Research Article

Higher HIF-1α Level in Cervical Cancer Worsen the Outcome of Radiotherapy in Stage IIIB Squamous Cell Carcinoma of the Cervix

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Abstract

Objective: To assess and evaluate HIF-1α levels as predictors of radiotherapy outcomes for patients with stage IIIB cervical cancer.

Methods: This retrospective cohort study was conducted in the Gynecology Oncology Division, Department of Obstetrics and Gynecology at FKUI, RSCM Jakarta. Biopsy data from 76 patients were analyzed to investigate HIF-1α levels using ELISA. Subsequently, these patients underwent complete radiotherapy, and outcomes were assessed using magnetic resonance imaging (MRI). Outcomes were categorized as positive response (disappearance of all lesions or a ≥30% decrease in the sum of the longest diameter compared to before radiotherapy) or negative response (lack of positive response criteria and a ≥20% increase in the smallest sum or new lesions). The one-year survival rate according to HIF-1α levels was also calculated. Data were analyzed accordingly.

Results: Among the 76 samples, 49 (61.8%) patients exhibited positive (complete and partial) responses, while 27 (38.2%) exhibited negative (progressive and stable disease) responses. The HIF-1α cut-off level ranged from 0.001 to 0.297 pg/mg, with the cut-off set at 0.019 pg/mg. We observed that higher HIF-1α levels worsened the outcomes of radiotherapy in patients with stage IIIB squamous cell carcinoma (SCC) cervical cancer (p = 0.044, RR= 1.909, 1.07- 3.75, 95% CI). A low HIF-1α level was associated with a better one-year survival rate (p=0.011).

Conclusion: Patients with stage IIIB squamous cell carcinoma cervical cancer and higher HIF-1α levels are at a 1.909-fold increased risk of experiencing negative radiotherapy responses compared to those with lower HIF-1α levels.

Keywords: cervical cancer, HIF-1α, radiotherapy response, stage IIIB SCC.

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INTRODUCTION

Cervical cancer is one of most prevalent cancers in women worldwide. During 2018, the number of cervical cancer cases worldwide has unalterable, namely 570.000 new cases and 311.000 death on 2018. The average age at which cervical cancer claimed a life was 59 worldwide; the range was 45 years (Vanuatu) to 76 years (Martinique). Out of 185 nations evaluated, 146 (79%) had cervical cancer in the top three malignancies affecting women under 45 years of age.[1] Due to the absence of symptoms in the early stages, cervical cancer is often diagnosed at an advanced stage. A study conducted at Dr. Cipto Mangunkusumo National General Hospital revealed that 41.6% of cervical cancer patients were diagnosed at stage III, with 71.6% of cases being of the squamous cell type.[2,3]

A common characteristic of cervical cancer, akin to other solid cancers, is oxygen deprivation (hypoxia) induced by aberrant tumor vasculature[4]. During the hypoxic state of the tumor, the tumor-specific immune response is modulated by the activation of Hypoxia-Inducible Factors (HIFs) and their downstream signaling pathways, including

CXCR4, M-CSFR, and CD47. Consequently, various immunosuppressive cytokines and growth factors are produced, promoting immune evasion and accelerating tumor progression.[5] Additionally, hypoxic tumor cells exhibit increased resistance to radiation. These cells initiate stress response mechanisms to adapt to low oxygen concentrations. Certain cells within the tumor respond adaptively to hypoxic stress by modifying their gene expression, leading to an aggressive phenotype and therapeutic resistance.[6] Cancer cells alter their metabolism in order to increase growth, survival, proliferation, and long-term survival. The unifying hallmark of this altered metabolism is enhanced glucose absorption and lactate fermentation. This process is observed even in the presence of fully functional mitochondria, and it is referred to as the Warburg Effect. Monocarboxylate transporter 4 exports lactate from cells as an end product of lactic acid fermentation following glycolysis. The lactate is subsequently taken up by cancer cells via the monocarboxylate transporter 1 (MCT1) and converted to pyruvate via the enzymatic activity of lactate dehydrogenase-B (LDH-B). Increased intracellular pyruvate levels limit the formation of alpha ketoglutarate, which stabilises and activates HIF-1, resulting in the activation of VEGF-dependent tumour angiogenesis and the acceleration of tumour growth.[7] Radioresistance caused by HIF-1-induced Warburg effect results in cancer cells that are difficult to treat and may result in tumour recurrence. HIF-1 activators represent a promising group of targets that could lead to the development of novel therapies.[7] As the consequences, the sensitivity of hypoxic tumor cells to radiation therapy will be decreased. In aggressive tumors, HIF-1α is generally more pronounced. It can be independent predictor of poor prognosis in certain types of cancer.[8]

The treatment method required is determined by the stage and extent of cervical cancer progression, which may comprise one or a combination of surgery, radiation, and chemotherapy.[9] However, failure rate of radiotherapy in cervical cancer patients is still unsettling, about 42% for stage III patients and 74% for stage IVA patients.[3] Prognostic factors of outcome for radiotherapy are related to patient age, stage of the disease, tumor size, histopathology of tumor, differentiation rate, and lymph node metastasis.[3] It is also affected by apoptosis factors and various biomarkers such as ki67, cell division cycle 6 (CDC6), maintenance protein 5 (MCM5), and c-myc.[3]

HIF-1 is the biomarker that need further study in order to determine their relation to radiotherapy outcome. It is one of the biomarkers regulated during hypoxia state of cells. Solid tumors must have experienced hypoxic state during its growth along with angiogenesis process.[10] As the consequences, it would also regulated HIF-1 during growth. HIF-1 consists of 2 subunits, HIF-1α and HIF-1ß. HIF-1α is a subunit which regulated by oxygen level and stimulates angiogenesis, erythropoiesis, and eventually apoptosis.[11] As a result, tumour therapies, such as radiotherapy, chemotherapy, and immunotherapy, can be less successful in a hypoxic tumour microenvironment (TME).[12]

Based on statements mentioned above, this study aims to assess and evaluate HIF-1α as a radiotherapy outcome predictor for stage IIIB cervical cancer patients.

METHODS

This cohort study included 76 patients with stage IIIB cervical cancer, all of whom had squamous cell carcinoma type and had not received any prior treatment before undergoing HIF-1α examination. Consecutive sampling was employed during patient selection. Exclusion criteria for the study encompassed patients with any other type of cancer besides cervical cancer and those who had undergone alternative forms of therapy such as surgery or chemotherapy. All patients received treatment at the Department of Radiotherapy, Faculty of Medicine, Universitas Indonesia. The study was conducted within the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, at Dr. Cipto Mangunkusumo National General Hospital.

HIF-1α level as independent variable was assessed using lab study before radiotherapy was initiated. Biopsy of cancer tissue was performed. Level of HIF-1α was assessed using ELISA method in Biochemical Laboratory Department of Biochemistry Faculty of Medicine, Universitas Indonesia. Due to absence of cutoff for significant HIF-1α level category for cervical cancer patients, HIF-1α level was assessed quantitatively and categorized into two groups, namely high and low level of HIF-1α using ROC (Receiver Operating Characteristic Curve) into two study groups in consideration of highest sensitivity, specificity, positive likelihood ratio, and accuracy possible.

Meanwhile, radiotherapy outcome was

determined using magnetic resonance imaging (MRI) which was assessed by radiologists in Dr. Cipto Mangunkusumo National General Hospital. Complete and partial response was then categorized as positive response while stable or progressive response was categorized as negative response.

The study was approved by the Faculty of Medicine, Universitas Indonesia. All human studies had been approved by the Research Ethics Committee (ethical number: 0944/UN2.F1/ETIK/2018). All patients who were included in this study had given their informed consent prior to their inclusion in the study.

This study use 5% error bound and 95% confidence interval limit, power of the test considered to be 90%. Collected data were then analyzed using SPSS for Macintosh version 22 software. The data was analyzed using chi square test.

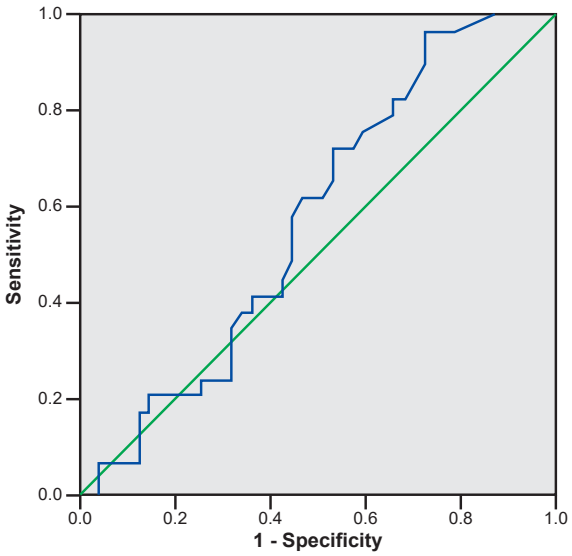
RESULTS

Seventy-six samples met the inclusion criteria for this study. Univariate tests were conducted to assess the characteristics of the study group subjects. The authors did not differentiate age since a normal distribution was observed, with a mean of 50.29 and a standard deviation of 8.6. Consequently, similarities were found in sociodemographic and clinicopathological characteristics among the study subjects, allowing for comparisons between groups. The characteristics among the groups in this study are presented in Table 1.

Table 1. Sociodemographic and Clinical Pathological Characteristics of Stage IIIB Cervical Cancer Patients

Subjects characteristics	n (%) (N = 76)
Age (years), mean (SD)	50.3 (8.6)
30–40	13 (17.1)
41–50	23 (30.3)
51–60	31 (40.8)
61–70	9 (11.8)
First sexual intercourse age (years)	
<20	33 (43.4)
≥20	43 (56.6)
Parity, mean (SD)	3 (0–8)
Diameter of tumor (cm), mean (SD)	5.35 (1.9–15.0)
Tumor differentiation	
Grade I	19 (25.0)
Grade II–III	57 (75.0)
Radiation therapy response	
Positive	49 (61.8)
Negative	27 (38.2)

In order to classify subjects into two study groups, HIF-1α level was categorized using ROC (Receiver Operating Characteristics) curve analysis based on HIF-1α positive response and negative response. Considering highest LR+ value of 1.36, sensitivity of 72.4%, specificity of 46.8%, and accuracy of 59.60%, cutoff point for HIF-1α was determined at 0.019 pg/mg. ROC curve of HIF-1α level can be seen in figure 1.



Afterwards, clinicopathologic and sociodemographic characteristics for each of the study groups were analyzed as shown in table 2.

Table 2. Clinicopathologic and Sociodemographic Characteristics Based on HIF-1α Level

Subjects characteristics	Positive response n (%)	Negative response n (%)	Total	*P-value*
Age (years), mean (SD)	50.45 (8.74)	50.03 (8.52)		0.059
First sexual intercourse age				0.449
<20	21 (63.6)	12 (36.4)	33 (43.4)	
≥20	26 (39.5)	26 (60.5)	43 (61.4)	
Diameter of tumor (cm)	5 (1.9–10.2)	6 (3.1–15.0)		0.337
Differentiation degree				0.054
Grade I	31 (66.6)	26 (89.7)	57 (75.0)	
Grade II–III	16 (34)	3 (10.3)	19 (25.0)	
Types of cancer				0.278
SCC	44 (63.8)	25 (36.2)	69 (90.8)	
Ceratinized SCC	3 (42.9)	4 (57.1)	7 (9.2)	
HIF-1α level (pg/mg)				0.044
≥0.019	23 (52.3)	21 (47.7)	44 (57.9)	
<0.019	24 (75)	8 (25)	32 (42.1)	

HIF-1α=hypoxia inducible factor-1 alpha; SD=standard deviation; SCC=squamous cell carcinoma

Chi square analysis was done to determine relationship between HIF-1α level and radiation therapy response. P value of the study was 0.044 which showed there was a significant relationship between HIF-1α level and radiation therapy response in stage IIIB SCC cervical cancer patients. Relative risk of this study was 1.909 (1.07 – 3.75, CI 95%).

DISCUSSION

In this study, a high level of HIF-1α was identified as one of the risk factors associated with a poorer outcome following radiotherapy, with a relative risk (RR) of 1.909 (95% CI = 1.07–3.75). This finding aligns with the pathophysiology of cervical cancer, as tumors with elevated HIF-1α levels face challenges in maintaining ATP levels during radiation therapy. Conversely, tumors in a hypoxic state tend to exhibit increased bioenergetic processes mediated by HIF-1α, leading to sustained tumor proliferation.[6,13]

The study concludes that a high level of HIF-1α is a risk factor for a poorer radiotherapy outcome, with a relative risk of 1.909 (95% CI: 1.07–3.75). This finding is consistent with the tumor's pathophysiology, as tumors with elevated HIF-1α levels struggle to maintain ATP levels during radiation therapy. Conversely, tumors in a hypoxic state tend to exhibit increased bioenergetic processes mediated by HIF-1α, which contributes to sustained tumor proliferation.[14]

In general, stage IIIB SCC cervical cancer patients in Dr. Cipto Mangunkusumo National General Hospital were about 50 years old, had born 3 children and had bad differentiation degree. This result is similar to a comparable study which shown that women aged more than 50 years is more prone to suffer from cervical cancer.[3] As mentioned before, we found that there are similarities in sociodemography and clinicopathology between subjects' characteristics.

Based on the bivariate analysis of each sample's characteristics, no significant relationship was found between patient characteristics and radiation therapy outcome in patients with stage IIIB squamous cell carcinoma (SCC) of the cervix. This result aligns with a previous study that demonstrated no significant association between patient age, overall survival, and relapse-free survival in cervical cancer patients.[10]

From this study, it can be concluded that HIF-1α promotes radioresistance in tumors. Three out of four functions of HIF-1α—apoptosis, metabolism, and proliferation—promote radiosensitization, while the fourth function, vascular protection, paradoxically promotes radioresistance for tumors. Despite having more functions that promote radiosensitization, it is concluded that the radioresistance function of HIF-1α is significantly more dominant. This is because an increase in HIF-1α levels leads to an upregulation of the transcription factor nrf2, which in turn regulates the production of enzymatic (endogenous) antioxidants such as superoxide dismutase (SOD), glutathione peroxidase, and catalase. The increase in enzymatic antioxidants can exacerbate radiation therapy responses.[15]

As the result, HIF-1α level influenced the

outcome of radiotherapy in cervical cancer patients. Along with increased HIF-1α level (cut-off point 0.019 pg/nm), the tumor became more resistant to radiotherapy. Based on this result, assessing HIF-1α level may give benefit to predict the success possibility of treatment before proceeding to radiotherapy. Limitation of this study was a single center of study. It is never attempted before to do multicenter research.

ACKNOWLEDGEMENTS

Authors would like to express highest gratitude to Department of Pathology Anatomy (Primariadewi Rustamadji, M.D., Ph.D.), Department of Radiotherapy (Sri Mutia Sekar Utami, M.D., Ph.D.), and Department of Radiology (Jacub Pandelaki, M.D., Ph.D.) Cipto Mangunkusumo Hospital. Special thanks to Cipto Mangunkusumo Oncology Division academical guidance and psychological supports, also to Hibah PITTA 2018 which provided the funding without any interest as return.

CONFLICT OF INTEREST

There is no conflict of interest in this study.

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