Prognostic capacity of preoperative levels of selected blood inflammatory markers for glioma patient's survival

(Historical cohort study)

Master of Public Health Thesis Project

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Abbreviation List

G - Glioma grade (grade of malignancy)

IDH - Isocitrate dehydrogenase (gene mutation)

VEGF - Vascular endothelial growth factor

KPS - Karnofsky Performance Status Scale

NLR - Neutrophil-to-lymphocyte ratio

RDW - Red cell distribution width

ESR - Erythrocyte sedimentation rate

CRP - C-reactive protein

PLT - Platelet count

NCN - National Center for Neurosurgery

WHO - World Health Organization

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Abstract

Background

Glioma is considered to be a rare type of cancer of the central nervous system (CNS) with the average age-adjusted incidence rate of 3.4 per 100, 000 population. Moreover, gliomas are the most common type of malignant brain tumors among adult population. They account for about 80% of all diagnosed brain tumors originating from brain parenchyma. Despite achievements in medical treatment and technologies glioblastoma patients still have poor prognosis with 3-5% of surviving patients after 12-15 months following standard therapy. In addition, recent studies highlight importance of blood, and molecular markers in predicting presence of gliomas.

Aims of the study

Primary aim of the study is to investigate the association of isocitrate dehydrogenase (IDH) mutation with the molecular markers of tumor tissue, and its association with the survival time of glioma patients.

Methodology

This is a historical cohort study that was performed using secondary data provided by National Center for Neurosurgery (NCN) (Astana, Kazakhstan). The participants of the study were between 2009-2012. Bivariate analysis was performed between variables survival status of patients (status) and each predictive variable. The Kaplan-Meier method was used to analyze median survival time between the patients, and to compare their overall survival length. IDH effect was tested by building a Cox-regression model, adjusting with each explanatory variable

Results

190 patients were included in the study. Mean age of the participants was 43±12.8 years, and there was approximately an equal distribution of gender of the participants: 107 patients were male (56.3%) and 89 were female (43.7%). The glioma grade (G) variable was found to

be significantly associated with survival time with the p=0.0433. Red Cell distribution width (RDW) was also significantly associated with survival status (p=0.011). Results of Cox-regression revealed no significant predictors in multivariate analysis. Unadjusted HR of dying among glioma patients with IDH versus without IDH was found to be HR 1.28 (95% CI: 0.790; 1.901). Final survival model included only IDH mutation, age and gender variables.

Conclusion

Similar to other studies, current study found a significant association between survival status of the patients compared to the stage of the glioma grade. It should be highlighted that significant associations were found between IDH and blood markers. For example, NLR (p=0.046), ESR (p=0.002), PLT (p=0.021), and RDW (p=0.0034) were significantly associated with IDH mutation genes among glioma patients.

No significant predictors of survival of glioma patients were found in this study, mainly due to lack of data. We suggest that absence of information on tumor size, tumor location, or important clinical interventions (such as chemotherapy) can be important confounding factors that should be taken into account in further studies.

1. Introduction

1.1 Background/ Literature Review

Glioma is considered to be a rare type of cancer of the central nervous system (CNS) with the average age-adjusted incidence rate of 3.4 per 100, 000 population (19, 20). Moreover, gliomas are the most common type of malignant brain tumors among adult population. They account for about 80% of all diagnosed brain tumors originating from brain parenchyma (31). It was reported that 15% of all CNS and primary brain neoplasms account for glioma. According to World Health Organization (WHO), malignant gliomas are subcategorized to III/IV grades, and these grades are assigned depending on certain clinical pathological characteristics, such as mitotic activity, vascular proliferation, nuclear atypia, and outcome of the treatment (4).

Although gliomas occur almost exclusively in the brain, they also might appear in the cerebellum, spinal cord, and in the brain stem. According to literature, 61% of all gliomas can be found on the four lobes of the brain: frontal (25%), temporal (20%), parietal (13%), and occipital (3%) (1). Historically, gliomas were considered to originate from glial cells, but modern evidences suggest that they might result from multiple cell types with neural-stem like properties (14).

Studies found that the average age for the development of glioblastoma is 64 years, but malignant glioblastoma might occur in any age groups, even among children (21). In addition, glioblastoma has 4 extreme degrees and malignancy, which more often occurs among people of working age after 40 years (7). Ellor, Pagano-Young, & Avgeropolous, 2014 argue that the risk of glioblastoma is higher in men comparing to women (risk ratio 1.6:1), and glioma rate was found to be higher among Caucasian ethnic group compared to other ethnicities.

Despite achievements in medical treatment and technologies, glioblastoma patients still have poor prognosis with 3-5% of surviving patients after 12-15 months following

standard therapy (10). Previous studies identified that factors such as: tumor size, assigned treatment, age, mental status, tumor grade, performance score, and surgical resection size were consistently identified as prognostic factors of glioma patients overall survival (9,12)

Studies revealed that blood-based biomarkers for any given malignant gliomas may give approximate information regarding health status of the patient, and about burden of the tumor (22). Dynamic characteristics of the marker testing can be described with ability to proportionally and perfectly reflect the tumor burden. Accurately assessed specific markers can be identical to unique characteristics of the tumor itself (e.g., circulating tumor specific DNA or tumor cells). However, changes of turnover rates of cells, variability in half-life of plasma markers, and obtaining different therapies after surgery might significantly affect marker characteristics in blood (20, 26).

Previous studies revealed that blood-based biomarkers are not always perfect. It becomes clear while taking into account routinely usage of blood-based biomarkers for other cancer types investigation. For instance, PSA (prostate-specific antigen) level can change dramatically in patients who had bacterial prostatitis, but markers still may provide essential information about cancer progression, and about tumor response (19,26). Several pilot studies revealed that circulating tumor DNA can be detected after blood tests among patients with malignant gliomas (15, 20, 26).

In addition, recent studies show that mutated IDH-1 DNA can also be found in the plasma of patients with IDH-1 positive gliomas. Therefore, researchers concluded that relationship exists between blood-brain barrier disruption, and IDH-1 DNA detectability (14, 26). For instance, results of meta-analysis of 165 studies conducted by Chen et al., 2016 confirmed association of IDH mutation with overall survival of glioma patients (27). Many recent studies interested in anti-angiogenic agents for investigational therapies of glioblastoma, pro-angiogenic proteins have mostly considered circulating proteins in glioma investigation. Vascular endothelial growth factor (VEGF) is relatively large protein weighing 38.3 kDA, and initial function of this factor in physiology is to stimulate growth of

endothelial cells. In addition, circulating levels of the VEGF was analyzed in several prospective studies of glioma, and they found to be significantly associated with glioma patients' survival (3, 26).

Numerous studies revealed the association of chronic inflammation with high susceptibility for cancer diagnosis and progression (32). Inflammatory blood markers such as: neutrophil-to-lymphocyte ratio (NLR), and platelet count (PLT), red cell distribution width (RDW) has been proposed for prediction of glioma patient's survival (3, 5, 32). The most studied blood count NLR was found to be relevant in many cancer studies (2, 29). Neutrophils and lymphocytes constitute a significant share of immune cells participating in inflammatory processes. In the context of cancer studies, NLR were proved to influence both pro-and antitumor procedures offering a dual mode of activation (28). Red cell distribution width (RDW) was considered as an important predictive blood parameter in various chronic inflammatory conditions and cardiovascular pathologies. However, there is limited number of studies that investigated importance of RDW in glioma patients' survival (5). Platelet count (PLT) is one of the convenient blood parameter that may serve as predictor for patient's survival. Initially, the main function of PLT is to ameliorate damaged tissues by evolving inflammatory cells. However, under chronic inflammation environment PLT stimulates growth of tumor, and studies found that PLT was highly correlated with PLT and NLR (32). It is believed that molecular markers can be useful for prognosis of glioma. Considering the availability of preoperative molecular and blood assays, such parameters can be essential to assess their effect on survival of glioma patients.

1.2 Risk factors and clinical presentation of disease

Results of different studies conducted to identify specific associative factors of glioblastoma with occupational and environmental exposure are still inconclusive. Wrench et al., in 2002 concluded that ionizing radiation is one of the few well-known risk factors for glioma development (33). Regarding other environmental exposures such as pesticides, vinyl

chloride, smoking, synthetic rubber manufacturing, and oil refining, these are found to be poorly associated with the development of glioma. It should also be highlighted that, potential risk factors as electromagnetic fields, formaldehyde, and nonionizing radiation produced by cell phones have not been found to lead to glioblastoma (4). The risk of glioma was found to be higher among specific genetic diseases, such as tuberous sclerosis, Turcot syndrome, Li-Fraumeni syndrome, however, heredity of the disease is counted to be less than 1% among glioblastoma patients (7, 5). Effects of serious head injuries or traumas were studied for a long time to investigate its relationship with gliomas (4,5). The results, however, are ambigious: some studies revealed a connection between head injury and meningioma, but no link between head trauma and glioma (5,6). Brain tumors are characterized to be more common among adult population. Nevertheless, people of any age group are susceptible to the development of the brain tumor (1). Regarding gender of patients, the risk of developing gliomas is generally higher among men relatively to women. However, there are specific types of brain tumors such as meningioma, which are more common among female population (16).

Presentation of patients with newly diagnosed glioblastoma may differ regarding the size and location of tumor, and anatomic characteristics of evolved part of the brain (5). Often patients are characterized by increased intracranial pressure syndromes, including focal or progressive neurological deficit, and headache. On average 25% of patients with glioma have reported seizure symptom, but this might increase up to 50% at later stages of brain tumor (20). Modern standard of treatment of glioblastoma represents prescription of antiepileptic drugs (AEDs) for patients reporting seizure. However, routine use of AEDs for glioblastoma treatment is not recommended (4). Overall, glioblastoma patients often report headache, confusion, memory loss, focal neurologic deficits, and change of personality or seizures (16).

1.3 Diagnostics and treatment

Treatment of the newly diagnosed glioma demands well-organized multidisciplinary approach. Primary diagnostic visualization of glioma includes magnetic resonance imaging (MRI), or computed tomography (CT) scan. Today standard therapies for treatment of glioma include maximal safe surgical resection, followed by concurrent radiation with temozolomide, and oral chemotherapy, and finally with adjuvant chemotherapy with temozolomide (4,15). Complete surgical resection and extensive methods of treating glioblastoma are difficult because of the frequent invasive characteristics of tumor and its location on mostly eloquent parts of brain responsible for control senses, speech, and motor function of patient. High level of invasiveness of tumor, surgical resection of the mass cannot be curative way, and cells of infiltrating tumor may remain within the surrounded brain by leading to further progression of disease or its reoccurrence (9). Novel technologies still have their limitations due to cost, need for specialized equipment and operators, and surgery suits.

1.4 Situation in Kazakhstan

Statistics of the incidence of glioblastoma in Kazakhstan are consistent with the worldwide data, with 3.0±0.04 rates per 100,000 people (30). According to Akshulakov et al., 2009, the number of patients registered with glioblastoma increased by 1.8 times between 1996 and 2007. According to statistical data for previous 10 years, highest rates of incidence for glioblastoma (from 4.8 to 5.8 per 100,000 population) occurred in Pavlodar, Kyzylorda, East Kazakhstan, and Almaty regions. Morbidity among males doubled within the last decade with the incidence rate 4.2±0.24 per 100,000 population, opposed to the beginning of the century 2.0±0.17 per 100,000 population. The incidence rate among women also increased from 1996 to 2007 (from 1.9 to 3.3 per 100,000 population) (2). *Brain tumors*

of children consistently is on the second place among structure of pediatric oncological pathology with the 11% share. A rate of pediatric glioblastoma within Post Soviet countries fluctuates between 0.12 in Kyrgyzstan up to 2.7 in Republic of Belarus (30). Overall, prevalence of glioblastoma among children in Kazakhstan also increased from 0.5 to 1.16 cases per 100,000 children population in the past 20 years. The highest rates of childhood glioblastoma can be seen in regions such as Pavlodar, Zhambyl regions, where rates achieved 3.0 cases per 100,000 population (30).

1.5 Aims of the study

Primary aim

- To investigate association of IDH mutation with the molecular markers of tumor tissue, and its effect on glioma patients survival.

Secondary aim

- To investigate prognostic significance of preoperative blood markers for survival of patients with glioma.
- To test each molecular marker for association with preoperative blood markers.
- To investigate whether there is an association between blood parameters, and molecular markers for survival of glioma patients.

It was already mentioned that many factors are associated with survival time of glioma patients. This study focuses on factors such as age, gender, level of glioma grade, survival time after enrollment to the study, presence of mutated genes among patients, Karnovski Performance Score, and complex of blood and molecular markers. Findings of the study may benefit professionals in clinical settings to offer better treatment interventions for patients with developed gliomas. *Importance of the study could be seen in its accessibility, and relatively low costs of prognosis central nervous system cancers*.

2. Methodology

This is a historical cohort study that is performed using secondary data provided by National Center for Neurosurgery (NCN) (Astana, Kazakhstan). The participants of the study were recruited through 2009-2012 from NCN. This is also a more analytical study, since in addition to presenting descriptive characteristics of the study cohort, it aims at analyzing the association of blood and molecular markers with the overall survival time of the patients after surgical treatment procedures.

Target population of the study includes patients diagnosed with glioma. *Sampling frame* includes patients who were surgically treated at the National Center for Neurosurgery between 2009-2012 in Astana. *Study population* includes glioma patients who met exclusion and inclusion criteria. The following inclusion criteria were applied to select the study population:

1. Patients who were treated at NCN from 2009 to 2012 years.

2. Patients with histopathological confirmed brain tumor (In accordance with WHO classification, 2007).

4. Patients with preoperative full blood count (FBC) analysis.

5. Absence of active infection and autoimmune disorders.

Exclusion criteria:

1. Patients with missing survival time information.
2. Patients with no outcome status, or unknown.

Rationale for choosing the study population

Current study focused on the patients with same characteristics and study population can be considered as homogenous. In addition, outcome variable was survival status of the patients (whether patient alive or dead) by the last follow up time. Out of 202 patients enrolled

in this study, only 190 patients were included to final data. The reasons of excluding 12 participants were absence of information regarding our outcome of interest, and repeated information resulting from typing error.

After defining final sample size, it was possible to calculate power of the test that will be used during the analysis. We assumed two sided significance level at 0.05, with sample size 190. Finally, power of the test was equal to 0.78.

3. Ethical Considerations

Ethical Committee of the NCN is approved a study with the document "IORG0008395". All ethical procedures were taken into account while collecting data, signed consent forms were obtained from patients or from their legal representative in case of patient's incapacity. Afterwards, the Institutional Research and Ethics Committee of Nazarbayev University School of Medicine reviewed and approved the study to analyze anonymized secondary data provided by NCN. Further safety measures were ensured by storing electronic data in a password-protected computer, and avoiding transmitting the data via email or other types of transmission devices. Data is planned to be used only in research purposes, and will be destroyed after three years.

4. Analysis

In this study statistical analysis was performed using statistical package STATA 12 (STATA Corporation, USA, Texas, 2012), and statistical significance was determined using p-value less than 0.05. After appropriate data management descriptive statistical analysis was performed characterizing numeric variables in terms of mean and standard deviation, while categorical variables were given in frequencies.

Some numeric variables such as age, and blood markers were categorized into groups considering classification in previous studies (21). Age of patients with glioma diagnosis categorized in two groups: those who are less than 45 years and those who are older than 45

years. There are 4 grades of glioma, but studies suggest excluding the first grade glioma from the study because of its rareness in a population (32).

Karnovski Performance Score was categorized into two groups: >70 meaning higher capability of functional status of patient, and <70 was considered as low degree of functionality. Blood counts such as NLR, ESR, PLT, and molecular markers as vascular endothelial growth factor (VEGF), and C-reactive protein (CRP) were categorized taken into account cutoff levels used in previous studies (32). RDW variable was considered in two categories: patients with RDW<14, versus RDW>14. The association between PLR variable and the outcome variable was tested among groups who have PLR<175 versus those with PLR>175. Studies suggest that association of VEGF with outcome of interest should be compared among three categories including: VEGF<7, VEGF≥7, and with no VEGF (21). CRP was also considered in two categories: patients with CRP<5, versus CRP≥5.

Pearson's product-moment correlation coefficient and Spearman's rank correlation coefficient were used to determine bivariate relationships between numeric variables.

Chi-square test was applied to examine bivariate associations between categorical variables. Variables were considered as a significant predictor if p-value was less than 0.05, and assumptions of the performed test were not violated (at least one observation in each cell). Log-rank test were performed to compare survival times between groups within categorical variables. Kaplan-Meier survival analysis method was used to determine median survival time, and to compare patients' overall survival length.

Before conducting Cox-regression, all of the listed assumptions were considered, and most of the assumptions were not violated by the study design. In addition, log(h(t)/h0(t)) is a linear function of X's assumption was tested with each numeric predictive parameter. Furthermore, it should be highlighted that main assumption for Cox-regression is the proportional hazard over the period of time. Significant variables and important confounding factors, and predictors that considered being important during the bivariate analysis were

included to our final model. The multivariate analysis was obtained using Cox proportional hazard regression and considering interactions, and avoiding multicollinear variables.

5. Results

Descriptive characteristics of the data are illustrated in Table1. The total sample size was 190 patients diagnosed with glioma. Mean age of patients was 43±12.8 years, and there was equal distribution of both genders: 56.3% males, 43.7% females. Those who were younger than 45 years were 53.1%.

One of the important variables in the data set was information on patient's glioma grade. There were no patients with first grade of glioma. 25 patients (13.1%) had second grade glioma diagnosis, 71 patients (37.4%) had third level of glioma grade, and 94 participants were confirmed with the fourth grade of glioma (49.5%). IDH (isocitrate dehidrogenase) mutation genes were present in 85 participants (49.1%), and 88 participants (50.9%) did not have IDH mutation. Karnovski Performance Score (KPS) average score of all participants was equal to 65.3±8.1. Almost two thirds of patients (62.1%) had KPS lower than 70. Average level of CRP was 4.6 mg/L, 133 patients (70%) had CRP below 5 mg/L. Average survival time in this cohort was 13.8±8.6 months, where at the end of the last follow up 91 (48%) of study participants died.

Bivariate analysis (log-rank test) was performed between variables survival status of patients (status) and with each independent variable. However, only two predictors: G (Glioma grade) variable was found to be significantly associated with survival time (p=0.0433), as well as RDW (Red Cell distribution width) variable (p=0.011). Surprisingly important risk factors mentioned in literatures such as age (p=0.7929), gender (p=0.4435), KPS (p=0.7208), NLR (p=0.1849) were not associated with survival in this study.

Further, results of chi-square test revealed that variables KPS (p=0.005), ESR (p=0.002), PLT (p=0.021), RDW (p=0.030), PLR (p=0.036), and NLR (p=0.046) were

significantly associated with IDH mutation. In contrast, variable G (Glioma grade) was not associated with the IDH mutation p=0.065 and 88 (46%) patients were IDH positive.

Kaplan-Meier method of survival analysis was used to compare overall survival time between the IDH positive and IDH negative patients. As illustrated in Graph 1, overall survival time is comparatively longer in IDH negative patients than in IDH positive patients. Among the patients with glioma both groups (IDH positive, and negative) have approximately equal median survival time about 25 months.

Results of Cox-regression revealed no significant predictors in multivariate analysis. Unadjusted HR of dying among glioma patients with IDH versus without IDH was found to be HR 1.28 (95% CI: 0.790; 1.901). Final survival model included only IDH mutation, age and gender variables.

6. Discussion

Primary aim of the study was to assess the importance of the IDH mutation in predicting survival for a given cohort of the patients. Previous studies found that IDH mutation genes are key process in the development gliomas and other type central nervous system tumors (29). Research conducted by Bleeker et al., in 2010 applied DNA samples obtained from frozen glioma specimens, and studied survival and enzyme activity. After adjusting for KPS, extent of surgical resection, chemotherapy, and dosage of received radiotherapy, IDH mutational status was found to be an independent risk factor of overall survival of glioma patients (28). However, in this study IDH was not significantly associated with outcome variable (survival status of the patient by the end of study). It is important to mention that bivariate analysis of risk factors with survival length (log-rank test) includes time element of survival analysis, which is why most of the predictive variables did not show association with outcome variable.

The bivariate analysis of IDH with other predictive variables found some interesting results. For example, KPS was significantly associated with IDH (p=0.005), meaning that

presence of the mutated genes can significantly affect preoperative physical condition of the patient. Regardless of the results on final model it should be highlighted that significant associations were found between IDH, and blood markers. There were not any studies investigating for the association of the IDH with blood parameters. For example, NLR (p=0.046), ESR (p=0.002), PLT (p=0.021), and RDW (p=0.0034) were significantly associated with IDH mutation genes among glioma patients.

As it was mentioned in previous parts glioma has poor prognosis and short survival time equal to 13-15 months on average (14,17,21). In current study median survival time found to be longer–22 months (95% CI 0.3408-0.6690). However, it does not necessarily mean that patients treated at NCN have longer survival time. Possible explanation might be absence of data when surgical intervention was applied or the time when patients acquired chemotherapy.

Similar to other studies, current study found significant association between survival status of the patients and stage of the glioma grade (11,13). Previous studies revealed that there is a significant difference in survival length considering glioma grades of the patient. Cox-Regression analysis showed that glioma grade is significant predictor of the survival time of the patients (p=0.017, and with 95% CI 0.2750-0.8830). In addition, significant difference was found in survival among all three stages of glioma grade (p=0.0464). However, tests revealed that glioma grade variable might be mediating variable, and its significance can be enhanced by its indirect effect on the outcome.

Several studies concluded that age and preoperative performance status (KPS) significantly affect the prognosis of the survival of the glioma patients (16,17, 8). However, current study did not find significant predictive characteristics of the KPS on survival ((p=0.7208).

Importance of the C-reactive protein for glioma patient's survival was mentioned in studies conducted by Matthias et al., (2013). However, current study did not find any

association between the survival of the glioma patients and CRP (p=0.07). In addition, we were interested to test an association between IDH mutation and CRP. In this case the analysis results also showed no association between CRP and IDH mutation (p=0.227).

Numerous studies proposed significance of the inflammatory markers for glioma survival (15). One of the routinely studied blood parameters is considered to be NLR (neutrophil-lymphocyte ratio). Meta-analysis of 100 researches including overall 40,559 sample size, revealed optimal cutoff value of 4.0 (23). Considering all suggestions of previous studies, we tested significance of following blood parameters: NLR, ESR, PLT, RDW. Results were as follows: during the bivariate analysis only RDW was significantly associated with outcome variable (p=0.0118). However, during the multivariate analysis RDW became less informative predictor, and was not included in the final model.

It should be mentioned that current study did not find association between survival status and age, which verify findings of previous studies (26). Research shows that glioma diagnoses is more common in men compared to women, and that men also have shorter survival time. However, this study no difference in length of survival was revealed between gender (p=0.06) groups (HR1.24, 95% CI: 0.788-1.980) (Table 4).

Despite the fact that current study did not find any significant predictors of survival for a given population, various types of sophisticated statistical analysis were used. Nevertheless, further studies are needed to assess effect of biological markers on glioma patient's survival.

7. Study limitations

Current study on survival analysis among glioma patients has several limitations that should be taken into account. Firstly, secondary data used in this study may have information bias, because current researcher did all analysis relying on correctness of the provided data. Secondly, it is believed that due to lack of the data current study did not find significant predictors for survival of glioma patients. Literature suggests that absence of information on tumor size, tumor location, or important clinical interventions (such as chemotherapy) can be important confounding factors (10,12,13).

8. Conclusion and recommendation

It was proved by previous studies that biological markers and their preoperative characteristics may serve as an important information for prognosis of the disease. Furthermore, available information on biological markers assists tailoring treatment strategy considering specific biological characteristics of patients. This study also recommends implementing further research on survival of the glioma patients by looking at the effect of the potential confounders such as size or volume of the tumor, tumor location, and information of whether patients acquired chemotherapy or not. The information from this study can be beneficial for treatment planning, prognosis, and it also opens a new avenue for further research in this sphere.

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APPENDIX LIST

Table1. Descriptive statistics of variables

Parameters	Patient number (%)
Age	
<45	101 (53.1)
≥45	89 (46.9)
Sex	
Male	107 (56.3)
female	83 (43.7)
G	
2	25 (13.1)
3	71 (37.4)
4	94 (49.5)
Survival status	
Alive	99 (52)
Died	91 (48)
KPS	
<70	72 (37.9)
≥70	118 (62.1)
IDH	
No mutation	85 (49.1)
mutation	88 (50.9)
NLR	
<4	134 (70.5)
≥4	56 (29.5)
ESR	
<15	135 (71.1)
≥15	55 (28.9)
PLT	
<350	183 (96.3)
≥350	7 (3.7)
VEGF	
no	11 (6.4)
<7	70 (40.4)
≥7	92 (53.2)
RDW	
<14	145 (76.3)
≥14	45 (23.7)
PLR	
<175	132 (69.5)
≥175	58 (30.5)
CRP	
<5	133 (70.0)
≥5	57 (30.0)

Table2. Bivariate analysis of each parameters with outcome variable (survival status)

Risk Factors	Survival status % (n)		p-value
	alive	died	
Age			
<45	62 (61.4)	39 (38.6)	0.7929
≥45	37 (41.6)	52(58.4)	
Sex			
Male	63 (58.9)	44 (41.1)	0.44356
Female	36 (43.4)	47 (56.6)	
G			
2	23 (92.0)	2 (8.0)	0.0433
3	47 (66.2)	24 (33.8)	
4	29 (30.85)	65 (69.15)	
KPS			
<70	24 (33.3)	48 (66.7)	0.7208
≥70	75 (63.6)	43 (36.4)	
IDH			
mutation	56 (63.6)	32 (36.4)	0.3510
no mutation	32 (37.7)	53 (62.3)	
NLR			
<4	72 (53.7)	62 (46.3)	0.1849
≥4	27 (48.2)	29 (51.8)	
ESR			
<15	84 (62.2)	51 (37.8)	0.7761
≥15	15 (27.3)	40 (72.7)	
PLT			
<350	97 (53.0)	86 (47.0)	0.1303
≥350	2 (28.6)	5 (71.4)	
VEGF			
no	6 (54.6)	5 (45.4)	0.1479
<7	35 (50)	35 (50.0)	
≥7	47 (51.0)	45 (49.0)	

RDW			
<14	76 (52.4)	69 (47.6)	
≥14	23 (51.1)	22 (48.,9)	0.0118
PLR			
<175	73 (53.3)	59 (44.7)	
≥175	26 (44.8)	32 (55.2)	0.1585
CRP			
<5	76 (57.1)	57 (42.9)	
≥5	23 (40.4)	34 (59.6)	0.0669

Table3. Bivariate analysis of each parameters with IDH (mutated genes)

Risk Factors	IDH % (n)		p-value
	Yes	NO	
Age			
<45	43 (48.3)	46 (51.7)	0.489
≥45	45 (53.6)	39(46.4)	
Sex			
Male	48 (49.5)	49 (50.5)	0.681
Female	40 (52.6)	36 (47.4)	
G			
2	14 (66.7)	7 (33.3)	
3	36 (57.1)	27 (42.9)	
4	38 (42.7)	51 (57.3)	0.065
KPS			
<70	25 (37.3)	42 (62.7)	
≥70	63 (59.4)	43 (40.6)	0.005
NLR			
<4	69 (55.7)	55 (44.3)	
≥4	19 (38.8)	30 (61.2)	0.046

ESR			
<15	75 (58.1)	54 (41.9)	
≥15	11 (29.7)	26 (70.3)	0.002
PLT			
<350	88 (52.4)	80 (47.6)	
≥350	0 (00.0)	5 (100.0)	0.021
VEGF			
no	4 (36.4)	7 (63.6)	
<7	34 (48.6)	36 (51.4)	
≥7	50 (54.4)	42 (45.6)	0.468
RDW			
<14	79 (54.5)	66 (45.5)	
≥14	9 (32.1)	19 (67.9)	0.030
PLR			
<175	73 (55.3)	59 (44.7)	
≥175	15 (36.6)	26 (63.4)	0.036
CRP			
<5	71 (53.4)	62 (46.6)	
≥5	17 (42.5)	23 (57.5)	0.227

Table4. Results of Multivariate (Cox-Regression)

Risk factors	Hazard Ratio	95% Confidence Interval
IDH	1.27	(0.784; 2.084)
Age	1.06	(0.677; 1.683)
Sex	1.24	(0.788; 1.980)
NLR	0.68	(0.412; 1.138)
ESR	1.3	(0.606; 2.808)
CRP	1.05	(0.520; 2.137)

Graph1. Kaplan-Meier method: comparison of survival time between IDH positive and IDH negative groups