RESEARCH ARTICLE

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Prognostic Significance of Neutrophil-Lymphocyte Ratio in Patients of High-Grade Glioma Undergoing Adjuvant Chemoradiation: A Prospective Study

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Abstract

Purpose: High-grade gliomas are highly fatal disease with poor prognosis despite multimodality management. Inflammatory biomarkers are widely used for prognostication in various solid malignancies to stratify high risk patients. The current research was conducted to investigate whether any change in neutrophil-lymphocyte ratio (NLR) during adjuvant chemoradiotherapy has any prognostic significance in high-grade glioma patients. **Materials and Methods:** Seventy-three biopsy proven high-grade glioma patients treated with adjuvant chemoradiotherapy were enrolled in this study. Haematological parameters were collected before treatment, weekly during treatment, and at 4th week after chemoradiotherapy along with baseline characteristics. Overall survival (OS) was determined using Kaplan-Meier curve. Variables found statistically significant in univariate analysis by Cox regression model were subjected to final multivariable analysis. **Results:** The median follow-up was around 17 months with a median OS of 17.2 months (95%CI 14.7-23). The best prognosis was seen in patients who had a baseline NLR< 3.5 with decline in NLR during treatment achieving a 1-year survival of 100% and median overall survival of 36.5 months. Patients who had baseline NLR ≥3.5 without a decline in NLR had worst prognosis with a 1-year survival of 25% (95%CI 9.4%-66.6%) and median OS of 7.1 months. On multivariate analysis, age [HR 1.025, p=0.040)], ECOG performance status≤1 [HR 0.089, p<0.001], extent of surgery [HR 0.305, p=0.001] and decline in NLR during treatment [HR 0.452, p=0.026] were found to be significant predictors of OS. **Conclusion:** This study demonstrated that NLR is a cost-effective biomarker that has prognostic significance in predicting overall survival for high-grade glioma patients.

Keywords: High grade-glioma- chemoradiotherapy- neutrophil-lymphocyte ratio- inflammatory biomarker

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Introduction

Malignant high-grade gliomas account for more than half of all adult primary brain tumours (Rock et al., 2012). The current management for Glioblastoma (GBM) is maximal safe resection followed by adjuvant chemoradiotherapy and maintenance chemotherapy (Stupp et al., 2005). Despite multimodality treatment, glioblastomas are highly fatal disease with dismal prognosis and a median survival of 14.6 months (Ohka et al., 2012).

The prognostic significance of various pretreatment factors has been established in many works of literature. Age at presentation, performance status, extent of tumor resection, tumour histology and location are established risk factors (Curran et al.,1993). Recent studies have integrated molecular markers like IDH-mutation, MGMT status and other novel markers to provide predictive and prognostic information (Wick et al., 2013). However, these molecular markers are costly and not readily available at cancer centres in resource constraint countries like India.

Weinberg and Hanahan proposed hallmarks of cancer that lead to cancer progression. Inflammation is one of the hallmark features that promotes cancer by supplying growth and survival factors, pro-angiogenic factors and extracellular matrix. Inflammatory factors are related to cancer initiation, progression, invasion and metastasis (Coussens and Werb, 2002). Peripheral blood neutrophil lymphocyte ratio (NLR) is a cost-effective and widely available biomarker of inflammatory reaction, and the elevated count is linked with poor prognosis in several malignancies like colorectal, hepatic, thoracic, ovarian, breast, pancreatic and myeloma (Cedrés et al., 2012; Xue et al., 2014; Zhang et al., 2017; Zhou et al., 2017; Liu et al., 2017; Zhou et al., 2018; Mu et al., 2018). A recent meta-analysis reported that increased NLR correlates

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with poor overall survival (OS) in glioma patients. But these studies were mostly retrospective with inconsistent parameters (lei et al., 2019).

With this background, we aimed to determine whether change in NLR during adjuvant chemoradiation treatment of high-grade glioma patients has any prognostic significance with regards to survival. The primary objective was to assess whether any change in NLR during chemoradiotherapy could affect overall survival. The secondary objective was to determine any correlation between other hematological parameters and overall survival. Steroids were presumed to increase the white blood cell count; therefore, we also analyzed any correlation between dexamethasone dose and NLR during treatment. Our study is the first prospective study done in a large number of high-grade glioma patients to assess the correlation of NLR with overall survival.

Materials and Methods

Patient Selection

This prospective observational study was conducted at a tertiary cancer centre in the North-eastern part of India. The period of patient accrual was from August 2020 to August 2021. The institutional ethical committee approved the study protocol via Ref. No. BBCI-TMC/Misc-01/MEC/183/2020. Adult patients (>18 years) with newly diagnosed biopsy proven WHO grade III or IV high-grade gliomas and who were fit to undergo chemoradiotherapy were included in this study. Patients were excluded if they had any autoimmune disease, haematological disorder, or any active infection. All patients provided written informed consent before enrolment in the study.

For every enrolled patient, Immunohistochemistry was done for molecular markers on their post-operative specimen. The tumor type and grade was defined based on histopathology findings and molecular markers viz.- IDH mutated/wild-type and 1p/19q co-deletion status as per the 2016 WHO classification of CNS tumors (Louis et al.,2016). H3K27 M-mutant tumors were termed as diffuse midline glioma.

The patients baseline characteristics were noted, including age, gender, performance status (Eastern Cooperative Oncology Group- ECOG) and body surface area (BSA). Patients were stratified based on the extent of surgical resection as follows: gross total excision, subtotal resection and biopsy using postoperative contrast-enhanced magnetic resonance imaging (MRI) of the brain.

Sample size

From the available data of retrospective literature on the primary objective investigated, for a single arm study design, with one-sided statistical significance of 5% and 90% power, a sample size of 67 patients was estimated. Considering 10% study dropouts, we finalized to accrue 73 patients into our study.

Radiotherapy Details

All study patients started chemoradiation within 6 weeks (or 4 weeks) of surgery. Simulation was done in the supine position using a 3-clamp thermoplastic brain mould. Contrast-enhanced CT images were obtained in the Phillips Big bore CT simulator with 3 mm slices from the vertex up to the C2 vertebra. The CT simulation and post operative MRI images were imported and registered to the Eclipse Version 15.6 treatment planning system (TPS) (Varian Medical Systems, Inc. Palo Alto, CA USA). The target volumes and organs at risk (OAR) for each patient were contoured by a single radiation oncologist in the Eclipse TPS and another radiation oncologist reviewed it. Target volumes were defined according to European Organisation for Research and Treatment of Cancer (EORTC) guidelines (Niyazi et al.,2016). The gross tumour volume (GTV) included the postoperative surgical cavity and/or gross residual enhancing lesion on T1-weighted MRI with gadolinium. The subclinical disease (CTV) was delineated by giving a 2 cm margin around GTV with the inclusion of oedema from a fluid-attenuated inversion recovery sequence (FLAIR). The planning target volume (PTV) was generated with margins according to the institution protocol. Treatment planning was done on the Eclipse TPS using a 6MV photon beam with a Varian Trilogy linear accelerator equipped with Millennium 120 MLC (Varian Medical Systems, Inc. Palo Alto, CA USA). The prescribed dose was 60 Gy in 30 fractions (2Gy per day for five days a week) with the Volumetric arc radiotherapy (VMAT) technique. In every case, the VMAT plan achieving 95% of the prescribed dose coverage of at least 95% volume of the PTV with maximum sparing of OARs was finalized for treatment. All patients received concurrent TMZ (75 mg/m2/per day) along with radiation followed by six cycles of maintenance TMZ (175mg/m2 for five days after every 28 days) according to the institution protocol.

Hematological Parameters

All patients underwent complete blood count investigation on a SYSMEX XN-1000 six parts cell counter (Sysmex Corporation, Kobe, Japan). NLR and platelet lymphocyte ratio (PLR) were obtained by dividing absolute neutrophil count (x10e9/L) or absolute platelet count (x10e9/L) with the absolute lymphocyte count (x10e9/L). Blood investigations were done at baseline (before starting chemoradiotherapy), weekly during treatment, and at 4th week after completion of concurrent chemoradiotherapy (CCRT). The change in NLR was calculated weekly during CCRT for every patient, e.g., NLR week 2nd minus week 1st, week 3rd minus week 2nd and so on until 4th week from completion of CCRT. The average of all the weekly change in NLR values was considered for interpretation.

When the average change was zero and higher for a patient, we designated it as "NO DECLINE in NLR"; whereas when the average change had a negative value, we defined it as "DECLINE in NLR".

Patients with symptoms of raised intracranial pressure received dexamethasone, and doses were recorded at every visit, i.e., baseline, weekly during treatment and at 4th week following completion of chemoradiotherapy. A time-weighted mean (TWM) was calculated representing the mean dose of dexamethasone taken by patients before the start of chemoradiotherapy till 28 days after completion

of RT, considering the daily dose received which was calculated by

$$\frac{\sum_{i=1}^{n} wixi}{\sum_{i=1}^{n} wi}$$

Where ω_i is the proportion of days each dose was taken from the start of RT till 28 days post-RT and x_i is dexamethasone dose.

Statistical Analysis

All enrolled patients were analyzed to assess significant risk factors for both primary and secondary endpoints. The variables were divided into continuous and categorical for statistical analysis. The primary outcome was overall survival, determined as the time from the date of initial histological diagnosis to the date of death. The Kaplan-Meier curve was used to determine overall survival. First, univariate Cox regression was done to evaluate prognostic factors in the patients. The variables found statistically significant in univariate Cox regression were taken in the final multivariable Cox regression model. The Pearson correlation test assessed the association between two continuous variables. P value < 0.05 was considered statistically significant. RStudio Desktop Version 2022.07.0+548 was used for statistical analysis. (Integrated Development for R. RStudio, PBC, Boston, MA, USA).

Results

Seventy-three patients were included in the study. Table 1 shows the baseline demographic profile of the patients. The median age of patients at diagnosis was 46 years (18 to 72). Patients' median BSA at diagnosis was 1.72 mg/m2 (1.24 to 1.83). Males and females were 68.5% and 31.5%, respectively. Only two patients (2.7%) had gross tumor resection (GTR) and IDH mutation was present in 23 (31.5%) cases. The majority of them had GBM histology (68.5%). Thirty seven percent of patients had grade III tumor and 63% had grade IV tumor. The mean (±SD) baseline dexamethasone dose was 2.74 (±4.89) mg whereas the mean (±SD) time weighted mean dexamethasone dose was 2.17 (±2.80) mg.

Table 2 provides the data on the hematological variables of the patients. The median (range) baseline values of the neutrophil count, lymphocyte count and platelet count were 7.17 (2.05-18.59) (×10e9/L), 2.03 (0.29 - 5.31) (×10e9/L) and 208 (94 - 572) (×10e9/L) respectively. The median (range) baseline values of NLR and PLR were 3.5 (1.04 - 37.33) and 110.66 (38.24 - 604.26), respectively. The median change of NLR per week, in the patient cohort between baseline and post treatment period was -0.007 (range -5.083 to 3.512) which was indicative of overall decline.

Patients were divided into two groups based on the change in median NLR. During treatment, 38 patients had a decline in NLR (as described in methodology above), whereas 35 patients did not have a decline in NLR. The median age of those who had a reduction in NLR (45.5 years) versus those who did not have a reduction in NLR (47 years) was not statistically different (p=0.938). There was a statistically insignificant difference in the proportion of reduction of NLR according to gender (Males 50% vs Females 56.5%: p-value=0.604).

The median follow-up in the study population was 16.73 months (3.27 - 46.83 months), with a median overall survival of 17.2 months (95% CI 14.7 - 23 months). Patients who had decline in NLR during the treatment had longer median overall survival compared to patients

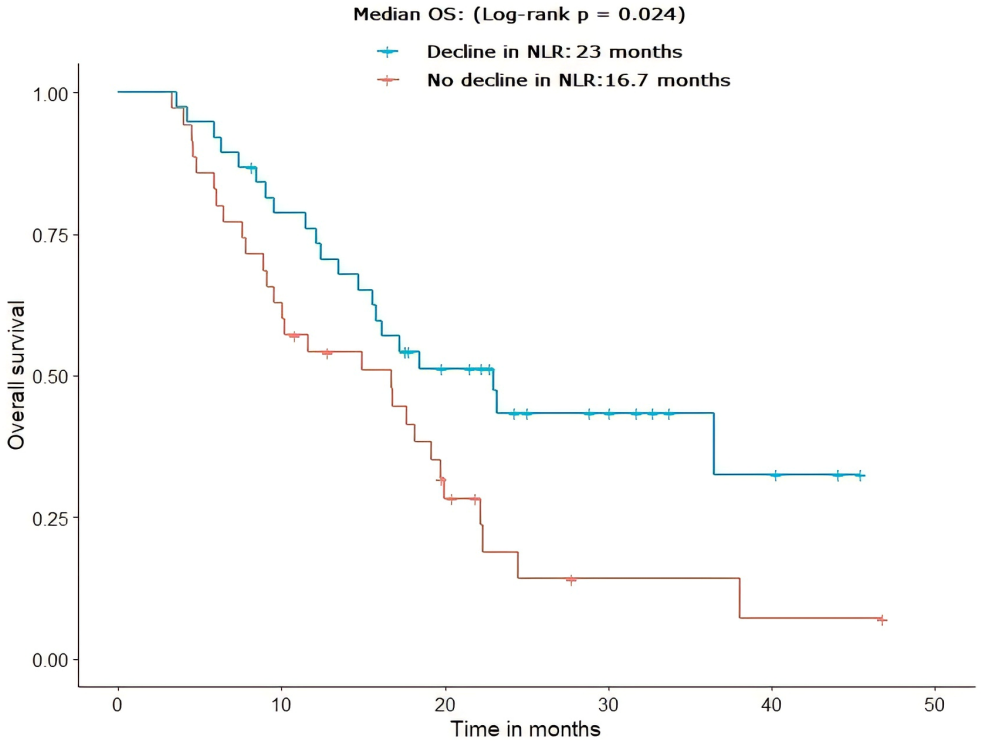

Figure 1. Kaplan-Meier Curve Showing Overall Survival of Patients According to Decline in NLR during Treatment

Table 1. Baseline Patient Demographics (N = 73)

Median age (years) (range)	46 (18 to 72)
Median body surface area (mg/m^2) (range)	1.72 (1.24 to 1.83)
Gender, n (%)	
Male	50 (68.5%)
Female	23 (31.5%)
ECOG PS, n (%)	
1	28 (38.4%)
2	25 (34.2%)
3	20 (27.4%)
Extent of surgery, n (%)	
Partial	45 (61.6%)
Biopsy	26 (35.6%)
GTR	2 (2.7%)
IDH status, n (%)	
Wild	50 (68.5%)
Mutant	23 (31.5%)
Type (Histology), n (%)	
Glioblastoma	50 (68.5%)
Anaplastic astrocytoma	16 (21.9%)
Oligodendroglioma	7 (9.6%)
Grade of tumor, n (%)	
III	27 (37.0%)
IV	46 (63.0%)
Mean (±SD) baseline dexamethasone dose (mg)	2.74 (±4.89)
Mean (±SD) time weighted mean dexamethasone dose (mg)	2.17 (±2.80)
Number of patients who used dexamethasone	35 (47.9%)

ECOG, Eastern Cooperative Oncology Group; GTR, gross tumour resection; STR, subtotal resection; IDH, isocitrate dehydrogenase

Table 2. Haematological Parameters from baseline and Rate of Change Post Treatment

Haematological parameters	Baseline value	Change at 2nd week	Change at 3rd week	Change at 4th week	Change at 5th week	Change at 6th week	Change at 10th week
	Median (Range)	Median (Range)	Median (Range)	Median (Range)	Median (Range)	Median (Range)	Median (Range)
Neutrophil count (×10e9/L)	7.17 (2.05 to 18.59)	-0.57 (-10.91 to 8.94)	0.05 (-5.53 to 14.46)	-0.12 (-11.64 to 6.31)	-0.19 (-5.33 to 8.55)	-0.09 (-7.03 to 3.51)	0 (-6.46 to 5.49)
Lymphocyte count (×10e9/L)	2.03 (0.29 to 5.31)	-0.11 (-2.23 to 8.26)	-0.12 (-7.91 to 0.99)	-0.06 (-1.3 to 5.7)	-0.12 (-1.25 to 5.56)	0 (-5.94 to 0.88)	0.01 (-1.02 to 7.67)
Platelet count (×10e9/L)	208 (94 to 572)	-12 (-425 to 207)	7 (-323 to 195)	-2 (-181 to 109)	1 (-138.85 to 161)	-3 (-105 to 124.85)	0 (-238 to 457)
NLR	3.5 (1.04 to 37.33)	-0.022 (-15.973 to 9.615)	0.256 (-23.739 to 98.894)	-0.04 (-96.578 to 8.472)	0.089 (-8.13 to 162.508)	0.015 (-163.786 to 23.164)	-0.246 (-31.7 to 11.263)
PLR	110.66 (38.24 to 604.26)	3.866 (-247.659 to 151.275)	9.614 (-328.333 to 1206.972)	4.408 (-1131.111 to 122.388)	7.929 (-194.399 to 716)	-3.371 (-452.119 to 671.849)	-5.425 (-771.273 to 251.794)

NLR, neutrophil lymphocyte ratio; PLR, platelet lymphocyte ratio

without a decrease in NLR [23 months versus 16.7 months, respectively; p = 0.024]. The overall survival for patients with a decline in NLR was 73.3% (95% CI 60.4% - 88.9%) and 43.4% (95% CI 29.3% - 64.2%) at 1 and 2 years respectively compared with 54.1% (95% CI 39.9% - 73.5%) and 18.9% (95% CI 8.6% - 41.6%) for patients who did not have a decline in NLR (Figure 1).

Patients were divided into following four groups on the basis of baseline NLR (median) and decline in NLR during chemoradiotherapy. Patients who had baseline NLR < 3.5 with a decline in NLR during treatment (n=14) had excellent prognosis with a one-year survival of 100% and median overall survival of 36.5 months. Patients who had baseline NLR <3.5 without a decline in NLR during treatment (n=23) had a one-year survival of 69.6% (95% CI 53.1% - 91.2%) and median overall survival of 19.2 months. Patients who had baseline NLR >=3.5 with a decline in NLR during treatment (n=24) had a one-year survival of 57.2% (95% CI 40.2% - 81.3%) and median overall survival of 14.7 months. Patients who had baseline NLR >=3.5 without a decline in NLR during treatment (n=12) had a one-year survival of 25% (95% CI 9.4% - 66.6%) and median overall survival of 7.1 months. Figure 2 represents the overall survival of these four groups.

Univariate analysis

Table 3. Univariate and Multivariable Analysis of Prognostic Factors for Overall Survival

Variables	Univariate analysis unadjusted HR (95% CI)	Multivariable analysis adjusted HR (95% CI)
Age	1.037 (1.015-1.06)	1.017 (0.991-1.044)
ECOG performance status <=1	0.086 (0.035-0.209)	0.112 (0.035-0.356)
Wild IDH	4.953 (2.277-10.77)	2.245 (0.810-6.224)
Grade IV tumor	3.528 (1.791-6.951)	1.062 (0.415-2.717)
Baseline neutrophil count <7.17	0.347 (0.191-0.63)	0.482 (0.178-1.309)
Baseline lymphocyte count <2.03	1.389 (0.789-2.444)	-
Baseline platelet count <208	1.47 (0.837-2.58)	-
Baseline NLR <3.5	0.512 (0.291-0.904)	0.593 (0.254-1.384)
Change in neutrophil count during treatment	1.27 (0.776-2.08)	-
Change in lymphocyte count during treatment	0.237 (0.039-1.455)	-
Change in platelets during treatment	1.004 (0.99-1.018)	-
Decline in NLR during treatment	0.519 (0.293-0.918)	0.380 (0.180-0.800)
Extent of surgery (Partial compared to biopsy)	0.373 (0.208-0.666)	0.310 (0.158-0.607)
Baseline dexamethasone dose	1.067 (1.013-1.123)	0.987 (0.921-1.059)
TWM dexamethasone	1.208 (1.09-1.339)	0.994 (0.871-1.135)
Body surface area	0.691 (0.07-6.824)	-

ECOG, Eastern Cooperative Oncology Group; GTR, gross tumour resection; STR, subtotal resection; IDH, isocitrate dehydrogenase; NLR, neutrophil lymphocyte; NLR, neutrophil lymphocyte ration; TMW, time-weighted mean

Each year of age increased death risks by 1.037 times (unadjusted HR 1.037; 95% CI 1.015–1.06). Patients with ECOG performance level ≤1 showed 91.4% (unadjusted HR 0.086; 95% CI 0.035–0.209) reduced death rates than those with category 2–3. Wild-type IDH patients had 4.953 times (unadjusted HR 4.953; 95% CI 2.277–10.77) greater death rates than mutant-type IDH patients. Compared to grade III tumours, grade IV tumours had 3.528 times (unadjusted HR 3.528; 95% CI 1.791–6.951) greater mortality rates. Partial surgery reduced mortality by 62.7% (unadjusted HR 0.373; 95% CI 0.208–0.666) compared to biopsy. Patients with baseline neutrophil count <7.17 had 65.3% (unadjusted HR 0.347; 95% CI 0.191–0.63) lower mortality risk than those with baseline neutrophil count >= 7.17. Patients with baseline NLR <3.5 had 48.8% (unadjusted HR 0.512; 95% CI 0.291–0.904) lower mortality risk than those with baseline NLR >= 3.5. Patients with NLR decline during treatment had 48.1% (unadjusted HR 0.519; 95% CI 0.293–0.918) lower mortality risk. With each mg increase in baseline dexamethasone, there were 1.067 times (unadjusted HR 1.067; 95% CI 1.013–1.123) higher hazards of mortality.

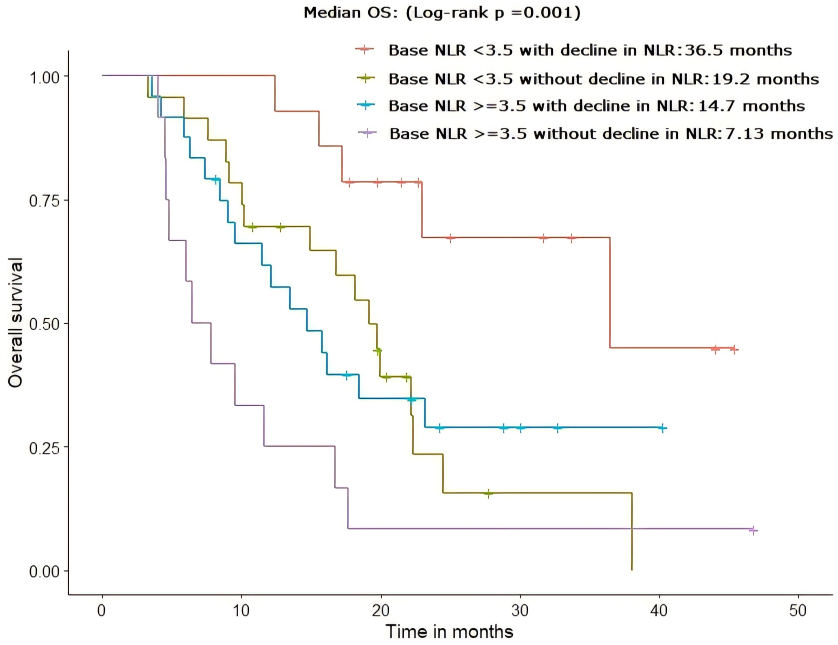

Figure 2. Kaplan-Meier Curve Depicting Overall Survival of Patients According to baseline NLR and Decline in NLR during Treatment

Each mg rise in TWM dexamethasone increased mortality by 1.208 times (unadjusted HR 1.208; 95% CI 1.09–1.339). Baseline lymphocyte count, baseline platelet count, change in neutrophil count during treatment, change in lymphocyte count during treatment, change in platelets during treatment, and body surface area were not found to be statistically significantly associated with overall survival.

Multivariable analysis

Age, ECOG performance status ≤1, extent of surgery, i.e., partial compared to biopsy, and decline in NLR during treatment were significant predictors of overall survival in multivariate analysis (Table 3). Patients with ECOG performance status ≤1 had 88.8% (adjusted HR 0.112; 95% CI 0.035–0.356) lower hazards of mortality as compared to patients with ECOG performance status 2 to 3. Patients with partial surgery had 69% (adjusted HR 0.310; 95% CI 0.158–0.607) lower hazards of mortality as compared to patients who underwent biopsy. Patients with a decline in NLR during treatment had 62% (adjusted HR 0.380; 95% CI 0.18–0.80) lower hazards of mortality as compared with patients without a decline in NLR during treatment.

Association between steroid and change in NLR

According to the test of collinearity, there was a weak correlation noted between dexamethasone dose and NLR (mean $\rho = 0.236$) in our study population. The median (IQR) change in NLR was -0.004 (-0.239, 0.780) among patients who used dexamethasone and -0.023 (-0.154, 0.125) among patients who didn't use dexamethasone; the difference was found to be statistically insignificant ($p>0.05$). Among patients who used dexamethasone, 51.4% had decline in NLR during treatment which was similar to the 52.6% patients in the subgroup that didn't use dexamethasone.

Discussion

This prospective study investigated the prognostic importance of NLR in patients with high-grade gliomas treated with adjuvant chemoradiation and attempted to validate the findings of earlier retrospective studies. In a systematic review by Templeton et al., (2014) NLR > 4 i.e. the median cut-off value was corelated with poor OS across all solid tumors. Bambury et al., (2013) also reported that NLR above 4 was an independent factor affecting overall survival (HR=1.932, 95% CI 1.011 - 3.694, p= 0.046). A Chinese study by Han et al., (2015) stated that pretreatment NLR was an independent factor of OS in multivariate analysis (HR = 1.050,95 % CI 1.003–1.100, p= 0.037) for Glioblastoma. Another study by Gan et al., 2019 on elderly patients with high grade glioma concluded that high NLR>3 was an unfavorable prognostic factor. The results of our study correlates and supports the findings of these previously published literature. However, Yersal et al., (2018) observed that GBM patients with NLR<4 had a superior OS (10.7 vs. 7.8 months) than NLR >4 patients, although the difference was not statistically significant (P>0.05).

In addition to the decline in baseline NLR, change in NLR during adjuvant chemoradiotherapy in high-grade glioma has also emerged as an independent prognostic factor in this study. At one year, the overall survival for patients with and without the decline in NLR was 73.3% (95% CI 60.4% - 88.9%) and 54.1% (95% CI 39.9% - 73.5%) respectively. Our result of this study is in accordance with a study by Mason et al., (2017) who stated that the 1-year OS were 72.9% (95% CI 66.3%-78.4%) and 58.8% (95% CI 50.8%-65.9%) in patients with and without a decline in NLR respectively. A systematic review and metanalysis by Lei et al., (2019) showed that low NLR had a good predictive value in both the univariate (HR: 1.69, 95% CI: 1.06–2.67) and multivariate analysis (HR: 1.31, 95% CI: 1.16–1.48) and that validates our finding also. In a retrospective study, Mathew et al., 2017 assessed the weekly change of NLR on GBM patients during chemoradiotherapy and reported that decline in NLR was an independent predictor of OS.

A high NLR signifies neutrophilia or lymphopenia or both. In this study, increase in baseline neutrophil count ($p\leq0.001$) was associated with poorer overall survival, although baseline lymphocyte count ($p=0.225$) did not show any significant correlation on univariate analysis. The association between white blood cell count and overall survival has been related to the tumour microenvironment. Neutrophilia plays a role in tumour progression and immunosuppression by suppressing the cytotoxic CD8 T-cells and NK cells and increasing suppressor CD4 T-cells (Petrie et al., 1985; Shau et al.,1988; Hirohata et al., 1995). On the other hand, lymphocytes have been associated with better treatment response by inhibiting the tumor proliferation and its invasion by inducing apoptosis and production of cytokine (Coussens and Werb, 2002; Mantovani et al., 2008). Han et al., (2015) reported a positive association between high pretreatment NLR >4 and high neutrophil count and CD3+T-cell infiltration in Glioblastoma. Liang et al., (2014) reported that neutrophilia was associated with increased tumour grade and resistance to anti-VEGF therapy.

Another haematological marker, the PLR was also investigated as a prognostic factor in our study but no correlation was found with overall survival. Our findings were similar with the results of Han et al, 2015 who also reported NLR to be superior to PLR as a prognostic factor in glioblastoma.

Characteristic variables like age, ECOG performance status and extent of surgery were also independent prognostic factors on multivariate analysis of our study results. There was no statistically significant difference in proportion of decline in NLR according to the gender (p-value=0.604) and age (p-value=0.938), although female patients (56%) showed greater proportion of decline in NLR. In our study no relationship was found between patient's body surface area (BSA) and NLR as well BSA and overall survival.

Various studies have shown that glucocorticoids increase the number of polymorphonuclear leukocytes in circulating blood, that plays a major role in inflammatory reaction (Nakagawa et al., 1998). Dexamethasone has been known to cause neutrophilia as well as lymphopenia

by suppressing migration of neutrophils to the site of inflammation and decreasing lymphocyte colony proliferation- which should lead to an increase in NLR (Johnson et al., 2022). A study by Montani et al., (1999) showed that dexamethasone induces cytolysis of T-cells by expressing anti-apoptotic factors. Contrary to what is postulated, our study results did not show any significant association between dexamethasone dose and change in NLR (p-value=0.236). The proportion of patients showing a decline in NLR during the study period was almost similar between the two subgroups based on dexamethasone use- suggesting that administration of systemic steroids did not significantly affect NLR in our study population. However, it's challenging to exclude steroids as a confounding factor in studies like ours, because there always remains a possibility that more sick patients with poorer prognosis would receive steroids in larger doses and for longer periods- thereby having high NLR at baseline with little or no decline in NLR over treatment period.

The results of this prospective study validate that the pretreatment NLR and the change of NLR during treatment can be used as independent prognostic indicators of survival in patients with high-grade glioma. This can be due to interaction between systemic and local inflammation which likely affects the clinical outcome. However, there are various other pathological and physiological factors that may impact NLR in a patient undergoing chemoradiation. In the era of molecular diagnosis, the standard prognostic markers should be molecular markers recommended by major clinical guidelines and societies. However, in developing countries like India, where molecular prognostication lags behind due to various logistical issues like availability and cost factors, this simple, inexpensive inflammatory marker remains a potent tool to prognosticate these patients.

Our results showed that NLR is a cost-effective biomarker that has prognostic significance in overall survival for high grade glioma patients. Future studies are required to investigate different subsets of T-cell that will help us to understand this underlying relationship, and hopefully the molecular mechanisms will also be explored.

Author Contribution Statement

The authors confirm contribution to the paper as follows: Conception and design: Prashasti Sharma, Partha Pratim Medhi; Analysis and Interpretation: Prashasti Sharma; Editing and reviewing: Partha Pratim Medhi, Mouchumee Bhattacharyya, Apurba Kumar Kalita, Jyotiman Nath; Overall Project Management: Partha Pratim Medhi, Prashasti Sharma

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Ethical approval

The study was approved by the institutional ethics committee of Dr. B. Borooah Cancer Institute, Guwahati. The institutional review board no is Ref. No. BBCI-TMC/Misc-01/MEC/183/2020.

Data Availability Statement

All data generated or analyzed during this study are included in this article and further enquiries can be directed to the corresponding author.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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