Circadian as a prognostic factor for radiation responses in patients with cervical cancer: A nested case-control study

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Abstract. The radiation response of cervical cancer is thought to be enhanced by the levels of melatonin due to its roles in the circadian cycle and cancer growth. In the present study, the roles of circadian rhythms and melatonin levels as prognostic factors for predicting the radiation response in patients with cervical cancer were examined. In this nested case-control study, patients with good and poor responses to radiotherapy were assessed in terms of the time-of-day radiation treatment was administered and further influencing factors. The radiation time was determined, as the subjects were either irradiated in the morning (06.00-10.00 am) or afternoon (04.00-06.00 pm). Data on tumour size and other biological parameters were collected and analysed by binary logistic regression. Among the 56 patients examined, most subjects had good radiation responses. Most patients were <50 years old with an initial body weight of >50 kg, no pain prior to radiation, low erythrocyte sedimentation rates, normal intravenous urography results, moderate or good differentiation on pathology and histo-pathologically non-keratinised cells. According to the multivariate analysis, the irradiation time as a surrogate of the circadian cycle (morning vs. afternoon), the initial haemoglobin (Hb) level and the clinical tumour size were significant predictors of the radiation response. The circadian cycle, tumour size and Hb levels may affect the radiation response in patients with cervical cancer. In addition, the morning group had better 5-year overall survival, but it was not significant, possibly due to the small cohort size. Further research is required to identify more relevant prognostic factors using different radiotherapy techniques [National Clinical Trial (NCT) no. NCT05511740, registration date, 08/20/2022].

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Introduction

Cervical cancer is a global malignancy burden among females; it ranks 4th in both incidence and mortality worldwide based on GLOBOCAN 2020 (1). It is estimated that lower-to-middle income countries account for ~84-90% of the global cervical cancer cases (2). Particularly in Indonesia, cervical cancer is the second most common and the second deadliest malignancy reported (1). It was also noted that the incidence rate of cervical cancer in Indonesia rose by ~17% between 1990 to 2017; however, the death rate remains relatively similar (3). Increased efforts are required to improve the treatment of cervical cancer.

Radiotherapy has an essential role in the treatment of cervical cancer. Concurrent chemoradiotherapy may yield a 5-year overall survival rate of almost 70% for locally advanced cervical cancer (4). In comparison with radiotherapy alone, chemoradiotherapy also leads to a significant 6% improvement in 5-year-survival (5). Adjuvant radiotherapy may decrease disease recurrence with a relative risk of 0.53 compared to no treatment. Although chemoradiation is the mainstay treatment for locally advanced cervical cancer, the results of this treatment modality remain unsatisfactory.

Based on radiobiology, the effect of radiation increases if the cell is in the radiosensitive phase when exposure is given. Radiotherapy failure occurs when the proportion of radioresistant cells is more significant, so that tumour proliferation cannot be prevented.

It is critical to remember that cancer cells are most sensitive to radiation during the G2/M phase of the cell cycle. However, clinical identification and assessment of cell kinetics to obtain the timing of G2/M phases are difficult and impractical (6). A previous study by our group examined the DNA content using flow cytometry to assess the proportion of G2/M and S phases and to analyse whether the proportion of sensitive phases had a circadian pattern (7). However, the study was not able to provide objective results due to the limited number of samples.

It is worthwhile to explore factors affecting radiotherapy and cellular kinetics, which may potentially increase radiosensitivity in cervical cancer. The circadian rhythm and the melatonin concentration are two such factors, which happen to be interrelated. The circadian cycle is a biorhythmic daily period in various body systems, featuring a specific, intricate, harmonious pattern. The circadian and cell cycles are two critical systems initially considered separate, but several studies have proven a close relationship between them (8-14).

Furthermore, melatonin protects cells against the side effects of radiation because it is a scavenger of OH-containing molecules (15). However, it has remained elusive whether the circadian rhythm and the level of melatonin may clinically affect the radiation response.

The current standard conventional radiation therapy guideline does not specify the timing for radiation treatment (morning, afternoon or evening) and there is no adjustment or preference for the timing of radiation among individual patients. Differences in response to radiation are expected in patients with cervical cancer between the morning and afternoon radiation groups due to the circadian rhythm and melatonin levels.

In the present study, it was hypothesised that the circadian cycle influences tumour radiosensitivity, including that of cervical cancer. A previous study by our group indicated that the melatonin concentration in patients with cervical cancer was significantly different when measured in the morning and in the afternoon (7). Based on these initial findings, a further study was performed and patients with cervical cancer were allocated into two groups: Patients who were irradiated in the morning and those irradiated in the afternoon. The pre-treatment melatonin concentration in the blood was measured exactly prior to irradiation to closely represent the melatonin concentration in the two groups.

The present study tested the hypothesis that a difference in radiation sensitivity of cervical cancer is present, depending on the time of day within 24 h. Based on previous findings, a study was designed to examine the effect of radiation administration at two different times in the circadian pattern on tumour response. The melatonin levels in each subject in the two groups were checked immediately prior to radiation to determine the melatonin levels corresponding to the time of radiation administration. Melatonin levels were examined three times in the irradiation period from the beginning of external radiation until the end of brachytherapy. It included the time-points prior to the initial irradiation, in the middle of the radiation period (at the 15-20th fraction) and after brachytherapy (7).

A case-control study was herein performed following on from the previous research, aiming to enhance the significance of the results and evaluate the 5-year survival rate. The present study aimed to identify prognostic factors, including the circadian cycle and melatonin levels, which may affect the response to radiation in patients with cervical cancer.

Patients and methods

Study population. The present study was conducted at the Radiotherapy Department at Cipto Mangunkusumo Hospital (Jakarta, Indonesia) in cooperation with the Department of Obstetrics and Gynaecology of the Faculty of Medicine, Universitas Indonesia-Cipto Mangunkusumo Hospital (Jakarta, Indonesia). The subjects were initially enrolled in this study between March 2012 and August 2014. The target population included patients with International Federation of Gynaecology and Obstetrics (FIGO) stage IIB-IIIB cervical cancer who received no previous treatment and had histopathologically confirmed squamous cell carcinoma. Patients with recurrent cancer, HIV-positive status, and chronic diseases, including diabetes mellitus and hypertension, were excluded. Subjects were patients who completed regular standard radiotherapy treatment either in the morning (06.00-10.00 am) or the afternoon (04.00-06.00 pm). According to World Health Organization tumour response criteria (16), the subjects were classified based on post-radiotherapy tumour response into subjects with a good response (complete and near-complete response/<1 cm) and poor response (partial response, progressive disease or stable disease). Poor response cases were assigned to the case group. The remaining patients with complete melatonin data were randomized into the control group. To ensure good statistical power in the study, a ratio of 1:3 between the case and control groups was used (Fig. 1) (17,18). All other possible confounding variables were denoted and included in the analysis.

The patients included were aged 25-70 years with a Karnofsky Performance Status >70, haemoglobin (Hb) levels >10 g/dl and had provided written informed consent to participate in the study. The standard treatment in this study was radiation alone or combined with chemotherapy. Radiotherapy was administered based on the protocol adopted by our department, which was five times a week (25x2 Gy) followed by intracavitary brachytherapy (3x7 Gy) once a week for three weeks. The decision to add chemotherapy or not was at the clinician's discretion. Patients were excluded if they did not complete at least a regular irradiation schedule comprising 20 sessions of external beam radiation therapy and two sessions of brachytherapy or if the patients received <25 fractions of the radiotherapy regimen. The response to radiation in the present study was assessed four weeks after the completion of brachytherapy. Blood samples for melatonin workup were taken in the morning or afternoon based on irradiation time. Melatonin was measured by ELISA using a melatonin kit (cat. no. IBL-RE54021; IBL International GmbH) and peripheral blood specimens were obtained before the start of the first session of radiotherapy (19).

Other variables investigated that may contribute to treatment response included age, time of radiotherapy, overall treatment time, Hb level, pathological findings, and initial clinical tumour size. Time of radiation was defined as when the external irradiation or brachytherapy was performed; each patient had been randomly assigned for irradiation treatment

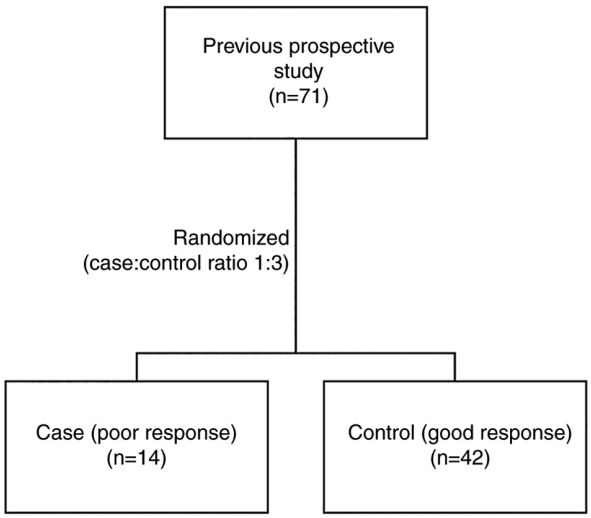

Figure 1. Flow chart of patient enrolment and grouping.

at 6-10 am for the morning group or at 4-6 pm for the afternoon group. The treatment was stationary and treatment time was according to the hospital's schedule. The time windows for the morning and afternoon groups were set according to the common melatonin level curve in the body. The overall treatment time was defined as the days between the first irradiation and the completion of brachytherapy. The Hb level was measured immediately before the initial irradiation treatment by using the standard Hb-cyanide spectrophotometric method at the Department of Clinical Pathology, Dr Cipto Mangunkusumo Hospital (Jakarta, Indonesia) and patients were divided into normal Hb (>10 g/dl) and low Hb (≤10 g/dl) groups. The pathologists performed a histopathological examination based on cell differentiation and keratinisation. The initial clinical tumour size was recorded at a gynaecological examination of the local tumour measuring the clinical tumour volume in the anteroposterior, latero-lateral and craniocaudal aspects in centimetres at the initial visit. Other variables, including age, the combination of chemotherapy, body weight, blood transfusion, erythrocyte sedimentation rate and intravenous pyelography results, were collected from medical records.

Statistical analysis. The statistical analysis was performed using SPSS version 20.0 software (IBM Corporation). The relationship between potential prognostic factors and tumour response after irradiation was investigated by performing a univariate analysis using the χ^2 test or Fisher's exact test. P<0.05 was considered to indicate statistical significance. In the univariate analysis, variables with P<0.25 were deemed suitable and included for multivariate analysis using binary logistic regression and Nagelkerke's R2 to identify the prognostic factors for radiation responses in patients with cervical cancer. Multicollinearity was assessed by correlation matrix. Kaplan-Meier curves were drawn, and log-rank tests were used to determine the 5-year survival rate.

Results

Characteristics of subjects in the two groups. The complete medical records of 71 patients with cervical cancer were collected between March 2012 and August 2014 in Dr Cipto Mangunkusumo Hospital (Jakarta, Indonesia). Most patients were <50 years old; 57.1% had poor response and 64.3% with good response. From the 71 patients, the proportion of subjects in each group was adjusted using a 1:3 ratio of cases/controls. A total of 14 patients with poor response were included in the case group, while the control group consisted of 42 of 57 patients with good response who were randomly selected from the control group. The clinical and laboratory data of the patients, including age, initial weight and the presence of pain, are presented in Table I. The tumour size was significantly related to the tumour response after irradiation (P=0.002).

A comparison between clinical tumour size and stage is included in Table II. Most patients were categorized into the stage III group, as they came for treatment after their symptoms developed into a more advanced stage. The patients with stage II and stage III were equally distributed based on clinical tumour sizes, as cervical cancer staging is determined by tumour size and parametrium invasion; thus, tumour size alone is insufficient to determine stages.

In addition, the comparison of tumour responses according to melatonin levels between the subjects irradiated in the morning and the afternoon was presented in Table III. The results suggested that the influence of melatonin levels on the tumour response in both groups was insignificant.

Potential factors affecting the radiation response. Univariate analysis was performed to identify variables significantly predictive of the response to radiation. As indicated in Table IV, tumour size (P=0.002) and transfusion during radiation (P=0.004) were significantly associated with the response to radiation. Other predictive variables significantly related to tumour response were the time of radiation (P=0.045) and post-treatment body weight (P=0.027).

Time of radiation affects the radiation response. According to the multivariate analysis, radiation time in the morning [adjusted odds ratio (OR)=8.70, 95% CI=1.25-60.73, P=0.023], normal initial Hb level (OR=13.53, 95% CI=1.38-132.25, P=0.017) and small clinical tumour size (OR=8.85, 95% CI=1.45-54.16, P=0.039) were associated with a significantly better tumour response to treatment at 4 weeks after brachytherapy. (Table V). The results of the multivariate analysis were based on this model with the Hosmer Lemeshow test (P=0.803), and Nagelkerke's R2 value was 0.441. It was revealed that the model was a good fit with 44.1% variability observed in the target variable. There were no multicollinearity assumptions among the independent variables based on the correlation matrix.

Relationship between time of radiation and 5-year overall survival. A total of 56 patients were included in the survival analysis. Among the subjects, three patients were lost to follow-up. The median follow-up duration was 11 months (interquartile range, 2.0-24.0 months). During the 5-year follow-up period, 25 subjects died. Based on the Kaplan-Meier curves (Fig. 2), the median survival time of the subjects irradiated in the morning was 24 months (95% CI: 6.30-41.70); meanwhile, the median survival time of the subjects irradiated in the afternoon was 20 months (95% CI: 0.00-44.06; P=0.121).

Table I. Subject characteristics (percentage based on column).

Variable	Radiation response		P-value
	Poor (n=14)	Good (n=42)	
Age, years			0.633[a]
≤50	8 (57.1)	27 (64.3)	
>50	6 (42.9)	15 (35.7)	
Initial body weight, kg			0.106[b]
≤50	2 (14.3)	17 (40.5)	
>50	12 (85.7)	25 (59.5)	
Pain			0.518[b]
No	8 (57.1)	29 (69.0)	
Yes	6 (42.9)	13 (31.0)	
Clinical tumour size, cm^3			0.002[a]
≤40 (small)	2 (14.3)	26 (61.9)	
>40 (large)	12 (85.7)	16 (38.1)	
Initial Hb level (g/dl)			0.058[b]
>10	10 (71.4)	39 (92.9)	
≤10	4 (28.6)	3 (7.1)	
ESR			0.097[b]
≤40	10 (71.4)	38 (90.5)	
>40	4 (28.6)	4 (9.5)	
Pathological differentiation			0.119[b]
Moderate/well	9 (64.3)	36 (85.7)	
Poor/moderate	5 (35.7)	6 (14.3)	
Pathological keratinisation			1.000[b]
No	13 (92.9)	39 (92.9)	
Yes	1 (7.1)	3 (7.1)	

[a]Chi-squared test; [b]Fisher's exact test. Values are expressed as n (%). Hb, haemoglobin; ESR, erythrocyte sedimentation rate; IVP, intravenous pyelogram.

Table II. Association between tumor size and stage.

Stage	Small tumor, ≤40 cm^3 (n=28)	Large tumor, >40 cm^3 (n=28)
Undefined	2 (7.1)	0 (0)
II	6 (21.4)	5 (17.9)
III	20 (71.4)	23 (82.1)

Values are expressed as n (%).

Table III. Relationship between melatonin levels and tumour response.

Group/melatonin levels, pg/ml	Good response	Poor response	P-value
Morning			0.298
≤13 (low)	10 (66.7)	5 (33.3)	
>13 (high)	10 (83.3)	2 (16.7)	
Afternoon			0.223
≤13 (low)	7 (63.6)	4 (36.4)	
>13 (high)	15 (83.3)	3 (4.3)	

Values are expressed as n (%). Fisher's exact test was used to calculate P-values.

In the Kaplan-Meier curves, there was a trend of irradiation in the morning being associated with increased survival, but overall, the difference was not significant.

Discussion

The present study aimed to prove the role of the circadian cycle in the treatment of cervical cancer by examining the effects of different timing of administering radiation therapy. Radiotherapy administered in the morning achieved a better tumour size reduction than radiotherapy administered in the afternoon. However, there was no significant difference in survival results between morning and afternoon radiation.

Table IV. Prognostic factors affecting the radiation response of patients (n=56) four weeks after brachytherapy.

Variable	Clinical response		P-value
	Good (n=42)	Poor (n=14)	
Time of radiation			0.045[a]
Morning	25 (59.5)	4 (28.6)	
Afternoon	17 (40.5)	10 (71.4)	
Chemotherapy			0.356[b]
Yes	23 (54.8)	11 (78.6)	
No	19 (45.2)	3 (21.4)	
Pathological keratinisation			1.000[b]
Yes	3 (7.1)	1 (7.1)	
No	39 (92.9)	13 (92.9)	
Differentiation status			0.119[b]
Moderate/well	36 (85.7)	9 (64.3)	
Poor/moderate	6 (14.3)	5 (35.7)	
Body weight after treatment, kg			0.027[a]
>50	22 (52.4)	12 (85.7)	
≤50	20 (47.6)	2 (14.3)	
Reduction of body weight >5 kg			0.089[a]
No	23 (54.8)	4 (28.6)	
Yes	19 (45.2)	10 (71.4)	
IVP			0.070[a]
Normal	26 (89.7)	19 (70.4)	
Abnormal	3 (10.3)	8 (29.6)	
Reduction of Hb			0.310[a]
No	10 (34.5)	6 (22.2)	
Yes	19 (65.5)	21 (77.8)	
Pre-radiation blood transfusion			0.080[a]
No	29 (69.0)	6 (42.9)	
Yes	13 (31.0)	8 (57.1)	
Blood transfusion during radiation			0.004[a]
No	30 (71.4)	4 (28.6)	
Yes	12 (28.6)	10 (71.4)	
Alignment with radiation time			1.000[b]
Yes	36 (85.7)	12 (85.7)	
No	6 (14.3)	2 (14.3)	
OTT			1.751[b]
On-time	29 (69.0)	9 (64.3)	
Not on time	13 (31)	5 (35.7)	

[a]Chi-squared test; [b]Fisher's exact test. Values are expressed as n (%). Hb, haemoglobin; ESR, erythrocyte sedimentation rate; IVP, intravenous pyelogram; OTT, overall treatment time.

This may be worth further investigating. The initial response in the cohort of the present study is certainly more representative of factors directly related to radiosensitivity, whereas survival may be influenced by numerous confounding factors, including disease severity, metastatic process, immune response, and side effects of treatment.

It has been proven that the difference in expression of genes between morning and afternoon is regulated by several CLOCK genes that work in accordance with circadian rhythms (20). The concept of circadian-related radiotherapy aims to deliver radiation with maximum synergy with the radiosensitive atmosphere provided by the time system from the 'internal' body clock and the world clock. In a study using zebrafish, Peyric *et al* (21) demonstrated cell cycle regulation by the circadian clock. The M phase of the cell cycle occurs rhythmically and

Table V. Factors affecting the tumour response after irradiation (n=56).

Variable	Clinical response		Adj. OR	95% CI
	Good (n=42)	Poor (n=14)		
Radiation time, morning vs. afternoon	25 (59.5)	4 (28.6)	8.70	(1.25-60.73)
Initial clinical tumour size, <40 cm^3 (small)	26 (61.9)	2 (14.3)	8.85	(1.45-54.16)
Initial Hb level, >10 g/dl (normal)	39 (92.9)	10 (71.4)	13.52	(1.38-132.25)

Values are expressed as n (%). Nagelkerke-R2=0.441; Hosmer Lemeshow test P=0.803. Adj. OR, adjusted odds ratio; CI, confidence interval; Hb, haemoglobin.

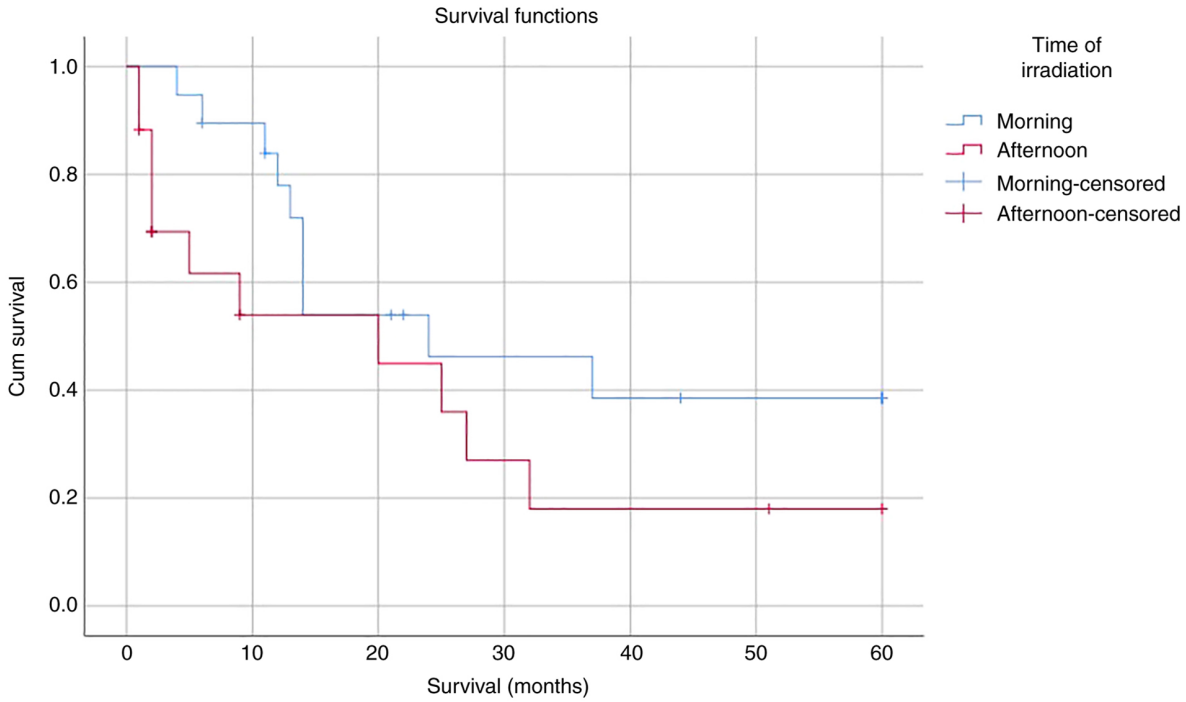

Figure 2. Kaplan-Meier survival curve of patients according to time of day of irradiation treatment (morning vs. afternoon). Cum, cumulative.

under circadian control (21). This may explain the better radiation response in the morning, as the probability of cancer cell death is higher when cells are in the G2M phase. Bjarnason *et al* (8) reported that mucous cells and human skin cells mainly divide in the evening between 6:00 pm to 12:00 am. Furthermore, three different studies by Klevec *et al* (22), Lakatua *et al* (23) and Smaaland *et al* (24) indicated that tumour-cell division occurs at an opposite time to that of healthy cell division. Based on these results, it may be assumed that cancer cells are more likely to be in the radiosensitive G2/M phase between 6:00 pm and 12:00 am, whereas normal cell proliferation occurs in the afternoon. This circadian pattern is evident in the melatonin hormone levels (22-24).

The levels of melatonin produced by the pineal gland depend on the circadian patterns: Gradually increasing from ~08.00 pm, reaching a peak at 03.00 am, gradually decreasing until 11.00 am, and reaching their lowest levels between 11.00 am and 08.00 pm (25-29). Certain studies revealed the different roles, functions and potential activities of melatonin, including its utility as a circadian biomarker, function in cancer development associated with circadian disruption (30,31), antioxidant activity and inhibition of cancer growth (15,32,33).

Prior studies reported the role and function of melatonin in cancer in the absence of radiation; to the best of our knowledge, no study has examined the effects of melatonin levels and the timing of radiotherapy (morning vs. afternoon) on the radiation response. Vijayalaxmi *et al* (33) suspected a role of pineal gland products in cancer development, particularly melatonin, which inhibited carcinogenesis in an *in vitro* study using MCF-7 breast cancer cells. This hormone was specifically demonstrated to increase the number of apoptotic cells and inhibit metastasis (34). The cancer-inhibiting effects of melatonin are influenced by various factors, including the melatonin concentration in culture media, the pattern of melatonin administration, the oestrogen receptor status (35,36), growth hormone levels in culture media and the rate of cell proliferation (35). Melatonin inhibits tumour transduction signals and the metabolic activity of cancer cells through MT1

receptor activity. Even though melatonin levels were noted to be higher in the morning compared to the afternoon, the present study does not sufficiently prove the effect of melatonin on the treatment response. This may be due to the difference in the concentration of melatonin among individuals, and thus, cut-off values for high or low concentrations of melatonin should be individualised. Such a study design may be able to negate factors with a low influence. The present study has not been able to provide sufficiently objective results due to the limited number of samples.

The present study illustrated that Hb levels affect the radiation response, in line with prior findings that anaemia and decreased Hb levels are prognostic indicators (7). Decreased Hb levels result in hypoxia, making cancer cells resistant to radiation. Oxygen increases radiosensitivity through direct and indirect effects; it is generally known that oxygenation increases the sensitivity of cells to radiation (6).

The tumour volume is an essential factor influencing the success of cervical cancer treatment. Lee *et al* (37) assessed the outcomes of 75 patients with stage IIB cervical cancer treated with chemoradiotherapy using MRI and overall survival was strongly related to the tumour volume. Specifically, the 5-year overall survival of patients with tumour volumes of 2.5-10, 10-50 and >50 ml were 75, 70 and 48%, respectively (38). This is consistent with the results of the present study that a smaller tumour size increases the success of therapy. However, the present study analysed the tumour size only, which was insufficient to determine the stage of cervical cancer.

In the present study, the tumour response was measured after 20-25 fractions of radiation, immediately after radiation and 2-4 weeks after radiation. This is in line with the time-points selected by Mayr *et al* (39), who performed MRI in 68 patients with advanced-stage IB2-IVB cervical cancer prior to radiation, after 10-12 fractions of radiation, after 20-25 fractions of radiation and 1-2 months after the completion of radiation. According to their research, the best time to perform MRI in the context of outcomes, namely the tumour regression rate, was after 25 fractions of radiation. Their study determined that this measurement most accurately predicted local control (84 vs. 22%, $P<0.0001$) and disease-free survival (63 vs. 20%, $P=0.0005$).

Based on preliminary research on patients irradiated in the morning (7), it was indicated that the melatonin concentration 2 h before radiation was high, even though its levels were already sharply declining. This phenomenon does not apply to patients irradiated in the afternoon. Although multivariate analysis did not indicate that melatonin levels affected clinical responses, it is possible that melatonin indirectly contributes to good responses, as the hormone influences variables that meaningfully predict response. It is also possible that the combination of radiation in the morning and melatonin levels influence the response to radiation.

One of the factors that may have induced bias in the present study was that during Hb level measurement, the patient's clinical status and blood transfusion status were not considered. The application of the study results may be generalised to patients with cervical cancer with FIGO stage IIB-IIIB who are indicated to receive radiotherapy.

The present study also did not implement the administration of radiotherapy during dawn (02.00-06.00 am), when the level of melatonin is theoretically the highest, as it is impractical in a clinical setting. It was observed that the circadian cycle has an essential role in radiosensitivity; however, the present study did not find any significant difference in melatonin levels between groups. Other factors related to the circadian cycle should be investigated that may support the increased radiosensitivity in the morning (06.00-10.00 am).

It was not the primary goal of the present study to find the highest levels of melatonin in an individual and then provide radiation at that time because, clinically, this would be difficult to apply. The present study simply aimed to prove the existence of a difference in radiosensitivity within a reasonable time in the daily clinical practice of radiation so that the results of the present study may be applied.

There are several limitations to the present study. The initial response may reflect a more specific intrinsic radiosensitivity that is unaffected by the stage and severity of the disease. Furthermore, this study did not assess numerous other factors that may influence the course of the disease, such as nutritional conditions, vitamin intake, immunity, and comorbidities, which may be associated with overall survival. The patients with comorbidities were excluded, so that it was not possible to further analyse this. Furthermore, the differences in adrenocorticotropic hormone levels between groups, which has an essential role in the circadian rhythm and may influence the tumour response, were not investigated (40). Those limitations should be considered in a future study.

In conclusion, the present case-control study is a further step to analyse the strength of the significance of the results of the study, following up with a 5-year survival analysis. While the study had a relatively small sample size, it pointed out that the circadian cycle may affect radiation treatment in cervical cancer. The circadian cycle, large tumour size and Hb levels affected the response of cervical cancer to radiation. Small tumour size, normal initial Hb level, and irradiation in the morning were associated with better tumour response after radiotherapy. However, the 5-year survival analysis indicated no significant difference between irradiation in the morning and afternoon, which was probably due to the small sample size and certain confounding factors related to overall survival that were not possible to control in this study.

Further research with a larger sample size is required to identify the optimal treatment for patients with radioresistant features. More sophisticated radiotherapy techniques such as Intensity-Modulated Radiation Therapy, hyper-fractionation and radiotherapy combined with chemosensitizers, and other methods should be applied to achieve a better treatment response. More accurate evaluations of the initial Hb level and tumour volume will be beneficial for designing treatment strategies and determining prognosis.

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

IR: Conceptualization, study design, data collection, data analysis, drafting and finalisation of the manuscript. SS, LN, ARH and SIW: Conceptualization, study design, supervision, finalisation of the manuscript. MM: Conceptualization, study design, data analysis, drafting and finalisation of the manuscript. SS and NCS: Conceptualization, finalisation of the manuscript. IR and SS checked and approved the authenticity of the raw data. All authors read and approved the final version before manuscript submission.

Ethics approval and consent to participate

This research was part of a previous prospective study approved by the Ethics Committee of the Faculty of Medicine Universitas Indonesia (Jakarta, Indonesia; no. 27/PT02. FK/ETIK/2010). All subjects provided written informed consent for participating in this study. The protocol was registered in the National Clinical Trial (NCT) registry (no. NCT05511740; registration date, 08/20/2022).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. Hull R, Mbele M, Makhafola T, Hicks C, Wang SM, Reis RM, Mehrotra R, Mkhize-Kwitshana Z, Kibiki G, Bates DO and Dlamini Z: Cervical cancer in low and middle-income countries. Oncol Lett 20: 2058-2074, 2020.
3. Wahidin M, Febrianti R and Susanty F: Burden of cervical cancer in Indonesia: Findings from the global burden of disease study 1990-2017. In: Proceedings of the 4th International Symposium on Health Research. Atlantis Press, Bali, 2020.
4. Pearcey R, Brundage M, Drouin P, Jeffrey J, Johnston D, Lukka H, MacLean G, Souhami L, Stuart G and Tu D: Phase III trial comparing radical radiotherapy with and without cisplatin chemotherapy in patients with advanced squamous cell cancer of the cervix. J Clin Oncol 20: 966-972, 2002.
5. Cohen PA, Jhingran A, Oaknin A and Denny L: Cervical cancer. Lancet 393: 169-182, 2019.
6. Joiner M and van der Kogel A (eds): Basic Clinical Radiobiology. 4th edition. Hodder Arnold, London, p375, 2009.
7. Ramli I: Influence of radiation patterns following circadian rhythm upon response of radiotherapy of uterine cervix cancer: Melatonin and cycle cell phase as biological marker and radiosensitivity (unpublished dissertation). Universitas Indonesia, Jakarta, 2019.
8. Bjarnason GA, Jordan RC, Wood PA, Li Q, Lincoln DW, Sothern RB, Hrushesky WJ and Ben-David Y: Circadian expression of clock genes in human oral mucosa and skin: Association with specific cell-cycle phases. Am J Pathol 158: 1793-1801, 2001.
9. Wood PA, Du-Quiton J, You S and Hrushesky WJ: Circadian clock coordinates cancer cell cycle progression, thymidylate synthase, and 5-fluorouracil therapeutic index. Mol Cancer Ther 5: 2023-2033, 2006.
10. Nagoshi E, Saini C, Bauer C, Laroche T, Naef F and Schibler U: Circadian gene expression in individual fibroblasts. Cell 119: 693-705, 2004.
11. Warman GR, Tripp HM, Warman VL and Arendt J: Circadian neuroendocrine physiology and electromagnetic field studies: Precautions and complexities. Radiat Prot Dosimetry 106: 369-373, 2003.
12. Arjona A and Sarkar DK: Circadian oscillations of clock genes, cytolytic factors, and cytokines in rat NK cells. J Immunol 174: 7618-7624, 2005.
13. Granda TG, Liu XH, Smaaland R, Cermakian N, Filipski E, Sassone-Corsi P and Lévi F: Circadian regulation of cell cycle and apoptosis proteins in mouse bone marrow and tumor. FASEB J 19: 304-306, 2005.
14. Lis CG, Grutsch JF, Wood P, You M, Rich I and Hrushesky WJ: Circadian timing in cancer treatment: The biological foundation for an integrative approach. Integr Cancer Ther 19: 1-22, 2003.
15. Karbownik M and Reiter RJ: Melatonin protects against oxidative stress caused by delta-aminolevulinic acid: Implications for cancer reduction. Cancer Invest 20: 276-286, 2002.
16. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Glabbeke MV, van Oosterom AT, Christian MC *et al*: New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 92: 205-216, 2000.
17. Biesheuvel CJ, Vergouwe Y, Oudega R, Hoes AW, Grobbee DE and Moons KG: Advantages of the nested case-control design in diagnostic research. BMC Med Res Methodol 8: 48, 2008.
18. Langholz B and Clayton D: Sampling strategies in nested case-control studies. Environ Health Perspect 102 (Suppl 8): 47-51, 1994.
19. Kennaway DJ: Measuring melatonin by immunoassay. J Pineal Res 69: e12657, 2020.
20. Hunt T and Sassone-Corsi P: Riding tandem: Circadian clocks and the cell cycle. Cell 129: 461-464, 2007.
21. Peyric E, Moore HA and Whitmore D: Circadian clock regulation of the cell cycle in the zebrafish intestine. PLoS One 8: e73209, 2013.
22. Klevecz RR, Shymko RM, Blumenfeld D and Braly PS: Circadian gating of S phase in human ovarian cancer. Cancer Res 47: 6267-6271, 1987.
23. Lakatua DJ, White M, Sackett-Lundeen LL and Haus E: Change in phase relations of circadian rhythms in cell proliferation induced by time-limited feeding in BALB/c X DBA/2F1 mice bearing a transplantable Harding-Passey tumor. Cancer Res 43: 4068-4072, 1983.
24. Smaaland R, Lote K, Sothern RB and Laerum OD: DNA synthesis and ploidy in non-Hodgkin's lymphomas demonstrate intrapatient variation depending on circadian stage of cell sampling. Cancer Res 53: 3129-3138, 1993.
25. Florez JC and Takahashi JS: Biological rhythm and the pineal gland. In: Greger R, Windhorst U, editors. Comprehensive human physiology: From cellular mechanisms to integration. Berlin, Heidelberg: Springer; 1996. p. 1199-1214.
26. Arendt J: Melatonin and the pineal gland: Influence on mammalian seasonal and circadian physiology. Rev Reprod 3: 13-22, 1988.
27. Jung B and Ahmad N: Melatonin in cancer management: Progress and promise. Cancer Res 66: 9789-9793. 2006.
28. Srinivasan V, Spence DW, Pandi-Perumal SR, Trakht I and Cardinali DP: Therapeutic actions of melatonin in cancer: Possible mechanisms. Integr Cancer Ther 7: 189-203, 2008.
29. Aulinas A: Physiology of the pineal gland and melatonin. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, *et al*, editors. Endotext (Internet). South Dartmouth (MA): MDText.com, Inc.; 2000 (cited 2022 Jul 26). Available from: http://www.ncbi.nlm.nih.gov/books/NBK550972/.
30. Brzezinski A: Melatonin in humans. N Engl J Med 336: 186-195, 1997.
31. Karasek M and Winczyk K: Melatonin in humans. J Physiol Pharmacol 57 (Suppl 5): 19-39, 2006.

32. Martín V, Herrera F, Carrera-Gonzalez P, García-Santos G, Antolín I, Rodriguez-Blanco J and Rodriguez C: Intracellular signaling pathways involved in the cell growth inhibition of glioma cells by melatonin. Cancer Res 66: 1081-1088, 2006.
33. Vijayalaxmi, Thomas CR Jr, Reiter RJ and Herman TS: Melatonin: From basic research to cancer treatment clinics. J Clin Oncol 20: 2575-2601, 2002.
34. Blask DE and Hill SM: Effects of melatonin on cancer: Studies on MCF-7 human breast cancer cells in culture. J Neural Transm Suppl 21: 433-449, 1986.
35. Hill SM and Blask DE: Effects of the pineal hormone melatonin on the proliferation and morphological characteristics of human breast cancer cells (MCF-7) in culture. Cancer Res 48: 6121-6126, 1988.
36. Sánchez-Hidalgo M, Guerrero JM, Villegas I, Packham G and de la Lastra CA: Melatonin, a natural programmed cell death inducer in cancer. Curr Med Chem 19: 3805-3821, 2012.
37. Lee DW, Kim YT, Kim JH, Kim S, Kim SW, Nam EJ and Kim JW: Clinical significance of tumor volume and lymph node involvement assessed by MRI in stage IIB cervical cancer patients treated with concurrent chemoradiation therapy. J Gynecol Oncol 21: 18-23, 2010.
38. Feldman J, Goldwasser R, Mark S and Schwartz J: A mathematical model for tumor volume evaluation using two dimensions. J Appl Quant Methods 4: 455-462, 2008.
39. Mayr NA, Taoka T, Yuh WT, Denning LM, Zhen WK, Paulino AC, Gaston RC, Sorosky JI, Meeks SL, Walker JL, *et al*: Method and timing of tumor volume measurement for outcome prediction in cervical cancer using magnetic resonance imaging. Int J Radiat 52: 14-22, 2002.
40. Nicolaides NC, Charmandari E, Chrousos GP and Kino T: Circadian endocrine rhythms: The hypothalamic-pituitary-adrenal axis and its actions. Ann NY Acad Sci 1318: 71-80, 2014.