Prospective Prediction of Treatment Response in High-Grade Glioma Patients using Pre-Treatment Tumor ADC Value and miR-222 and miR-205 Expression Levels in Plasma

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ABSTRACT

Background: Treatment response in High-grade Glioma (HGG) patients changes based on their genetic and biological characteristics. MiRNAs, as important regulators of drug and radiation resistance, and the Apparent Diffusion Coefficients (ADC) value of tumor can be used as a prognostic predictor for glioma.

Objective: This study aimed to identify some of the pre-treatment individual patient features for predicting the treatment response in HGG patients.

Material and Methods: In this prospective study, 18 HGG patients, who were candidated for chemo-radiation treatment, participated after informed consent of the patients. The investigated features were the expression level of miR-222 and miR-205 in plasma, the ADC value of tumor, Body Mass Index (BMI), and age. Treatment response was assessed, and Least Absolute Shrinkage and Selection Operator (LASSO) regression was used to obtain a model to predict the treatment response. Mann-Whitney U test was also applied to select the variables with a significant relationship with patients' treatment response.

Results: The LASSO coefficients for miR-205, miR-222, tumor's mean ADC value, BMI, and age were 3.611, -1.683, 2.468, -0.184, and -0.024, respectively. Mann-Whitney U test results showed miR-205 and tumor's mean ADC significantly related to treatment response (P-value<0.05).

Conclusion: The miR-205 expression level of the patient in plasma and tumor's mean ADC value has the potential for prognostic predictors in HGG.

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Keyword

MicroRNAs; ADC Map; Regression Analysis; LASSO Model; Glioma

Introduction

In adults, gliomas are the most common type of brain tumors divided into four histopathological grades as follows: 1) grades I and II, known as low-grade glioma, and 2) grades III and IV, recognized as high-grade glioma (HGG) [1,2]. HGG Features such as local invasion, genomic instability, rapid cell proliferation, high angiogenesis, resistance to apoptosis and drug delivery, and the propensity for necrogenesis often lead to poor response to treatment [3,4]. In addition, a treatment protocol

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is not successful for all HGG patients because of the genetic and cytologic heterogeneity of HGG tumors [3,5,6]. Therefore, each patient's treatment planning must be based on individual biological information. For example, oncologists have used molecular biomarkers to prescribe the most effective chemoradiation therapy regimens [3], e.g., the expression of microRNAs (miRNAs) associated with glioblastoma progression can be used as a prognostic predictor for glioblastoma [4]. MiRNAs are non-coding RNA molecules of 18-22 nucleotides for regulating post-transcriptional gene expression and affecting many prominent features of malignant tumors such as cell proliferation, angiogenesis, invasion, and metastasis [4,7,8]. MiRNAs are also important regulators of drug and radiation resistance [4]. Studies have shown that miR-222 is overexpressed in GBM patients. In addition, in GBM patients, expression of miR-205 decreases, resulting in increasing the grade of malignancy. In glioma, miR-205 acts as a tumor suppressor by inhibiting proliferation, reducing invasion, and increasing apoptosis [9,10]. Therefore, information about the expression of dedicated miRNAs before chemo-radiation therapy may help predict the treatment response.

A close relationship between miRNA expression and radiation resistance has also been reported [4]. Since miRNAs are released into the bloodstream and are quite stable [11], circulating miRNAs can be used as non-invasive biomarkers in the diagnosis, prognosis, or monitoring of cancer [3,11,12]. The first circulating miRNA studied for prognosis was mir-21, in patients with diffuse large B-cell lymphoma [13]. Since the standard treatment for glioma after surgery is radiation and chemotherapy with temozolomide, and miRNAs effects on developing chemo-radiation therapy resistance, studies have examined the potential role of miRNAs in resistance to these treatments [4,14]. A study showed that miR-222 is overexpressed in GBM patients and had several targets involved in gliomagnesis, such as apoptotic pathways [4]. In addition, miR-205 is downregulated in glioma patients and could be used as a prognostic biomarker in glioma patients [9].

Diffusion-weighted imaging (DWI) is a form of Magnetic Resonance (MR) imaging in which the random Brownian motion of water molecules within a voxel of tissue or Apparent Diffusion Coefficients (ADC) value is measured and also used to obtain information about tumors environment. Studies have shown that ADC values decrease with increasing degree of malignancy and cellularity of tumor [15,16]. Tumor ADC value can also be used independently as a quantitative predictor of progression-free survival for glioma patients [15]. Because of the different responses to treatment in HGG patients, depending on the genetic and individual characteristics, identifying some features that can predict the treatment response can aid the physicians in designing individualized treatment protocols. This study aims to identify accessible characteristics of HGG patients for applying as potential prognostic predictors. For this purpose, several features, including absolute levels of miR-205 and miR-222 in plasma, tumor ADC value, age, and Body Mass Index (BMI) were collected from HGG patients before starting the chemo-radiation therapy. This data and the treatment response data were used to design a LASSO regression model for predicting response to the chemoradiation therapy of HGG patients.

Material and Methods

Patients

This prospective study protocol was approved by the human ethics board of Isfahan University of Medical Sciences, Isfahan, Iran. From December 2018 to November 2020, patients with pathologically confirmed HGG (grade III and IV), who were candidates for chemo-radiation treatment with MR imaging information of T1, T2, FLAIR, and DWI sequences, were included in this study, after informed consent of the patients or their companion. Patients with other types of brain tumors or brain

metastases were excluded, i.e., twenty-one patients were included. Three patients died before starting radiation therapy. Finally, 18 patients: 8 females and 10 males with the median age of 52.44±13.7 years (range 27-72) and median Karnofsky performance scale (KPS) score of 7.38 participated in absolute quantification of two miRNAs (miR-222 and miR-205) levels in plasma.

Before chemo-radiation therapy, blood samples were collected in K2-EDTA, containing tubes, and centrifuged for 12 mins at 1500×g within 2 h after collection. The supernatant was removed to RNase-free tubes and miRNA isolation from fresh plasma was performed.

Mirnas Isolation and Quantitative Reverse-Transcriptase Polymerase Chain Reaction (qRT-PCR)

MiRNAs were extracted from 3 mL plasma from the patients using GeneAll® Hybrid-RTM miRNA isolation kit (Seoul, KOREA 138-859) according to the manufacturer's protocol.

Purified miRNA samples were stored at -70 ℃. MiRNA concentration and purity were then quantified using the Epoch Microplate Spectrophotometer (BioTek- China).

cDNA of miRNAs was synthesized using 10 µL of the total RNA according to the Reverse Transcription System kit (ZistRoyesh, Iran) with miR-specific stem-loop primers [17]. Real-time Polymerase Chain Reaction (PCR) was performed with the ZistRoyesh kit (Tehran, Iran) using 10 µL of 2x qPCR SYBR green master mix with reactions prepared in duplicate. ABI Applied Biosystems™ Thermal Cycler (Thermo Scientific, USA) was used with the cycling conditions included an initial 15 min incubation at 95 ℃, followed by 40 cycles of 95 ℃ for 30 s and 56 ℃ for 1 min. In addition, a dilution series of known template concentrations (101-1012 (molecule/µL)) was prepared for absolute quantification of miRNA levels in plasma, and real-time PCR was performed. Each miRNA and sample determined the average cycle threshold (Ct). The log of each known concentration in the dilution series (x-axis) was plotted against the CT value for that concentration (y-axis). These standards measured the absolute concentration of the miRNAs in the patient's samples using their Ct values.

Treatment

According to the standard treatment guidelines and patients' conditions, surgery followed by chemo-radiation therapy and then adjuvant chemotherapy with temozolomide was prescribed. The dose of temozolomide for concurrent chemoradiation therapy was 75 (mg/m^2/day, for no more than 7 weeks) and for adjuvant chemotherapy was 150-200 mg/m^2/day on 5 days every 28 days for at least 6 maintenance cycles.

In Milad hospital, Isfahan, for each patient, a 3-dimensional conformal RT treatment plan was performed using a Prowess Panther DAQ treatment planning system, commissioned for the 15-MV photon beam of a Siemens Artiste linear accelerator (Siemens Medical Solutions, Muenchen, Germany).

Total doses of 60-70 Gy were delivered to each patient during 5-6 weeks over 5 days per week (dose/fraction=1.8-2 Gy). In this study protocol, two phases of radiation therapy were performed where Planning Target Volume (PTV_1) and PTV_2 received approximately 45 and 16 Gy, respectively. In the initial plan, PTV_1 included clinical target volume (CTV_1) and 0.3-0.5 cm margin while CTV_1 was defined as postoperative peritumoral edema and 2 cm margin, followed by a boost field with enhanced tumor and 2.3-2.5 cm margin as PTV_2 (according to RTOG 0525 and RTOG 0825) [18].

The clinical assessment of the patients, Karnofsky performance scale (KPS), was scored at three points (before chemo-radiation therapy, one and three months after receiving radiation therapy). Based on the general condition of patients, KPS was scored from 0 to 100, i.e., 100 and 0 are considered perfect performance status and death, respectively.

Three months after the chemo-radiation

therapy course, treatment was evaluated by an experienced radiation oncologist and an experienced radiologist using KPS and the standard measure of changes in the volume of enhanced and non-enhanced anatomic lesions in MR images according to Response Assessment in Neuro-Oncology (RANO) criteria [19]. In RANO criteria, treatment response was divided into four types, including complete response, partial response, stable diseases, and progression. Despite a few studied patients, they were divided into two groups: the patients who responded to treatment and those who did not. Patients that indicated partial response were included in the response group and those with disease progression were included in the non-response group. Moreover, patients with stable disease were included in the response group, providing their KPS score improved after treatment. However, reducing the KPS score post-treatment led to the involvement in the non-response group.

MR Imaging

All patients underwent MRI using T1W (pre- and post-contrast), T2W, Fluid Attenuated Inversion Recovery (FLAIR), and DWI sequences in three-time points, before treatment planning, one month, and three months after radiation therapy. Imaging was done using 1.5 T MRI units (Siemens MAGNETOM Aera or Siemens MAGNETOM Avanto or PHILIPS, Ingenia).

A free open-source toolkit, ITK-SNAP, version 3.6.0 (www.itksnap.org) was used to extract the mean Apparent Diffusion Coefficients (ADC) values of the tumor region in the ADC map. After importing the ADC map of each patient into the toolkit, the tumor was delineated and the mean of ADC values was obtained. Figure 1 shows an example of tumor contouring in the ITK-SNAP toolkit based on T1 images.

Statistical Analysis

Different statistical methods could be used depending on the sample size and the type of independent and dependent variables to investigate the predictive effect of several patient characteristics. Since the sample size was very small compared to the number of independent variables, LASSO method was used, i.e. the number of variables is controlled by defining the penalty function on the sum of the absolute values of the model coefficients. Then coding was conducted using RStudio software (1.4.1103) with the Glmnet package [20] and LASSO model coefficients were obtained for the related variables; deviance of the model was also obtained. Finally, the Mann–Whitney U test using SPSS software (IBM Statistics 26) was applied to find the variables with a significant relationship with the response to treatment.

Results

Treatment was assessed, and the patients were divided into response and non-response groups. Ten patients were included in the response group, and eight did not respond to the treatment. Further, a complete response was not observed among the patients. The absolute expression levels of miR-222 and miR-205 in the plasma of patients were obtained based on the number of molecules per microliter of plasma, using real-time PCR and the standard curves, and the results are shown in Figure 2. The optimal lambda value and the coefficients of the LASSO regression are shown in Table 1. Mann-Whitney U non-parametric test was also applied to evaluate the significance of the relationship between dependent and independent variables as seen in Table 2.

Discussion

In this study, the relationship between the treatment response and several characteristics of patients before the treatment was investigated using LASSO regression. The characteristics studied for each patient include the expression level of miR-205 and miR-222 in plasma, the mean ADC value of tumor, age, and BMI. The LASSO coefficient for miR-205 with a positive value was larger than those of

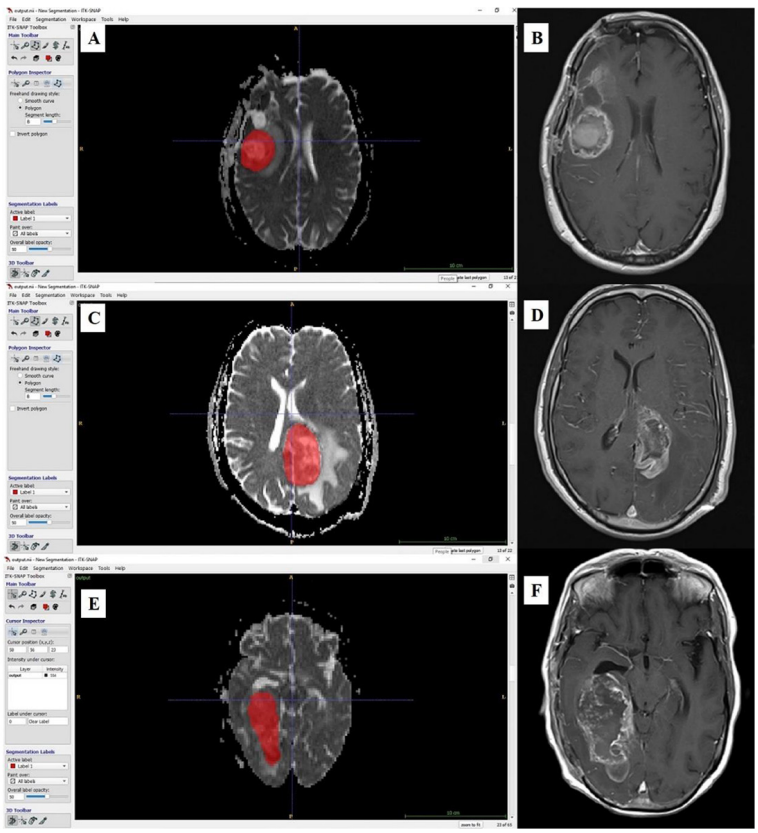

Figure 1: Tumor delineation in Apparent Diffusion Coefficients (ADC) map. In order to calculate the tumor mean ADC value in ADC map of each patient, tumor was delineated using ITK-SNAP toolkit (left images), based on T1 images (right images) and the mean ADC value was obtained.

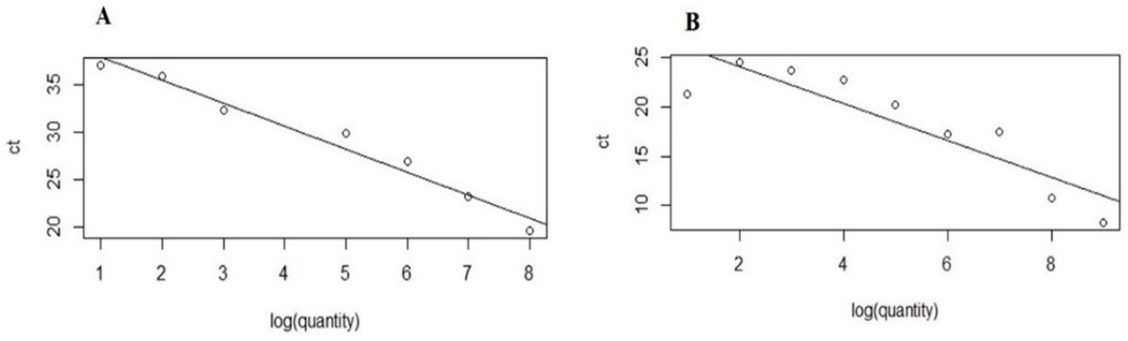

Figure 2: Standard curves of A) miR-205, B) miR-222. The log of each known concentration in the dilution series (x-axis) was plotted against the Ct value for that concentration (y-axis). From these standards, the absolute concentration of the miRNAs in the patient's samples was measured using their Ct values.

other variables, i.e., the expression level of this miRNA is directly related to the response to treatment. Vascular Endothelial Growth Factor (VEGF) is a direct target of miR-205, resulting in miR-205 acting as a glioma tumor suppressor [10]. Studies have shown that downregulation of miR-205 in tissue specimens, glioma cell line, and serum is associated with poor prognosis in patients with glioma [9,21-23]. On the other hand, the LASSO

Table 1: Results of Least Absolute Shrinkage and Selection Operator (LASSO) regression modeling for variables associated with treatment response

Variables	Lasso coefficients
miR-205	3.611
ADC	2.468
miR-222	-1.683
BMI	-0.184
age	-0.024
Intercept	4.656
Deviance (model)	11.988
lambda	0.039

ADC: Apparent Diffusion Coefficients, BMI: Body Mass Index

coefficient for miR-222 was negative, indicating that the survival of glioma patients decreases with increasing the expression of this miRNA. Some studies have acknowledged the role of miR-222 in increasing resistance to chemotherapy and radiation therapy [4,24,25]. This miRNA is known as an MGMT-regulating miRNA in glioblastoma that one of the miR-222 targets is the P53 Upregulated Modulator of Apoptosis (PUMA) gene, regulating cell death. Under normal circumstances, PUMA binds to BCL-2 and CVL-x, leading to apoptosis; however, increased miR-222 expression and downregulation of PUMA improve cell survival [26,27]. According to the results, the second feature associated with treatment outcome was the tumor ADC value that the LASSO coefficient of this feature was also positive, i.e., patients with a higher level of tumor ADC value are more likely to respond to the treatment. The amount of ADC decreases with increasing malignancy and the grade of disease [15] consistent with the results obtained in this study. The LASSO coefficients for BMI and age were -0.184 and -0.024, respectively, indicating that both are inversely related to the response to treatment, although age had a very weak relationship. LASSO regression modeling does not provide a *P*-value for the model and only provides model deviance. Here, the model deviance was 11.988 and the Null deviance was 24.73. Mann-Whitney test results were used to confirm the LASSO regression coefficients. For this purpose, *P*-value obtained for each variable in the Mann-Whitney test were compared with LASSO regression coefficients of the variables in terms of their correlation degree with treatment response. As shown in Tables 1 and 2, these two results are completely consistent. For example, the *P*-value for the miR-205 expression variable was the lowest and its LASSO coefficient was the largest. A limitation of this study

Table 2: Results of Mann-Whitney non-parametric test

Variables	Response	N	Mean	St. Error Mean	*P*-value
miR-205	Non-response	8	0.030	0.026	0.009
	response	10	0.200	0.097	
ADC	Non-response	8	0.387	0.130	0.043
	response	10	0.733	0.084	
miR-222	Non-response	8	0.291	0.136	0.068
	response	10	0.076	0.074	
BMI	Non-response	8	27.350	1.812	0.083
	response	10	23.920	0.968	
Age	Non-response	8	58.000	4.101	0.203
	response	10	48.000	4.705	

ADC: Apparent Diffusion Coefficients, BMI: Body Mass Index

is its small sample size, while regression models with larger sample sizes have more stable coefficients and a much higher validity. So, more study with a larger number of data is needed before clinical application.

Conclusion

The prognostic predictors for chemo-radiation treatment response of HGG patients were studied using the LASSO model and Mann-Whitney test. Based on the results, patients' miR-205 expression level in plasma and tumor's mean ADC value significantly correlated with treatment response.

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Authors' Contribution

M. Heidari, under the supervision of P. Shokrani, made a contribution in collecting patient information, performing experimental works, measuring data, and preparing the manuscript. Admission of patients to the study, their treatment, and their treatment evaluation were done by AR. Amouheidari and S. Hemati. AR. Amouheidari also collaborated in the tumor contouring of the patients. H. Khanahmad and I. Rahimmanesh provided guidance and participation in the genetic part of the study. P. Jafari had cooperation in statistical data analysis and coding for Lasso modeling. The final version of the manuscript has been approved by all authors.

Ethical Approval

This study protocol was approved by the human ethics board of Isfahan University of Medical Sciences, Isfahan, Iran (Ethical code: 1397.198).

Informed consent

Patients were included in the study after informed consent of them or their companion.

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Conflict of Interest

None

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