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ORIGINAL RESEARCH ARTICLE

The correlation between osteopontin level and radiation response of malignant gliomas at Cipto Mangunkusumo Hospital

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***Abstract*:** Osteopontin is an endogenous molecular marker for tumor hypoxia, and hypoxia is one of the factors that determine the aggressiveness of the disease. The purpose of this study is to determine the correlation between osteopontin levels and radiation response in malignant glioma. A retrospective cohort study was conducted on 15 malignant glioma patients who underwent radiation therapy from July 2004 to May 2015 at the RSUPN Dr. Cipto Mangunkusumo Hospital. Osteopontin levels were measured from paraffin-embedded tissue using a commercial ELISA kit. Tumor volume was calculated using computed tomography (CT) scan and magnetic resonance imaging (MRI) images, based on three-dimensional volume measurements. Tumor response was evaluated by comparing pre- and post-radiation tumor volumes using CT scan and MRI images. The mean osteopontin level was 0.49 ± 0.45 ng/mL and the mean percentage change in tumor volume was 8.59 ± 54.22%, with a 60% enlargement in tumor volume. A progressive disease was found in 26.7% of patients. There was a weak but insignificant negative correlation (r = –0.39, p = 0.146) between the level of osteopontin and radiation response. In contrast, there was a strong but insignificant positive correlation (r = +0.68, p = 0.219) between the level of osteopontin and radiation response in the patient group that used the chemosensitizer temozolamide.

***Keywords*:** osteopontin; malignant glioma; radiation response

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Introduction

Malignant glioma is a malignancy of the brain that most often occurs in adulthood, comprising approximately 78% of the total central nervous system malignancies in adults[1]. It is associated with high morbidity and mortality, and the current standard treatment involves the use of surgery, radiation, and chemotherapy with temozolomide[2]. An optimal therapy for glioblastoma results in a survival time of about 12–15 months, even though tumors often recur within 6.9 months[3]. It is known that hypoxia is one of the factors responsible for the failure of treatment in a variety of malignancies. Hypoxia affects malignant glioma tumor cells by activating the genes involved in tumor cell's adaptation to stress. Hypoxia is associated with aggressive tumor cell growth, metastasis, and poor response to treatment. Cancer cells will be 2–3 times more radioresistant in hypoxic conditions[4].

Osteopontin (OPN) is a phosphoglycoprotein and

glycosylated protein that is secreted into the extracellular matrix and causes tumor progression as well as perseverance of solid tumors. It is also one of the endogenous molecular markers for tumor hypoxia as the amounts of osteopontin in tumors are associated with tumor oxygenation. High osteopontin expression is found in various tumor malignancies such as malignant gliomas, hepatocellular carcinoma, lung cancer, stomach cancer, melanoma, and renal clear cell carcinoma[5-7]. Osteopontin is mainly found in microvascular tumor cells and plays an important role in angiogenesis. It is one of the genes that regulates hypoxia and promotes the ability of tumors to invade brain tissue by causing cell adhesion and migration of tumor cells[8]. The effect of osteopontin varies in different cell types, depending on the expression of integrins and signaling pathways that can be activated. Denhardt *et al.* showed the anti-apoptosis effects of osteopontin through interactions at cell receptors[9]. Increased expression of vascular endothelial growth factor, osteopontin, and integrin avβ3 are associated with angiogenesis in glioblastoma[10]. Osteopontin has the potential to be a therapeutic target and might also serve as a prognostic factor in assessing the results of treatments.

Materials and methods

Fifteen patients who were histopathologically diagnosed with malignant glioma from 2004 to 2015 at the Cipto Mangunkusumo Hospital were enrolled in this study. The diagnosis of malignant glioma is established based on anatomical pathology results, and consists of anaplastic astrocytoma, oligodendroglioma, and glioblastoma multiforme (grades III and IV). Patients underwent post-surgery radiotherapy at the hospital's Department of Radiotherapy and the response to treatment was evaluated by comparing the pre-and post-radiation tumor volumes that were measured using computed tomography (CT) scan and magnetic resonance imaging (MRI) images. The response to radiation therapy was assessed by measuring the changes in tumor volume using CT scan and MRI images of the head.

Measurements were taken at least two weeks post-surgery and two weeks prior to radiation (Volume 1). This volume was then compared to the measurements taken after radiation therapy (Volume 2). The imaging volume was then measured using the following formula: (sagittal × axial × coronal) longest perpendicular to each other and multiplied by 0.52 as the multiplier. The response to radiation therapy was measured using the following formula: (Volume 1 – Volume 2/Volume 1) × 100%. The sample characteristics of patients enrolled in the study are shown in **Table 1**. The measurement of osteopontin levels involved several procedures, starting with the deparaffinization of paraffin embedded tissue samples (30 μm) and a subsequent protein extraction for the enzyme-linked immunosorbent assay (ELISA) procedure[11]. The ELISA test for osteopontin detection was conducted using commercial kits from Cloud-Clone (SEA899Hu, Ucsn Life Science Inc., Wuhan, China).

Table 1. Sample characteristics of patients enrolled in this study

Characteristic	Malignant glioma (n = 15)	
	n	%
Gender		
Male	6	40.0
Female	9	60.0
Age (year)		
⩽50	10	66.7
>50	5	33.3
KPS (%)		
⩽70	6	40.0
>70	4	26.7
No data	5	33.3
Tumor grade		
Gr 3	4	26.7
Gr 4	11	73.3
Total dose (Gy)		
<60	4	26.7
⩾60	11	73.3
Dose per fraction(Gy)		
1.8 Gy	3	20.0
2 Gy	12	80.0
Temozolamide		
Yes	5	33.3
No	10	66.7
Follow up (mean)	11.33 (4–17) weeks	

Abbreviations: KPS = Karnofsky Performance Status; Gy = gray, unit of radiation dose, expressed as absorbed energy per unit mass of tissue

Ethics statement

This study was approved by the Ethics Committee of Medical Faculty, University of Indonesia.

Results

Table 1 shows the characteristics of patients enrolled in this study. Fifteen patients (six males and nine females) diagnosed with malignant glioma were included in the study. Apart from gender, the table also lists age (⩽50 or

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⩾50), Karnofsky Performance Status (KPS), tumor grade (III or IV), total dose, dose per fraction (Gy), and the use of temozolomide. The follow-up period ranged from 4 to 17 weeks. Meanwhile, **Table 2** shows the osteopontin concentration and tumor response measurements. The mean concentration of osteopontin was found to be 0.49 ± 0.45 ng/mL. The response to radiation therapy was analyzed by measuring the pre-and post-radiation tumor volumes using CT scan or MRI imaging. The mean tumor volume before and after radiation is 82 ± 50.48 mm^3 and 82.49 ± 82.84 mm^3, respectively, with a mean change in volume of 8.59% ± 54.22%. Up to 60% of patients who underwent radiation therapy experienced a reduction in tumor size, while 40% of patients experienced tumor enlargement (**Table 3**).

Table 2. Osteopontin concentration and tumor response measurements

Variable	*n* (15)
Osteopontin concentration (mean)	0.49 (range 0.29–2.09) ng/mL
The initial tumor volume (mean)	82.00 (range 36.42–212.14) mm^3
The final tumor volume (mean)	82.49 (range 7.21–336.66) mm^3
changes in tumor volume (mean)	8.59 (range 75.64–80.20)%

Table 3. Radiation response is based on the change (in percentage) in tumor volume and tumor progression

Radiation Response	*n* (15)
Changes in tumor volume	
Volume decreases	9 (60.0%)
Volume increases	6 (40.0%)
Tumor progression	
progressive	4 (26.7%)
not progressive	11 (73.3%)

According to the World Health Organization's criteria for solid tumor response evaluation, a progressive disease (PD) is characterized by an increase of 25% in tumor size on repeat imaging. Using this criterion as a benchmark, 26.7% patients were categorized as having a progressive disease, thus excluding the remaining 73.3% of patients. Variable factors that influence the success of radiation therapy is shown in **Table 4**. There is a strong but statistically insignificant positive correlation between radiation response and the prescription of temozolomide. The relationship between osteopontin levels of malignant glioma patients before and after radiation shows a weak negative correlation, with a correlation coefficient of −0.39 and a *p* value of 0.146. The

Table 4. Factors affecting radiation response

Variable	Radiation Response (*n* = 15)		*P*
	Volume decreases	Volume increases	
Gender			
Male	5	1	0.287
Female	4	5	
Age (year)			
⩽50	6	4	0.999
>50	3	2	
KPS (*n* = 10)(%)			
⩽70	4	2	0.524
>70	1	3	
No data (*n* = 5)			
Tumor grade			
Gr 3	3	1	0.462
Gf 4	6	5	
Total dose (Gy)			
<60	1	3	0.235
⩾60	8	3	
radiation techniques			
3dCRT	8	5	0.999
IMRT	1	1	
Temozolamide			
Yes	5	0	0.042
No	4	6	

Abbreviations: dCRT = definitive chemoradiation; IMRT = Intensity Modulated Radiotherapy

regression formula used to measure the radiation response is 31.74 to 47.22 × OPN value, as shown in **Figure 1**. An analysis on the use of temozolomide revealed that there was a weak but statistically insignificant negative correlation between radiation response and the group that was not prescribed temozolomide. The correlation coefficient was −0.38, with a *p* value of 0.284 (as shown in **Figure 2**). In contrast, a statistically insignificant but

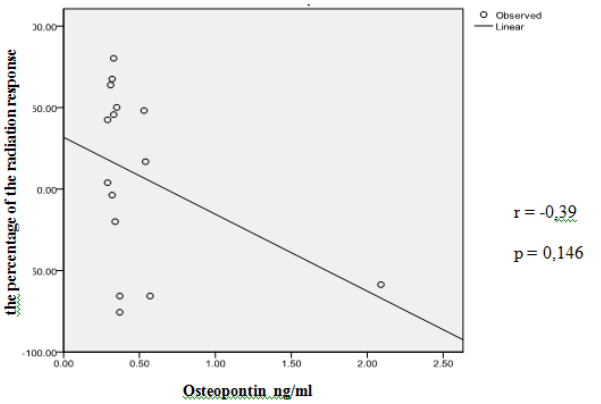

Figure 1. Correlation between osteopontin concentration and tumor response

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strong positive correlation was observed for the group that was prescribed temozolomide. The correlation coefficient is +0.68 with a *p* value of 0.219, as shown in **Figure 3**.

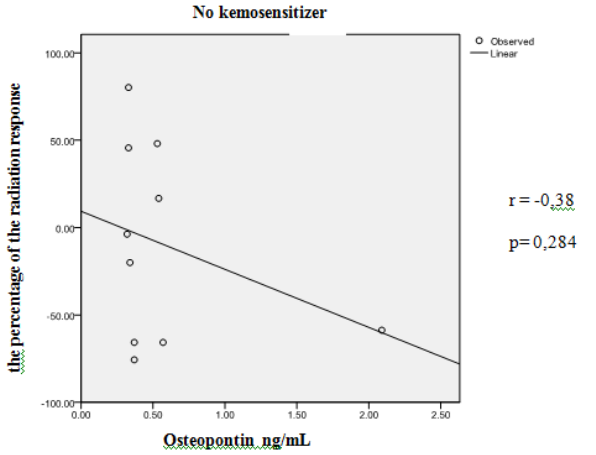

Figure 2. Correlation between osteopontin concentration and tumor response in patients who only received radiation treatment

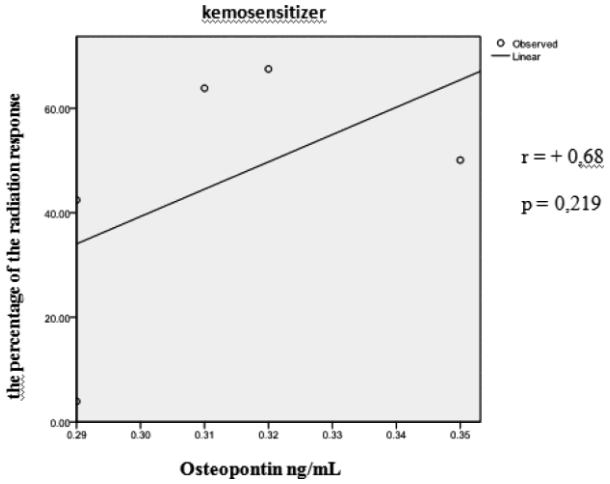

Figure 3. Correlation between osteopontin concentration and tumor response in patients that were treated with concurrent chemoradiation (TMZ)

Discussion

A commercial ELISA kit was used to quantify osteopontin concentrations obtained from malignant glioma tissues. The results in **Figure 1** show that there was a weak negative correlation between the levels of osteopontin and the radiation response. However, the negative relationship observed in the study was found to be statistically insignificant (r = −0.39 and p = 0.146). No statistical significance was found in this study owing to the small sample size. Patients were grouped based on the use of temozolomide. The group that did not receive temozolomide (**Figure 2**) had a weak negative correlation with the radiation response, but the correlation was not statistically significant. A negative correlation indicates that a higher level of osteopontin concentration results in a poorer radiation response or alternatively, the tumor volume increases after undergoing radiation (r = –0.38 and p = 0.28). Saitoh *et al.* have reported that the expression of osteopontin in human glioma cells may correlate with the malignancy grade of the gliomas[12]. This supports the results of the current study although both studies used different methods to quantify the osteopontin levels in tumor samples. A study conducted by Mirna *et al.* showed that the osteopontin level correlates negatively with a decrease in tumor size when patients with locally advanced rectal cancer were given neoadjuvant chemoradiation treatment[13]. In the patient group receiving temozolomide (**Figure 3**), a positive but statistically insignificant correlation was observed (r = +0.68 and p = 0.219). This is probably due to the small number of samples involved in this study. A strong positive correlation means that despite increased levels of osteopontin, the administration of temozolomide continues to increase the radiation response. It is possible that temozolomide has the ability to negate the effects of elevated levels of osteopontin. Existing preclinical data support the positive interaction between the administration of temozolomide and radiation[13]. Temozolomide has been shown to increase the effects of radiation on glioma tumor cells and inhibit their growth by inducing cell cycle arrest in the G2/M phase of the cell cycle, which is highly radiosensitive[14-16]. In general, temozolomide and radiation inhibit tumor cell invasion by inhibiting integrin and increasing the degree of radiation-induced double-stranded DNA breaks, resulting in tumor cell death; however, this mechanism occurs only when temozolomide is given concomitantly with radiotherapy[17-18]. There are two primary limitations to this study. Owing to the limited number of paraffin blocks available for assessment, the study was limited to a small sample size. Furthermore, it was difficult to assess the tumor response after radiation because of a variation in imaging modality before and after radiation, resulting in a less than ideal ratio of the tumor size.

Conclusion

A weak but insignificant negative correlation was observed between the levels of osteopontin and radiation response. There was also a strong but insignificant positive correlation between the levels of osteopontin and radiation response in patients who had been prescribed temozolomide. However, larger sample sizes must be used in future studies to conclusively prove that there is a correlation between the levels of osteopontin and radiation response.

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