoma, uterine cervical neoplasms

Cervical cancer is the fourth most prevalent cancer in women around the world, with approximately 528,000 new cases each year.[1] Meanwhile, stage IIIB (advanced localized stage) is also the most prevalent stage, consisting of 46.4% of all advanced cases found.[2] Most cervical cancer patients in Indonesia are diagnosed with the advanced stage, causing the mortality rate of cervical cancer patients to remain high and treatment outcomes to be disappointing. To date, standard therapy for stage IIIB cervical cancer is chemoradiation consisting of external beam radiotherapy, intracavitary brachytherapy, and chemotherapy administered concurrently using cisplatin.[3,4] Based on the Indonesian Society of Gynecologic Oncology data, complete response was achieved by 46% of all patients at the Cipto Mangunkusumo Hospital in 2016.[2] However, there are only a few known factors for predicting radiation therapy outcome in cervical cancer.

Manganese superoxide dismutase (MnSOD) is an enzyme believed to protect cells from toxic products produced by oxygen metabolism. This antioxidant enzyme is essential for the survival of aerobic organisms and is an antiapoptotic agent against

ionizing radiation and oxidative stress.[5] MnSOD, which is produced in the mitochondria, reduces the amount of a free radical, such as superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2). Furthermore, the H_2O_2 is eliminated by glutathione peroxidase (GPx) and catalase by being converted into H_2O and molecular oxygen (O_2).[6] MnSOD also plays a role in the development of cancer and has been reported to be found in several types of cancer cells. Low expression of MnSOD is associated with a high rate of tumor growth.[7] Under oxygenated conditions, MnSOD is thought to decrease the effects of oxygen and may be involved in the induction of radiation resistance in both healthy tissue and malignant neoplasms.[8] Currently, no factor is available to predict the clinical response to radiation therapy for stage IIIB patients. MnSOD, as the front guard in blocking free radicals from radiation, is thought to be a predictor for clinical response to radiation therapy in stage IIIB cervical cancer. This study was aimed to assess and evaluate whether MnSOD activity can be used as a predictor of radiation therapy responses in patients with stage IIIB squamous cell carcinoma (SCC).

METHODS

This study is a retrospective study determined to assess the activity of MnSOD using spectrophotometric technique and evaluate whether MnSOD activity can be used as a predictor of radiation therapy responses in patients with stage IIIB SCC or not.

Samples were obtained before patients underwent radiation therapy to measure the activity of MnSOD in cancer tissues. Samples were then preserved in liquid nitrogen and regulated using phosphate salt. The activity of MnSOD was obtained biochemically using RanSOD® kit and spectrophotometry with McCord and Fridovich method. MnSOD activity was then determined by calculating the inhibition rate of reaction between hydrogen peroxide, oxygen, and xanthine oxidase.

Radiation therapy response was determined by using magnetic resonance imaging (MRI) study on patients three months after completion of 25 times external radiation regiments. Complete response was defined as no primary tumor lesion found on MRI and no clinical symptoms found in patients, partial response was defined as more than 30% reduction in tumor size, progressive response was defined as more than 20% enlargement in tumor size, while a stable response was defined as a response other than stated above.

This study used 5% error, and 95% confidence intervals, and the power of the test was considered to be 90%. Among all patients with stage IIIB SCC cervical cancer in Cipto Mangunkusumo Hospital, 76 subjects were matched with inclusion criteria.

The inclusion criteria for this research are adult patients with stage IIIB SCC cervical cancer who had undergone a complete radiation therapy regimen in the Department of Radiotherapy, FMUI–Cipto Mangunkusumo Hospital Jakarta from January 2016 to December 2017. While the exclusion criteria are patients having additional cancer besides cervical cancer, had undergone any other modality of therapy such as surgery or chemotherapy, or did not complete the full regimen of radiation therapy.

The study was approved by the Faculty of Medicine, Universitas Indonesia. All human studies were approved by the Research Ethics Committee with ethical clearance number 033/UN2.F1/ETIK/2018. All patients who were included in this study had given their informed consent before their inclusion in the study.

Collected data were analyzed using Statistical Package for the Social Sciences (SPSS) for Macintosh v. 20. Data were collected from previous research to obtain outcomes of radiation therapy for each patient.[9] Sociodemographic and clinicopathologic characteristics of patients were analyzed descriptively. Due to the absence of a cut-off for significant MnSOD activity in cancer patients, the cut-off was determined using a receiver operating characteristic (ROC) curve and to classify subjects into two study groups for consideration of highest sensitivity, specificity, positive likelihood ratio, and accuracy. Bivariate analysis between MnSOD activity and radiation therapy response was done to find the relationship between variables using the Chi-square method. The relationship between MnSOD activity and radiation therapy response was then analyzed statistically.

RESULTS

A total of 76 patients met the inclusion criteria and were further analyzed. A univariate test was performed to assess the general characteristics of the study

Table 1. Sociodemographic and clinicopathologic characteristics of stage IIIB cervical cancer patients

Subjects' characteristics	Frequency, n (%) (n = 76)
Age (years), mean (SD)	50.29 (8.6)
First sexual intercourse age (years)	
<20	33 (43.4)
≥20	43 (56.6)
Parity, median (min–max)	3 (0–8)
Diameter of tumor (cm), median (min–max)	5.35 (1.9–15.0)
Tumor differentiation	
Good	19 (25)
Bad	57 (75)
Radiation therapy response	
Complete–partial	49 (61.8)
Stable–progressive	27 (38.2)
MnSOD activity (U/ml), median (min–max)	8.85 (0.748–31.30)

MnSOD=manganese superoxide dismutase

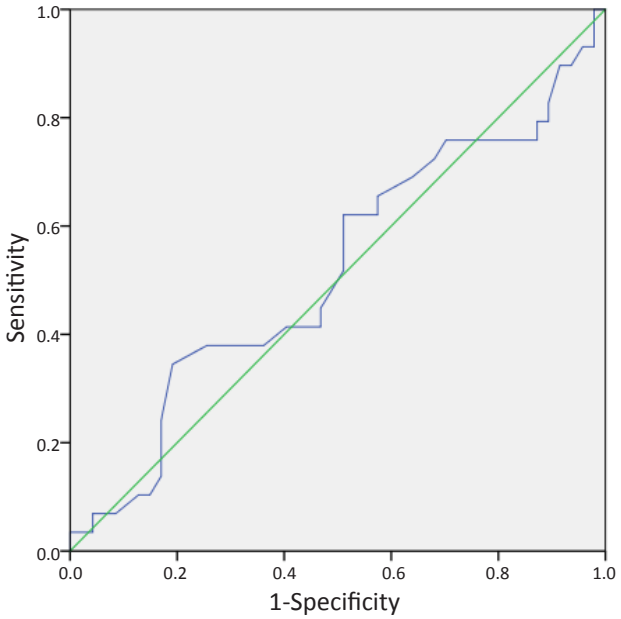

Figure 1. Receiver operating characteristic curve of MnSOD activity

subjects' sociodemographic and clinicopathologic variables (Table 1).

MnSOD activity was categorized using ROC curve analysis to classify subjects into two study groups. A cut-off limit of ≥13 U/ml was set considering likelihood ratio (LR+) value of 1.81, sensitivity of 34.5%, specificity of 80.9%, and accuracy of 57.70% (Figure 1).

Categorization of subjects based on MnSOD activity, sociodemographic, and clinicopathologic characteristics for each study group were analyzed and are shown in Table 2.

A bivariate analysis was performed on each sociodemographic and clinicopathologic characteristic of subjects to find significant relationship between characteristics of subjects and the radiation therapy response. Results of this bivariate analysis can be found in Table 1. Based on these results, there was no significant relationship between subjects' characteristics and radiation therapy response.

A Kaplan-Meier study was also done in this study to compare the mortality rate of stage IIIB SCC cervical cancer patients between patients with high MnSOD activity and patients with low MnSOD activity. Based on this study, patients with lower MnSOD activity (<13 U/ml) had a median survival rate of 355 days (324–385 days) and patients with a higher MnSOD activity (>13 U/ml) had a median survival rate of 280 days (176–383 days) (Figure 2).

DISCUSSION

Cervical cancer is the fourth most prevalent cancer among women in the world, with stage IIIB being the most common stage. To date, the standard therapy for stage IIIB cervical cancer is chemoradiation and chemotherapy, but only a few known factors about predicting radiation therapy outcome in cervical cancer exist. MnSOD is thought to be a predictor for clinical response to radiation therapy in stage IIIB cervical cancer. MnSOD also plays a role in the development of cancer and has been found in several types of cancer cells.[6,7]

In this study, it can be found that higher level of MnSOD activity is related to both poorer radiation therapy outcomes and worse 1-year survival rates for stage IIIB SCC cervical cancer patients. Generally, stage IIIB SCC cervical cancer patients in Cipto Mangunkusumo Hospital from January 2016 to December 2017 were about 50 years old and had three children. This data is similar to a study conducted by Aziz which shows that the risk of having cervical cancer is higher in patients aged 50 years or older with an odds ratio of 2.53.[10] Subjects were then categorized into two study groups, with MnSOD activity of <13 U/ml in 55 subjects (72.4%) and ≥13 U/ml in 21 subjects (27.6%). No previous research has examined the relationship between MnSOD activity and radiation therapy outcome in SCC, therefore no comparison can

Table 2. Clinicopathologic and sociodemographic characteristics based on MnSOD activity

Subjects characteristics	MnSOD activity <13 U/ml, n (%) (n = 58)	MnSOD activity ≥13 U/ml n (%) (n = 18)	*p*
Age, mean (SD)	50.25 (8.635)	50.22 (9.073)	
First sexual intercourse age (years)			
<20	32 (55.2)	11 (61.1)	
≥20	26 (44.8)	7 (38.9)	
Parity, median (min–max)	3 (0–8)	3 (0–6)	
Diameter of tumor (cm), median (min–max)	5.0 (2.3–11.0)	5.3 (1.7–15.0)	
Keratinization			
No	55 (94.8)	14 (77.8)	
Yes	3 (5.2)	4 (22.2)	
Differentiation degree			
Good	13 (22.4)	6 (33.3)	
Bad	45 (77.6)	12 (66.7)	
Radiation therapy outcome			0.035
Complete–partial	38 (69.1)	9 (42.9)	
Stable–progressive	17 (30.9)	12 (57.1)	

MnSOD=manganese superoxide dismutase

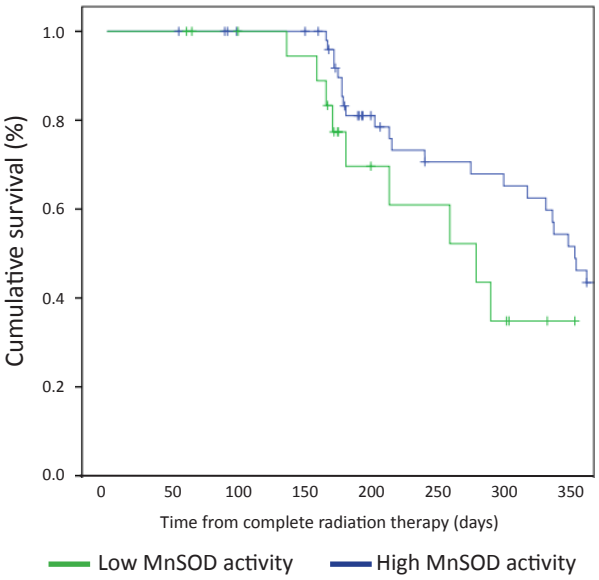

Figure 2. Survival curve for different MnSOD activities

be made with this data. Nelson et al[7] explained that low expression of MnSOD was associated with a high rate of tumor growth. High tumor mitotic activity is found to respond better to radiation therapy.[11]

Based on this study, it is known that higher MnSOD activity is related to worse radiotherapy outcome in stage IIIB cancer patients. There was no previous research studying the relationship between MnSOD and radiation therapy outcome. However, the results of this study can be explained by the theory that MnSOD is the first line in fighting against free radicals produced by the radiation process by eliminating superoxide radical toxins and protecting cells from the damaging effects of ionizing radiation by reducing O_2^- into H_2O_2. Furthermore, H_2O_2 is eliminated by GPx and is catalyzed into H_2O and O_2.[8] As this process happens, the amount of free radicals that are supposed to eliminate cancer cells decreases, so the radiation therapy is not as effective. Thus, the higher the activity of MnSOD, the worse the radiation therapy response based on the results stated in Table 2. Application of these results to MnSOD activity before radiation therapy can be used to predict the response in stage IIIB cervical cancer patients.

One-year survival analysis was also done in this study using the Kaplan-Meier method. From this analysis, it was concluded that patients with lower MnSOD activity (<13 U/ml) generally have a 1-year better survival with a median survival rate of 355 days (324–385 days) than patients with higher MnSOD activity (>13 U/ml) who have lower median survival rate of 280 days (176–383 days). This result is similar to that of a study reported by Nakano et al[8] in Japan, in which tumors that expressed MnSOD had lower survival rates than those with negative MnSOD. The study was performed using the immunohistochemistry method.[8]

In conclusion, this study found that higher levels of MnSOD activity are related to poorer radiation therapy

outcomes and worse 1-year survival rates for stage IIIB SCC cervical cancer patients. From this conclusion, it is thought that the manipulation of MnSOD activity can be utilized to improve radiation therapy outcomes. One option to manipulate the level of MnSOD is to use carbogen and nicotinamide to decrease the amount of cell hypoxia.[12] Another available alternative is to use nelfinavir, a human immunodeficiency virus protease inhibitor, which can increase the level of ROS in the mitochondria by reducing MnSOD through a process that remains unknown.[13]

Conflict of Interest

Authors declare that there is no conflict of interest in this study.

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REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86.
2. Staging Cervix [Internet]. Indonesian society of gynecologic oncology (INASGO). 2015.
3. Di Saia P, Creasman W. Clinical gynecologic oncology. 8th ed. Philadelphia: Elsevier Inc.; 2012. 98 p.
4. Marth C, Landoni F, Mahner S, McCormack M, Gonzalez–Martin A, Colombo N. Cervical cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow–up. Ann Oncol. 2017;28(suppl_4):iv72–83.
5. Miao L, St Clair DK. Regulation of superoxide dismutase genes: implications in disease. Free Radic Biol Med 2009;47(4):344–56.
6. Matés JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology 2000;153(1–3)83–104.
7. Nelson KK, Ranganathan AC, Mansouri J, Rodriguez AM, Providence KM, Rutter JL, et al. Elevated SOD2 activity augments matrix metalloproteinase expression: evidence for the involvement of endogenous hydrogen peroxide in regulating metastasis. Clin Cancer Res 2003;9(1):424–32.
8. Nakano T, Oka K, Taniguchi N. Manganese superoxide dismutase expression correlates with p53 status and local recurrence of cervical carcinoma treated with radiation therapy. Cancer Res 1996;56(12):2771–5.
9. Kusuma F, Andrijono, Nuranna L, Prijanti AR, Sekarutami SM. Levels of Survivin, Telomerase and Cytochrome C as Predictors of Therapeutic Response in Patients with Stage IIIB Squamous Cell Carcinoma of Cervix. PhD [Dissertation]. Jakarta: Faculty of Medicine Universitas Indonesia; 2017.
10. Aziz MF. Gynecological cancer in Indonesia. J Gynecol Oncol. 2009;20(1):8–10.
11. Catheryn M. Yashar. Clinical Gynecologic Oncology. 8th ed. Philadelphia: Elsevier Inc.; 2012. 98 p.
12. Sekarutami SM, Gondhowiardjo S, Aziz F, Sregar NC, Harahap A. Effects of hypoxia on angiogenesis and proliferation–correlation with tumor response in patients with cervical cancer treated with combined radiation and carbogen=nicotinamide. Ejc Suppl. 2011;1–23:22–3.
13. Xiang T, Du L, Pham P, Zhu B, Jiang S. Nelfinavir, an HIV protease inhibitor, induces apoptosis and cell cycle arrest in human cervical cancer cells via the ROS–dependent mitochondrial pathway. Cancer Lett 2015;364(1):79–88.