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Relationship between phosphatase and tensin homolog (PTEN) expression in high grade glioma and histopathologic findings

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ABSTRACT

Introduction. Malignant glioma is resistant brain tumor to treatment and considered by aggressiveness that fails successful resection. Molecular pathways including PTEN related with tumor behavior.

Materials and methods. We studied the immunohistochemichal expression of PTEN in 32 brain surgical resection specimens with different histologic grades. All samples were from pathology department of Sina hospital in Tehran-Iran from 2020-2021.

Results. From 32 patients, 21 (65.6%) had no PTEN staining. There was significant relationship between high age and no PTEN expression and average age with no PTEN staining was 54.05±14.01 years (P value: 0.044). There was significant relationship between necrosis in histopathologic evaluation with no PTEN staining (P value: 0.016). Also, there was no significant relationship between sex, tumor's size, Tumor's site, tumor's grade, and primary or secondary type of tumor with PTEN staining (P value > 0.05).

Conclusion. PTEN expression related to histopathologic features such as necrosis and age. PTEN can be introduced as an important prognostic indicator in high grade.

Keywords: PTEN, glioma, prognosis

INTRODUCTION

Malignant glioma is the most frequent primary brain tumor in adults, however the prognosis for patients with these tumor is poor even with developments in diagnosis and standard therapies such as surgery, radiation therapy, and chemotherapy. Development in the treatment of glioma now be influenced by excessive degree on a better understanding of the biology of these tumors (1). The recent World Health Organization (WHO) guidelines categorize three malignancy grades of glioma base on histologic findings that predict patient survival. While glioblastoma multiforme (WHO grade 4 glioma) is related with a consistently weak outcome, survival differs noticeably between patients with low grade glioma (WHO grade 2) and high grade glioma (WHO grade 3 and 4) (2). Glioblastoma multiforme (GBM) is an extremely mortal brain tumor presenting as one of two subtypes of high grade glioma with distinctive molecular profiles. The primary GBM subtype presents by means of a high-grade disease and the secondary GBM subtype develops from the slow evolution of a low-grade disease (3). Tumorigenesis is a multistep happen dependent on a consecutive gathering of genetic and epigenetic mutations in the glial cells (4). Genomic profiling has demarcated GBM subgroups and recognized alterations in central signaling pathways including the RTK/RAS/PI3K/PTEN, P53/ARF/MDM2, and RB/

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CDKN2A pathways (5). PTEN (phosphatase and tensin homologue deleted on chromosome ten) is a tumor suppressor gene involved in an extensive range of human cancers, comprising glioblastoma. PTEN in the PI3K/Akt signaling pathway is a major negative regulator (6). PTEN role in the expansion of diverse biologic structures of malignant gliomas, such as loss of cell-cycle control and uninhibited cell proliferation, escape from apoptosis, brain invasion, and abnormal angiogenesis (7). Tumor resistance and immune escape could show vital characters in tumor development and pose difficulties for immunotherapy (8). Despite the status of PTEN in tumorogenesis in brain, a distinctive connection with survival is still restricted (9). We aimed to evaluated the PTEN status in different grade of glioma and its relationship with histopathologic and prognostic factors.

MATERIALS AND METHODS

Study population and setting

In the current study, we assigned 32 patients with high grade glioma which were obtained via surgical resection between 2020 and 2021 from electronic registry of Department of Pathology, Sina Hospital affiliated to Tehran University of Medical Sciences. Each patient must have had all of the following data recorded to be included: demographic information, tumor size, tumor site, tumor grade, primary or secondary type, presence or absence of necrosis, and formalin-fixed paraffin-embedded primary tumor samples for IHC analysis. This study was cross sectional. Cases with incomplete information or patients who underwent neoadjuvant therapy were excluded. Gliomas interpretation for the enormous majority of malignant brain tumors that are categorized according to histopathologic features into low and high grade. High grade gliomas description for 60–75% of all gliomas, and comprise World Health Organization (WHO) grade III anaplastic astrocytoma and grade IV glioblastoma multiforme (GBM) (10). Increasing malignancy of gliomas relates with a cellular proliferation and angiogenesis leading to inadequate blood supply, hypoxic regions and eventually to the creation of necrosis, a representative of glioblastoma (Figure 1,2) (11). Glioblastomas could progress de novo (primary glioblastomas) or over development from low grade astrocytomas (secondary glioblastomas) (12).

Immunohistochemical analysis

The Department of Pathology of Sina Hospital retains particular archival tumor blocks from each patient. Two expert pathologists assessed H&E-stained sections from these blocks to highlight areas of tumors, accurately.

IHC was performed in 3 μm formalin-fixed, paraffin-embedded sections to percentage the expression of PTEN. PTEN expression was investigated using a mouse monoclonal anti-PTEN antibody (1:50 dilutions, clone 6H2.1; Master Diagnóstica, Spain). Sections were cut from each block, deparaffinized with xylene, rehydrated in a series of decreasing concentration of ethanol solutions, and then antigen retrieval was achieved in Cell Conditioning Solution CC1 (pH 7.4, 95°C; Ventana Medical Systems) for 60 minutes, and lastly retrieval solutions were cooled for at least 30 minutes at room temperature (25°C). Then, in order to inhibit endogenous peroxidase, the slides were treated for 10 minutes with 3% H_2O_2 in methanol. Slides were incubated with monoclonal antibody for 120 minutes at room temperature and were labelled with the Envision Detection System from DAKO. Color reaction product was developed with 3,3'-diaminobenzidine, tet-

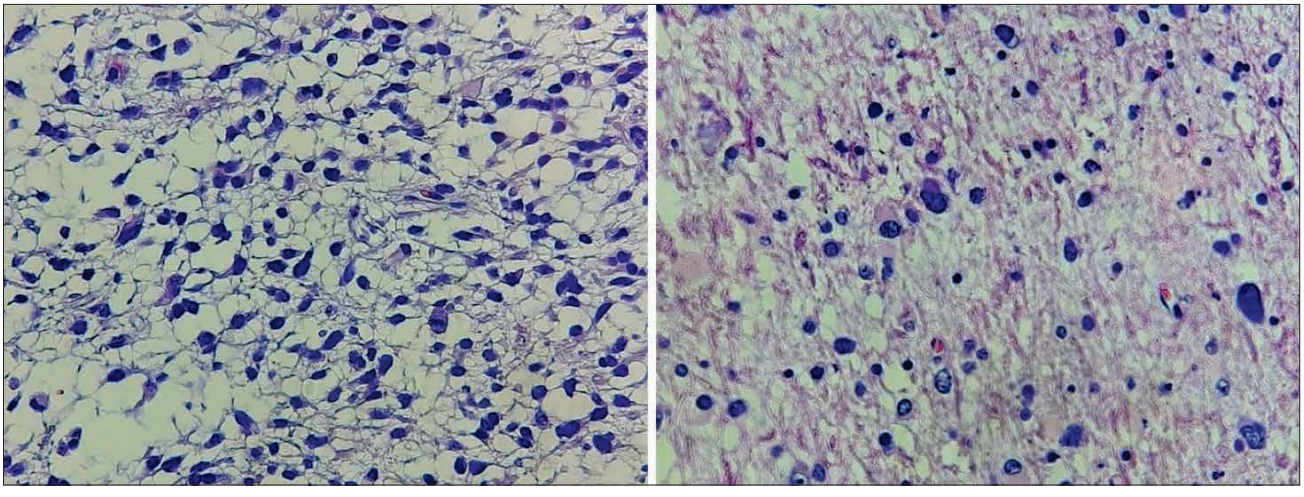

FIGURE 1. Histopathologic examination showed hypercellular neoplastic glial tissue with pleomorphism and atypical nuclei, compatible with anaplastic astrocytoma (WHO grade 3) (H&E, x200)

rahydrochloride (DAB plus; DAKO Glostrup, Denmark) as a substrate, and nuclear contrast was achieved with hematoxylin/ammoniacal water counterstaining. Formalin-fixed, paraffin-embedded sections from normal stroma of prostatic tissue were used as PTEN positive controls. Negative controls were performed by replacing the primary antibody with PBS/nonimmune mouse serum. The IHC reports were conducted using similar articles.

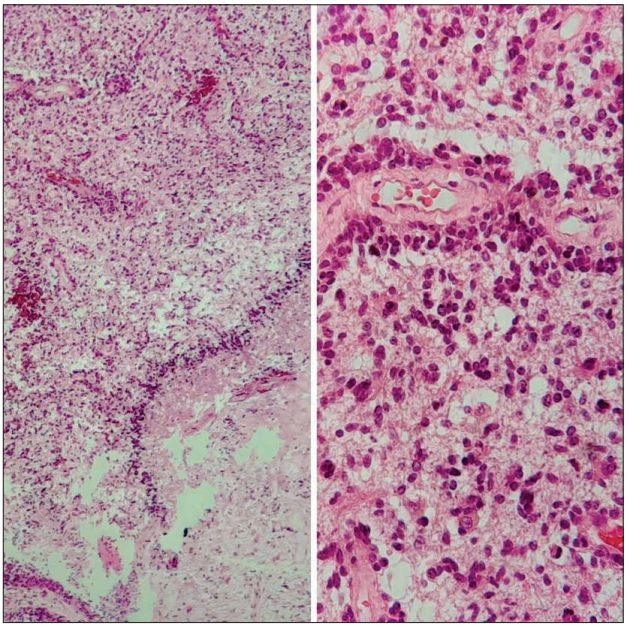

FIGURE 2. Histopathologic examination showed hypercellular neoplastic glial tissue with vascular proliferation, necrosis, and pleomorphism, compatible with glioblastoma multiforme (WHO grade 4) (H&E, x100 and x200, respectively)

The scoring system used for PTEN expression assessments was according to a semi-quantitative scoring method classifying the percentage of staining as follows: score of 0 designated no positive cells; score of 1 designated 1-33% of cells staining positive; score of 2 designated 34-66% of cells staining positive; score of 3 designated >66% of cells staining positive. Stains were reflected positive if a score of 2 or higher was given (13) (Figure 3).

Statistical analysis

For statistical analysis, results were presented as mean ± standard deviation (SD) for quantitative variables and were calculated by absolute frequencies and percentages for categorical variables. Quantitative variables were also compared with T-test or Mann-Whitney U test. The association between the variables was examined using the Pearson's or Spearman's correlation test. For the statistical analysis, the statistical software SPSS version 23 for windows (SPSS Inc., Chicago, IL) was used. P values of 0.05 or less were considered statistically significant.

RESULTS

The database query returned 32 patients with mean age of 50 years (22-75years) including 21(65.6%) male and 11(34.3%) female. Out of 32 high grade gliomas, 26(80.2%) was GBM and 6(18.8%) was anaplastic astrocytoma which out of from them, 29(90.6%) was primary and 3(9.4%) was secondary subtype. Necrosis presented in 25(78.1%)

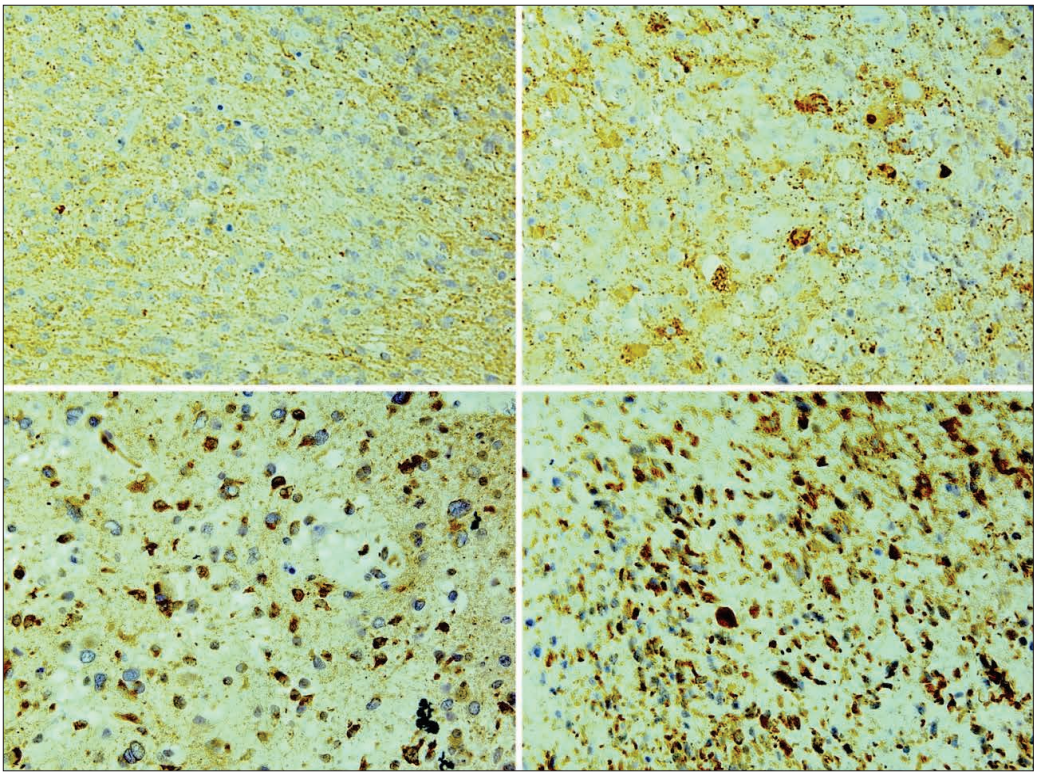

FIGURE 3. Immunohistochemical examination showed PTEN staining grading: grade 0(left upper), grade +1(right upper), grade +2(left lower), grade +3 (right lower)

of high grade gliomas. Average of tumor size according to received specimen for pathology assessment was 3.9±1.89 cm in greatest diameter. Tumor location in right temporal had higher occurrence than other lobes (31.2%). PTEN staining was 11(34.4 %) positive and 21(65.6%) negative (Table 1).

TABLE 1. Frequency of PTEN expression according to stain grading

PTEN staining	No staining	+1	+2	+3
Frequency	12(37.5%)	9(28.1%)	9(28.1%)	2(6.2%)

PTEN staining in 66.7% of male and 63.6% of female was negative. PTEN staining with sex showed no significant correlation (P value: 0.508). Patients' age with no PTEN staining was 54.5±14.1 years and with PTEN staining was 42.9±14.6 years which showed significant correlation between no PTEN staining with high age (P value: 0.044). From of GBMs, 73.1% and from of anaplastic astrocytomas, 33.3% had no PTEN stating which showed no significant correlation between lack of PTEN staining and tumor's grade (P value: 0.148). 69.0% of primary high grade glioma and 33.3% secondary high grade glioma had no PTEN staining which showed no significant correlation between lack of PTEN staining and primary or secondary subtype of glioma (P value: 0.266). There was significant relationship between presence of necrosis in tumor specimen on histopathology assessment with PTEN staining (P value: 0.016) (figure 1). Also, there was no significant relationship between tumor's site and size with PTEN staining (P value > 0.05).

DISCUSSION

The average age of patients who diagnose with primary high grade glioma is 55 years. GBMs may also originate from an existing astrocytoma that has undergone development to a higher grade. These secondary high grade gliomas are subsequently described by a elongated clinical progression and are established in a younger patient population (mean age: 40 years) (14). In our study, Average age of the patients was 54.05±15.00 years and 90.6% of tumors was primary.

Deletions of all or fragment of chromosome 10 are the most common genetic alterations in high-grade gliomas. The PTEN gene on chromosome region 10q23 and has been concerned as a target of modification in gliomas and also in other malignancies such as those of the breast, prostate, and kidney (15). PTEN has functions of a protein tyrosine phophatase which dephosphorylates both phophotyrosyl and phosphoseryl/threonyl remains. Expression of PTEN in glioma cells effects in growing suppression and inhibition of migration, dissemination and focal adhesion formation (16). The tumor suppressor PTEN effects the phosphoinositide 3-kinase pathway negatively for cell survival by dephosphorylating the phospholipid substrates phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate (17). Ermoian et al. study showed decreased PTEN expression is universal between high grade gliomas and could act a role in the progress of low grade gliomas. PTEN inactivation in gliomas indicates a predominantly aggressive clinical performance (18). Sano et al. study exhibited a

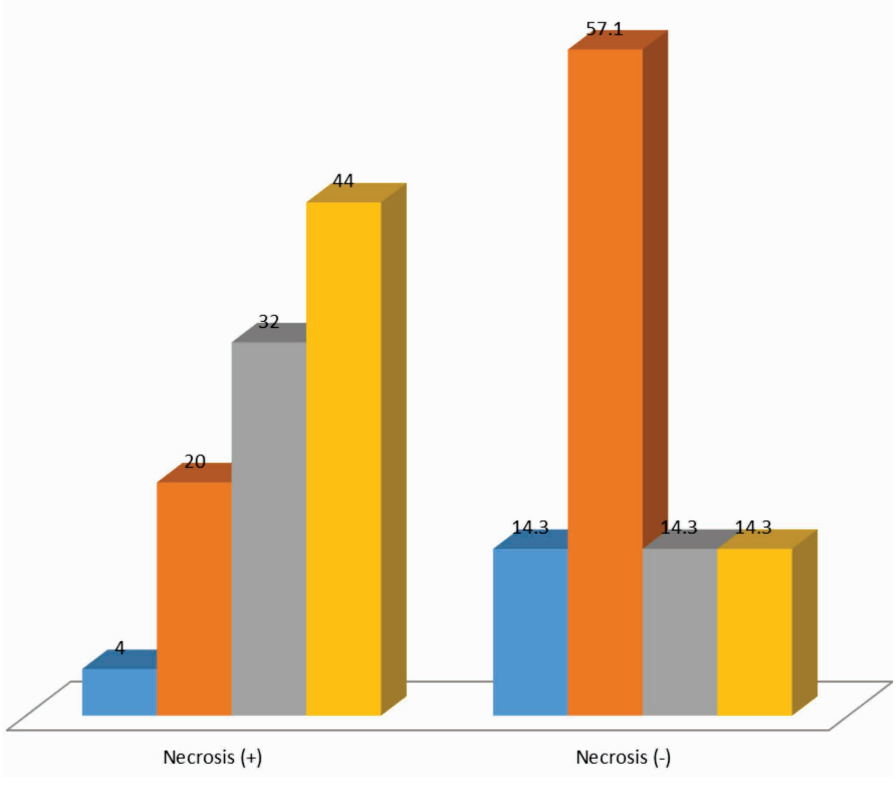

FIGURE 4. Relationship between presence or absence of necrosis with PTEN staining grading. There was correlation between presence of necrosis and no PTEN staining. (blue: +3, orange: +2, gray: +1, yellow: no staining)

meaningfully better outcome for patients whose tumors expressed high levels of PTEN (19). Recent studies recommend an investigative impact of PTEN gene alterations as a molecular indicator for poor prognosis in high grade glioma. Also, the probability of discriminating targeting of PTEN mutant tumor cells by definite pharmacologic inhibitors of members of the PTEN/PI3K/Akt pathway releases new views for a targeted molecular therapy of malignant gliomas (7). PTEN mutations were established in a higher proportion of high-grade tumors, the relationship between tumor grade and mutation would be important (20), but in our study no significant relationship between tumor grade and PTEN mutation was seen. Multivariate analyses have constantly recognized age, tumor grade and histopathology features, and amount of surgical resection are amongst the most important predictive factors influencing survival (21). Our study established age and necrosis in histopathology assessment of resected specimen related to PTEN mutation and prognosis. Moreover, in some studies, several factors including tumor grade, age, and sex, were not identified as prognostic factors for the survival of patients with gliomas. Some authors believed that an important link could be discovered if the sample size was greater than the sample in previous study, since the PTEN mutations were only found (22). Rasheed et al. study showed there was no noticeable connection between occurrence of PTEN mutation and survival; but, there was a trend for PTEN mutations to happen in older age patients (23), which was similar to our study. Between the histological items of GBM evaluation, necrosis has been established to be a dominant predictor of poor patient prognosis which are due to loss of phosphatase and tensin homolog (PTEN), and ionotropic glutamate receptor activity lead to AKT pathway activation, nutrient overconsumption and necrosis (24). In our study, necrosis had significant connection with PTEN mutation and as result, PTEN could be used as prognostic factor. Also, Rong et al. results show that PTEN loss and hypoxia up-regulate tissue factor expression and stimulate plasma clotting by glioma cells, proposing that these mechanisms may cause thrombosis and necrosis in GBM (25). PTEN loss is reason of move to an enhanced speed of clinical advancement subsequent from hypoxia induced neoangiogenesis. So, necrosis and angiogenesis are indications of poor prognosis (26). Abounader et al. informed that the loss of PTEN discovered in high-grade gliomas may meaningfully contribute to their malignancy (27). Also, increased glycolytic energy production in PTEN loss glioma is detected may support to describe the aggressive behavior (28). Tumor markers that predict survival and treatment response are being identified with confidence from developing array molecular technologies (29). The high grade gliomas display intrinsic resistance to most medical treatments, backing to their poor prognosis (30). Once all of these molecular analyzes, including the screens for PTEN mutations, convert clinically useful in terms of cost and efficacy, genetic testing should turn into a routine section of high grade glioma assessment (31). Our results indicated that presence of PTEN mutations play a prognostic role in Iranian patients. However, supplementary studies are required to clinically confirm this signature already its use as a routine investigative method.

CONCLUSIONS

PTEN has role in regulation of normal and malignant progenitor cell differentiation, self-renewal and tumorigenic potential. Recent data designates that PTEN mutation is accompanying by poor prognosis in high grade glioma patients and could be as prognostic factor. Nevertheless, this result is causing from information in limited studies, possibly issue to selection bias, and therefore well showed, high-quality randomized controlled trials are necessary.

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