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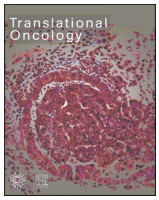

Original Research

Clinical analysis of the efficacy of radiation therapy for primary high-grade gliomas guided by biological rhythms

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ABSTRACT

Objective: High-grade glioma (HGG) patients frequently encounter treatment resistance and relapse, despite numerous interventions seeking enhanced survival outcomes yielding limited success. Consequently, this study, rooted in our prior research, aimed to ascertain whether leveraging circadian rhythm phase attributes could optimize radiotherapy results.

Methods: In this retrospective analysis, we meticulously selected 121 HGG cases with synchronized rhythms through Cosinor analysis. Post-surgery, all subjects underwent standard radiotherapy alongside Temozolomide chemotherapy. Random allocation ensued, dividing patients into morning ($N = 69$) and afternoon ($N = 52$) radiotherapy cohorts, enabling a comparison of survival and toxicity disparities.

Results: The afternoon radiotherapy group exhibited improved overall survival (OS) and progression-free survival (PFS) relative to the morning cohort. Notably, median OS extended to 25.6 months versus 18.5 months, with $P = 0.014$, with median PFS at 20.6 months versus 13.3 months, with $P = 0.022$, post-standardized radiotherapy. Additionally, lymphocyte expression levels in the afternoon radiation group 32.90(26.10, 39.10) significantly exceeded those in the morning group 31.30(26.50, 39.20), with $P = 0.032$.

Conclusions: This study underscores the markedly prolonged average survival within the afternoon radiotherapy group. Moreover, lymphocyte proportion demonstrated a notable elevation in the afternoon group. Timely and strategic adjustments of therapeutic interventions show the potential to improve therapeutic efficacy, while maintaining vigilant systemic immune surveillance. A comprehensive grasp of physiological rhythms governing both the human body and tumor microenvironment can refine treatment efficacy, concurrently curtailing immune-related damage—a crucial facet of precision medicine.

Introduction

Glioma stands as the most prevalent and aggressively malignant primary brain tumor [1–3]. Among these, high-grade gliomas (HGG), including WHO grade 3–4 astrocytoma and glioblastoma (GBM), exhibit potent invasive potential devoid of clear boundaries, resulting in a grim prognosis and posing challenges for complete surgical resection [4]. The conventional postoperative glioma treatment protocols encompass radiotherapy coupled with concurrent temozolomide chemotherapy, enduring as the global norm for glioma patients [5]. Nonetheless, there has been a conspicuous absence of significant enhancements in clinical outcomes in recent decades [6]. The median overall survival time for

Abbreviations: CI, Confidence interval; CTV, Clinical target volume; GBM, Glioblastoma; GHNXMU, General Hospital of Ningxia Medical University; GTV, Gross tumor volume; HGG, High-grade glioma; HR, Hazard ratio; IDH, Isocitrate dehydrogenase; IMRT, Intensity-modulated radiotherapy; KPS, Karnofsky Performance Score; MGMT, Methylguanine-DNA methyltransferase; OS, Overall survival; PFS, Progression-free survival; PSQI, Pittsburgh Sleep Quality Index; PTV, Planning target volumes; RT, Radiotherapy; TMZ, Temozolomide.

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glioblastoma merely spans 14.6–20.9 months, with a meager 5 %–14 % 5-year survival rate [7–11]. Furthermore, survivors often grapple with enduring neurological impairments [12]. Hence, the imperative task remains the amelioration of glioma patient survival rates and mitigation of treatment-associated side effects. Despite the prevailing focus on new drug discoveries and molecular targets, substantial clinical headway has eluded realization [13–15]. Certain medical practitioners are endeavoring to refine conventional radiotherapy regimens by leveraging the tenets of biological rhythms, with the anticipation of forging novel treatment strategies [16].

In antecedent investigations, we ascertained disparate biological rhythms between glioma cells and peritumoral normal cells, with the rhythm-regulating genes Per2 and Per1 evincing close ties to glioma invasion and proliferation. These biological rhythms exhibit robust pulsatile attributes. Our research further unveiled that cell sensitivity to radiation varies in tandem with the cell cycle, with the gap 2 (G2) and mitosis (M) phases displaying heightened radiosensitivity, while the synthesis (S) phase exhibits lesser susceptibility [17],which are similar to previous studies [18–21]. Elevated Per2 expression in glioma tissue correlates with augmented X-ray sensitivity, a phenomenon not manifesting in normal tissues. Exploiting this divergence could potentially optimize treatment by administering radiotherapy when malignant cells evince relative radiosensitivity, while preserving the relative radioresistance of normal cells. In subsequent study [22], we discerned that in a murine glioma model with synchronized rhythms, evening radiotherapy yielded diminished glioma cell proliferation rates and augmented tumor cell apoptosis in comparison to morning radiotherapy, suggesting the capacity of biological clock genes to heighten radiosensitivity. Moreover, the presence of biological clock gene expression was noted in glioma patients, concomitant with the degree of malignancy. Hence, we conjecture that animal simulation of human biorhythms aligns with tumor cell proliferation and apoptosis in the human body.

Truly, across the preceding decades, several experimental and clinical investigations have showcased affirmative connections between the circadian clock and drug responses in cancer patients [23–25]. However, the integration of patient biorhythms into glioma radiotherapy strategies has remained infrequently documented. This necessitates the exploration of novel glioma treatment paradigms in the temporal-spatial realm, aimed at discerning optimal individualized treatment approaches or mitigating the amplification of radiotherapy-associated side effects.

Material and methods

Patient recruitment

After the approval of the Medical Ethics Committee of the General Hospital of Ningxia Medical University (GHNXMU), we reviewed the medical records and electronic documents of all patients who received glioma treatment in our center from May 2012 to May 2020. The present investigation involved a comparative analysis of the circadian rhythms exhibited by all enrolled participants, encompassing parameters such as sleep timing, meal patterns, heart rate, and activity schedules, aimed at detecting biorhythmic concordance between the two study groups. Subsequently, 211 patients diagnosed with primary high-grade glioma, who exhibited synchronized daily rhythms as per the Pittsburgh Sleep Quality Index (PSQI), were selected for further study. The treatment time of each patient in the cohort was allocated to morning or afternoon treatment by the radiation oncologist according to the time they arrived at the hospital for treatment, and standardized radiotherapy regimens were adopted. The above measures were coupled with strict inclusion criteria (Fig. 1) to avoid selective bias. The ethical approval for this study was secured from the Clinical Research Ethics Committee of GHNXMU.

Radiation therapy

The treatment planning process was executed utilizing the Pinnacle Planning System v.9.8 (Philips Medical Systems, Milpitas, CA, USA). This involved integrating a contrast-enhanced planning CT scan with postoperative MRI images encompassing post-contrast T1, FLAIR/T2, and DWI sequences. Precise delineation of treatment volumes was undertaken by a pair of radiation oncologists. The gross tumor volume (GTV) was demarcated to encapsulate the residual contrast

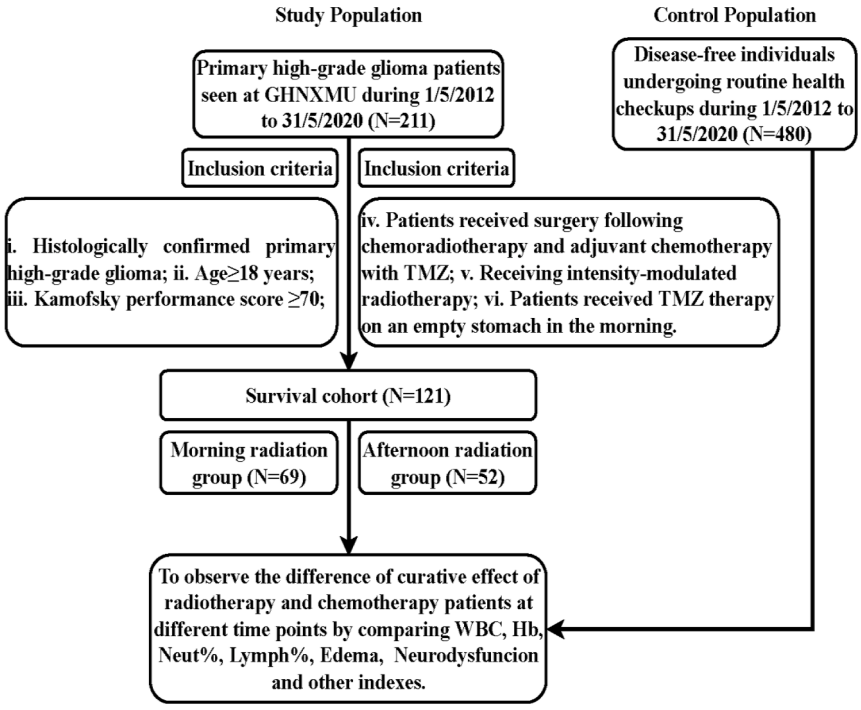

Fig. 1. CONSORT diagram describing the flow of patient enrollment. GHNXMU, general hospital of Ningxia medical university; TMZ, temozolomide; WBC, white blood cell; Hb, hemoglobin; Neut, neutrophil; Lymph, lymphocyte.

enhancement as well as the surgical cavity. Subsequently, the GTV was expanded by 2 cm to formulate the clinical target volume (CTV). The CTVs were further expanded by 0.3 cm to derive the planning target volumes (PTV). Radiation therapy was administered through the application of IMRT. The prescribed dosage entailed 60 Gy distributed across 2 Gy per fraction for the PTV.

Chemotherapy

Synchronous chemotherapy involved the administration of temozolomide at a dosage of 75 mg/m^2/day, commencing on the initial day of radiotherapy and extending for a duration of 6 weeks, concluding concurrently with radiotherapy. Subsequent adjuvant chemotherapy was initiated one month following concurrent chemoradiotherapy. In the first cycle of adjuvant chemotherapy, temozolomide was administered at a dosage of 150 mg/m^2/day. If well-tolerated, the dosage was escalated to 200 mg/m^2/day starting from the second cycle. Each cycle consisted of 5 consecutive days of treatment followed by a 28-day rest period.

Statistical analysis

The evaluation of biorhythmic attributes in the enrolled cohort was conducted through Cosinor analysis. Categorical variables were assessed using the χ^2 test, while continuous variables were evaluated using Student's t-test, under the assumption of equal variance. Nonparametric tests were employed when variables did not adhere to a normal distribution. *P* values were computed using a two-tailed test. Survival analysis encompassed the Kaplan–Meier method, with comparisons effectuated via the log-rank test. Additionally, survival assessments incorporated Cox regression analyses, yielding estimated hazard ratios (HRs) accompanied by 95 % confidence intervals (CIs). OS was defined as the duration from the initial surgery to the occurrence of mortality. PFS was delineated as the interval from the primary surgical intervention until the onset of disease progression (confirmed through radiologic evaluation) or death. For multivariate analysis, the Cox-proportional hazards model, coupled with a backward stepwise methodology, was employed. Statistical significance was predicated on a threshold of $P < 0.05$.

Results

Presentation of exclusion criteria and methodology for patient selection in the two groups

The study cohort comprised patients diagnosed with primary HGG at the GHNXMU between January 5th, 2012, and May 31st, 2020 ($N = 211$). Patients meeting the following exclusion criteria were excluded: those not presenting with de novo HGG ($N = 20$), individuals who did not undergo radiotherapy (RT) and Temozolomide (TMZ) treatment ($N = 27$), and patients who were lost to follow-up post-surgery ($N = 25$). Subsequently, among the remaining primary HGG patients who underwent surgical intervention along with RT and TMZ therapy ($N = 139$), those with incomplete vital status data ($N = 12$) and those harboring IDH1/2 mutations ($N = 6$) were excluded. Ultimately, the cohort was refined to 121 patients with primary HGG who fulfilled the necessary admission criteria. This group of patients was further categorized into two distinct time-based radiation administration schedules: the morning radiation group (07:00–12:00, $N = 69$) and the afternoon radiation group (13:00–18:00, $N = 52$) (Fig. 2). The clinical characteristics of patients were analyzed in Supplementary Table1.

Synchronization of biorhythms in two groups

We meticulously conducted the PSQI scoring on a cohort consisting of 268 patients. Notably, there were no individuals who scored between 0 and 5, while 32 patients recorded scores within the range of 6 to 10. A significant portion of the cohort, encompassing 211 patients, achieved scores ranging from 11 to 15, and a smaller subset of 25 patients attained scores ranging from 16 to 21. Recognizing the crucial significance of synchronizing biorhythms, our subsequent analysis distinctly focused on the specific score range of 11 to 15. By employing predefined inclusion and exclusion criteria, we refined our selection to a subset of 121 cases (Supplementary Table 2). This subset served as the foundation for our comprehensive evaluation of daily biorhythm characteristics. The outcomes derived from our Cosinor analysis unmistakably revealed significantly synchronized rhythms within the domains of heart rate ($P = 0.003$), sleep time ($P = 0.000$), and activity time ($P = 0.009$) among the enrolled patients (Fig. 3).

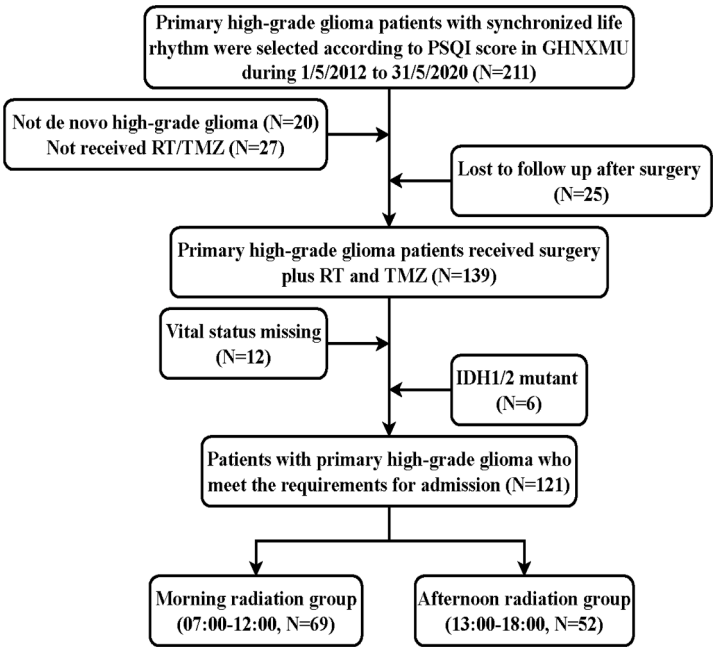

Fig. 2. Presentation of exclusion criteria and methods for patients in the group. PSQI, Pittsburgh sleep quality index; GHNXMU, general hospital of Ningxia medical university; RT, radiotherapy; TMZ, temozolomide; IDH, isocitrate dehydrogenase.

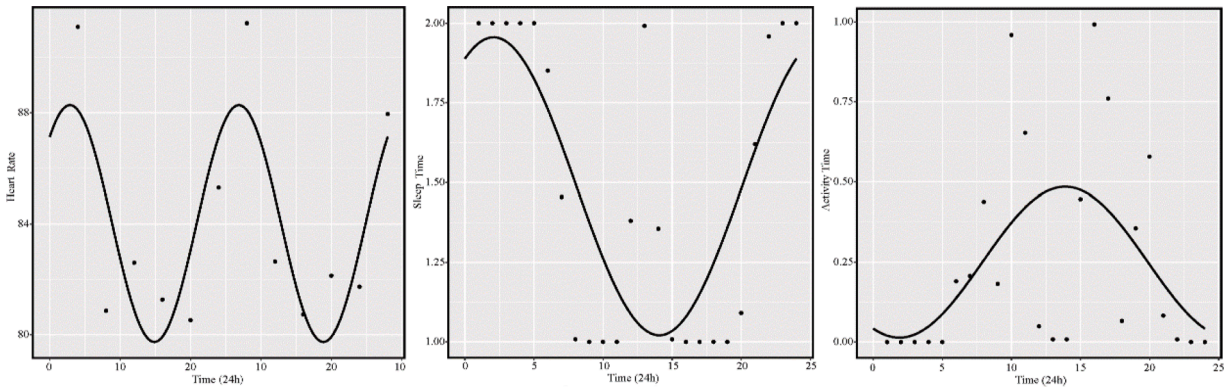

Fig. 3. Cosinor analysis of heart rate, sleep time and activity time biological rhythm in the case group.

Follow-up and survival

Following up on data until July 2021, a total of 72 patients (78.3 %) in the study cohort had succumbed to their conditions. The median duration of post-treatment surveillance was 17.5 months (range: 5.3–84.2 months), while for the surviving patients, this period extended to 25.4 months. The median overall survival (OS) observed was 21.5 months, with a 95 % confidence interval ranging from 18.9 to 24.0 months. The two-year and five-year OS rates were calculated at 34.7 % and 10 %, respectively. In terms of progression-free survival (PFS), the median duration was 16.4 months, encompassing a 95 % confidence interval from 13.8 to 19.1 months. The two-year and five-year PFS rates were found to be 16.6 % and 6.6 %, respectively. Interestingly, a comparative analysis between patients receiving afternoon radiation and those undergoing morning radiation demonstrated improved OS and PFS in the former group. Specifically, the median OS was notably longer in the afternoon radiation group (25.6 months) as opposed to the morning radiation group (18.5 months), yielding a statistically significant difference ($P = 0.014$). Similarly, the median PFS for the afternoon radiation group was extended to 20.6 months compared to 13.3 months in the morning radiation group ($P = 0.022$), as illustrated in Fig. 4. There was no difference in OS and PFS between the morning and afternoon groups in gender (Fig. 5).

Levels of lymphocyte expression in two groups after radiotherapy

After standardized radiotherapy, the levels of lymphocyte expression in the afternoon radiation group 32.90(26.10, 39.10) were significantly higher than the morning radiation group 31.30(26.50, 39.20), $P = 0.032$ (Fig. 6).

The adverse events following standardized radiotherapy

The presence of edema exhibited no statistically significant disparity between the two groups, with a P of 0.450. Similarly, the manifestation of neurodysfunction among the two groups demonstrated no substantial contrast, with a P-value of 0.087. Furthermore, an absence of significant divergence was observed in terms of white blood cell count ($P = 0.280$), hemoglobin levels ($P = 0.670$), and neutrophil percentage ($P = 0.260$), all of which serve as markers reflecting bone marrow suppression within both groups subsequent to radiotherapy (Supplementary Table 3).

The molecular pathology of the high-grade glioma following operation

The MGMT promoter methylation status was available for 78 (40.5 %) patients, and 28 (23.1 %) showed IDH1 positive. There was no significant difference in IDH1 ($P = 0.653$) (Supplementary Figure 1) and MGMT ($P = 0.724$) (Supplementary Figure 2) following operation in two groups.

Discussion

Currently, the globally recognized therapeutic approach for post-operative glioma patients remains the combination of standard radiotherapy and temozolomide [26]. This regimen stands as the gold standard treatment for initial glioma management, with radiotherapy

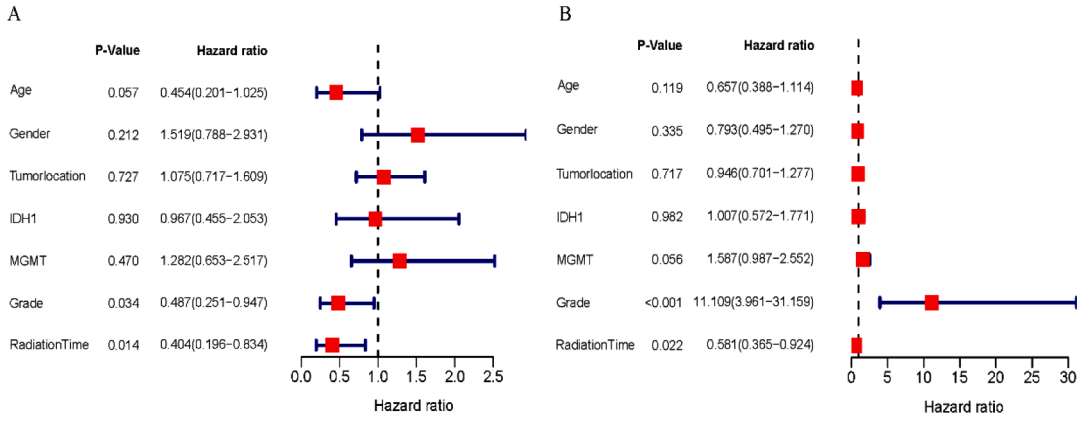

Fig. 4. Multivariate analysis of overall survival and progression-free survival. (A) Multivariate analysis of overall survival, and the median overall survival for the afternoon radiation group was 25.6 months compared to 18.5 months in the morning radiation group ($P = 0.014$). (B) Multivariate analysis of progression-free survival, and the median progression-free survival for the afternoon radiation group was 20.6 months compared to 13.3 months in the morning radiation group ($P = 0.022$). Multivariate analyzed by Cox regression analysis).

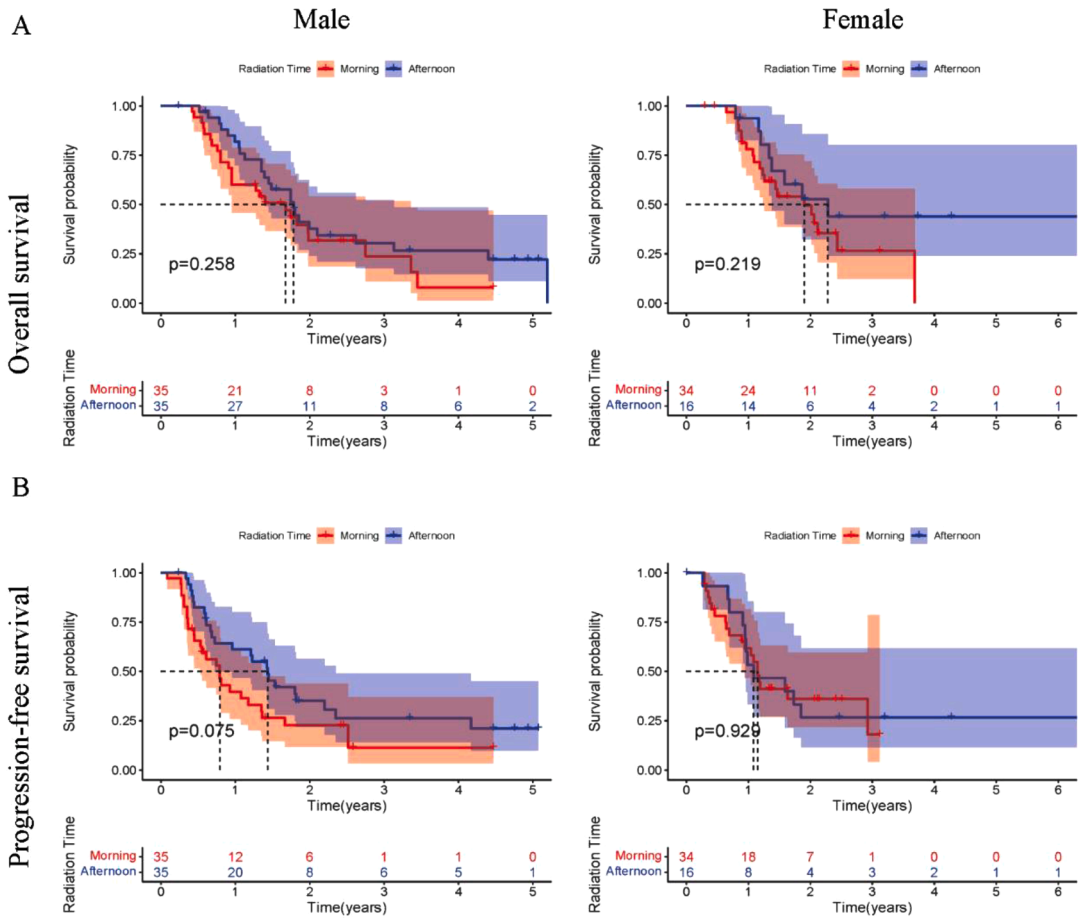

Fig. 5. Kaplan-Meier curves of overall survival and progression-free survival according to the time of the day of gender. (A) Overall survival of morning radiation group and afternoon radiation group after radiotherapy. (B) Progression-free survival of morning radiation group and afternoon radiation group after radiotherapy.

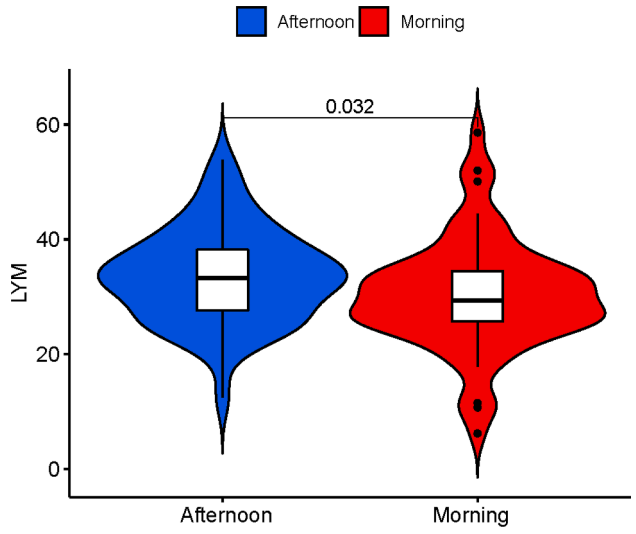

Fig. 6. Levels of lymphocyte expression in morning radiation group and afternoon radiation group after radiotherapy.

occupying a pivotal role. Despite the passage of recent decades, discernible enhancement in clinical outcomes has yet to be achieved. However, radiotherapy is not without its attendant severe adverse effects, such as cerebral edema, epileptic events, and neurological impairments. A broader array of strategies is imperative to optimize treatment protocols, striving for maximal therapeutic benefit, extension of progression-free survival, overall survival, and enhancement of patients' quality of life.

Emerging from a confluence of studies, compelling evidence underscores the pivotal role of circadian rhythm disturbances in the etiology and progression of gliomas [17,22,27-30]. The circadian biorhythm configuration in humans encompasses a network of intrinsic 24-hour oscillators governing cyclic physiological processes. Correspondingly, individual cells exhibit their own circadian rhythms, intricately linked with cellular proliferation and metabolism, underpinned by the orchestration of biological clock genes. Consequently, the body's circadian biorhythmic system hinges not only on these clock activities but also on the harmonious alignment of internal clocks with environmental cues. Research reveals that discrepancies between these internal and external processes can precipitate circadian rhythm disruptions, fostering the genesis and advancement of gliomas. Chronotherapy, a pioneering modality, administers anticancer interventions in synchronization with a patient's diurnal rhythm. Earlier investigations have demonstrated varying impacts of circadian-aligned radiotherapy and chemotherapy on efficacy, survival, and toxicity across diverse malignancies [31–34]. Within neuro-oncology, one study illustrated morning-administered gamma knife radiosurgery for brain metastatic non-small cell lung cancer resulted in improved local control [35]. Likewise, temporal delivery of whole-brain radiotherapy exhibited a significant association with overall survival in elderly female patients with brain metastases [36]. The influence of chronotherapeutic administration of TMZ on GBM patients was also reported by Damato et al., noting morning dosing correlated with prolonged overall survival

in MGMT methylated GBM cases [37].

Previous investigations [17] have indicated intrinsic biorhythms within glioma cells interlinked with the cell division cycle. The circadian rhythm intrinsic to glioma cells is intertwined with their radiosensitivity. Consequently, our findings suggest a coordinated relationship between glioma proliferation and apoptosis rates, underscored by their interaction with individual biological rhythms. To elucidate the prognosis of HGG treatment across distinct temporal segments, we conducted an exploration of therapeutic efficacy. Employing the PSQI, we identified patients whose daily patterns diverged from the normative populace, facilitating an understanding of sleep-wake cycles and cognitive functions. These circadian rhythms influence a wide range of physiological cycles, such as diurnal variations in blood pressure, heart rate, hormonal levels, respiratory patterns, exercise capacity, clotting mechanisms, and more [38]. Incorporating Cosinor analysis of heart rate, sleep, and activity timing – a novel application in glioma patients – enabled the detection of significant biorhythmic traits, ameliorating potential sample errors and elevating the precision of experimental outcomes. These findings illuminate the semblance of biorhythmic patterns between the two groups. Abundant research underscores the regulatory role of clock genes in these physiological activities. A prior investigation confirmed that heightened Per2 expression markedly attenuates stemness marker expression, diminishes self-renewal capacity, and induces G2/M phase cell cycle arrest in vitro glioma stem cells (GSCs). Furthermore, Per2 was identified as a suppressor of malignant attributes in GSCs [17], ultimately influencing radiotherapy sensitivity.

Our statistical analysis corroborates that afternoon radiation correlates with enhanced overall survival and progression-free survival compared to morning radiation. These clinical findings align with results from prior animal experiments, suggesting a plausible connection between bodily biorhythms and tumor cell proliferation cycles. Our previous observations indicated a surge in Per2 expression following X-irradiation, inducing G2/M arrest and sensitizing glioma cells to X-irradiation-induced apoptosis [17]. The current study suggests that Per2 potentially curtails GSC invasiveness and stemness, potentially accounting for G2/M arrest seen in Per2-overexpressing GSC cell lines [22]. Opting for divergent temporal radiotherapy administration within the context of standardized treatment significantly improved OS and PFS. In a previous study [39], the results showed no statistical difference in OS and PFS, which may be related to its small sample size, lack of rhythm synchronization of patients, and the presence of potential selection bias. Therefore, we collected the data of all glioma patients in our center during the 8-year period. According to the strict inclusion criteria, 121 patients with rhythmic synchronization were selected from 211 patients with high-grade glioma. The treatment time of each patient was determined by the radiation oncologist. They were randomly divided into morning group and afternoon group. This may make the results of this study more applicable.

Retrospective analysis confirmed that the biological rhythm of the body affects the effect of radiotherapy [40]. In this study, after standardized radiotherapy, the levels of lymphocyte expression in the afternoon radiation group were significantly higher than the morning radiation group. Lymphocytes, as an indicator of immune function in the body, play an important role in immune surveillance during tumor progression and metastasis. Their cytotoxicity and ability to induce tumor cell apoptosis can control tumor growth. Numerous studies have shown that radiotherapy can induce a decrease in lymphocytes, leading to a significant reduction in patient survival rates. In a systematic review and meta-analysis, Pim J.J. Damen et al. demonstrated that lymphopenia induced by radiotherapy in solid tumor patients is negatively correlated with overall survival [41]. Previous research has shown that severe treatment-related lymphopenia following radiotherapy in non-small cell lung cancer and pancreatic cancer is an independent predictor of low patient survival rates [42,43]. For progressed solid tumors, lymphopenia induced by chemoradiotherapy is also an independent factor associated with low survival rates [44]. This study found that the proportion of lymphocytes in the afternoon radiotherapy group was significantly higher than that in the morning radiotherapy group, corresponding to higher overall survival and progression-free survival rates in the afternoon radiotherapy group. This seemingly results from the upregulation of Per2 expression during the afternoon period [45], as studies suggest that Per2 gene knockout corresponds with notable splenic lymphocyte reductions [46]. The onset of glioma is intrinsically linked to circadian clock gene aberrations. Notably, the expression levels of core clock genes in high-grade gliomas markedly surpass those in low-grade gliomas and non-gliomas [47]. Research has pinpointed Per2 as a profoundly altered circadian gene in the malignant transformation of gliomas. Our previous findings documented diminished Per2 expression in glioma specimens when contrasted with adjacent non-glioma tissues.

Glioma prognosis hinges on multifactorial determinants. In the present study, there existed no substantial dissimilarity in age, sex, brain edema, neurological dysfunction, WBC, HB, and Neut% between the two groups. Although this is partially due to the limited number of cases synchronized with biorhythms enrolled in this study, it also underscores that the intensified radiotherapy regimen did not amplify radiotherapy's adverse effects. Molecular pathological indicators, encompassing MGMT methylation and IDH1, also exhibited no marked variation between the two patient groups. This observation could stem from the study's relatively diminutive sample size and suggests that the influence of molecular pathology on cell proliferation regulation might be less discernible. Aberrant circadian rhythms have been implicated in precipitating sleep disorders [27]. During treatment, sleep disturbances were evident in both groups without significant intergroup variance.

These challenges might arise from the study's limited sample size and the lack of comprehensive research into harnessing human biorhythmic indices to guide molecular-level tumor radiotherapy. Envisaging the identification of optimal windows of radiosensitivity within the timeframes differentiating normal and neoplastic cells, the dual goals of heightened therapeutic efficacy and attenuated side effects remain paramount. Recent research has revealed that Chinese medicine lycium barbarum extract, specifically lycium barbarum glycopeptide (LbGP), upregulates circadian clock gene Per2 expression via the PKA/CREB pathway [48]. In forthcoming clinical trials, glioma patients will be administered LbGP oral liquid prior to radiotherapy, aimed at enhancing the radiosensitivity through Per2 upregulation. The anticipation is that these endeavors will culminate in the discernment of optimal tumor-specific temporal rhythms for radiotherapy sensitivity, consequently furnishing essential groundwork and clinical data for chronoradiotherapy advancement.

Conclusions

The present investigation has discerned a noteworthy enhancement in both OS and PFS within the afternoon radiation group when compared with the morning radiation group. Furthermore, the afternoon radiation group exhibited a substantial elevation in lymphocyte expression levels compared to the morning radiation group. Chronoradiotherapy, predicated on the synchronization of biological rhythms within the body and tumor cells, has exhibited its potential to amplify therapeutic efficacy while concurrently mitigating adverse effects. Although a certain interplay between the host's biorhythmic patterns and those of tumor cells is evident, the precise molecular underpinnings remain elusive. Anticipated advancements encompass the establishment of a novel approach for precise glioma therapy targeting, informed by the principles of biological rhythm theory and guided by molecular clock mechanisms.

Data statement

The data supporting the conclusions of this article are provided in this article and the supplementary material. In addition, all data from

this study can be obtained from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Zhanfeng Niu: Investigation. **Zhihua Yang:** Methodology, Data curation. **Shengyu Sun:** Investigation. **Zhong Zeng:** Formal analysis, Data curation. **Qian Han:** Formal analysis, Data curation. **Liang Wu:** Investigation. **Jinbo Bai:** Investigation, Formal analysis. **Hailiang Li:** Investigation. **Hechun Xia:** Writing – original draft.

Declaration of competing interest

The authors declare no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2024.101973.

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