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Investigation of Nestin protein expression in Iraqi patients with high-grade Glioma

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Abstract

Gliomas are tumors of the brain parenchyma that are classified histologically based on their resemblance to different types of glial cells. In Iraq, as in many countries, gliomas pose a significant health challenge, the spread of glioma disease refers to its incidence and prevalence within the population progressively. **Material and methods:** Fixed paraffin-embedded Glioma brain tumors of 64 samples, thirty-four as patients (21 male and 13female) and thirteen(benign) as control (10 male and 20 female) were collected from the Neurological Hospital in Baghdad, samples date from December 2022 to July 2023. Diagnosis and grading of Glioma (high grade) in the study were done by immunohistochemistry technique by Nestin marker to investigate its expression in samples through a scoring system where it was, 0 negative, 1 light, 2 moderate, and 3 strong. **Results:** The result showed non-significant P≤0.01 between male and female inpatient p-value (0.277), with mean (3.29±0.56, 3.69±0.13, respectively), noticed a high significant P≤0.01 between patient and control p-value (0.0001) in both sex, it also showed highly significant difference P≤0.01 in the percentage of patients distribution according to scores, and the highest percentage for male and female was at score 2 and 3, in percentages for male (42.86) and female (30.77, 69.23, respectively). **Conclusion**: The results confirm that Nestin is associated with high-grade Glioma.

Keywords: Cancer, Glioma, Nestin, Immunohistochemistry.

Introduction

Iraq's cancer incidence varied from 1999 to 2007 but then started to rise significantly thereafter [1]. The ability of cancer cells to spread from a primary lesion to distal organs is the main cause of mortality for cancer patients. A variety of biological mechanisms aid in the spread of cells from primary tumors [2]. varying tumor cells have varying rates of tumor cell proliferation and tumor growth maintenance [3]. Tumor cells that lose their capacity to multiply, self-renew, and develop into tumor cells provide the tumor's phenotypic marker [4,5]. Cancer cells can take on new characteristics that facilitate their motility, metastasis, and reproduction. To

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control the differentiation of cancer cells, the tumor environment is essential [6].

Gliomas are tumors of the brain's supporting tissue that start in the glial tissue, which provides support for the brain's nerve cells [7]. Gliomas, or brain parenchymal tumors, are categorized histologically according to how closely they resemble certain glial cell types. The three primary forms of glial tumors are ependymomas, oligodendrogliomas, and astrocytomas [8].

Glioma is categorized into four classes by the World Health Organization (WHO) according to the severity of malignancy. Grades I–II gliomas are referred to as low-grade gliomas (LGGs), whilst grades III–IV gliomas are referred to as high-grade gliomas (HGGs). The precise categorization of HGGs and LGGs before malignant transformation plays a crucial role in treatment planning [9].

The etiology of glioma and other brain cancers is yet unclear. However, other risk factors have been found, including advanced age, ionizing radiation exposure (such as radiation treatment), and glioma in the family history [10]. Although 25 risk factor loci and a number of uncommon hereditary mutations have been found, only ionizing radiation has been shown to cause glioma in certain families [11].

GBM is regarded as hypercellular anaplastic lung cancer. High mitotic activity glioma cells frequently experience necrosis and microvascular proliferation [12]. The differentiation between primary and secondary GBM, which appear rapidly and without any indication of less malignant precursor lesions, aids in clinical and biological studies. This subtype, which makes up 80% of GBM cases, typically affects older individuals (mean age of 62 years). Secondary tumors from low-grade (WHO grade II) or anaplastic (WHO grade III) astrocytomas grow more slowly in these patients. This subtype is more common in individuals with a mean age of 45 years or younger [13].

Since its discovery in 1985, the biological function of Nestin—which has a molecular weight of 177.4 Kd and 1621 amino acids—has been thoroughly investigated. Nestin was first shown to be involved in cytoskeletal structure and to be a marker for neural stem/progenitor cells. Nestin's presence is necessary for NSCs to self-renew [14]. Nestin has recently been found as a protein that is needed throughout the body, challenging the notion that it is limited to tissue regeneration areas. It is concentrated in endothelial cells throughout all adult vascular beds [15,16]. Nestin expression is a crucial indicator of continuing angiogenesis and CSCs in tumors since it is present in both cancer cells and newly developing tumor vasculature [17]. In some cancers, nestin is linked to metastasis and rapid growth. Because Nestin regulates proliferation, invasion, and stomata formation, it may be a suitable molecular target treatment for glioblastoma cells [18]. Numerous studies have demonstrated that reducing Nestin activity suppresses the growth, invasion, and movement of Glioblastoma cells [19]. Immunochemistry is the study of antibodies, antigens, and the interactions between them. It looks at the molecular processes that underpin immune system activity. The application of this method in the diagnosis and prognosis of illnesses and cancers is made possible by the creation of locus-specific monoclonal antibodies [20], Unlike many other laboratory techniques, IHC allows one to assess a molecule's expression pattern in a microenvironment without damaging the histologic architecture [21].

Materials and Method

A total of sixty-four paraffin-embedded tissue blocks, thirty-four high-grade Gliomas (13 female and 21 male) in mean age (44.94±2.99), with their data (gender, age, grade of Glioma), and, thirty samples (benign) were taken as control groups (20 female and 10 male), the average age of all sampler is(13-70) year, all data were collected from Histology Laboratory related to the Neurological Hospital in Baghdad, the sampling date was from December 2022 to July 2023. The research was approved by the ethical committee. One section of the paraffin-embedded Glioma tissue blocks was

stained with Hematoxylin and eosin-stained (H&E) and studied the histopathological diagnosis, whereas an additional section was stained with IHC by Nestin monoclonal antibody (my bio sours company).

The tumors were characterized histologically using hematoxylin and eosin-stained sections to examine cell density, mitosis, vascular proliferation, and necrotic tissue. The use of the IHC technique on other paraffin sections was used to diagnose glioma using this advised technique.

Immunohistochemistry staining protocol

The immunohistochemistry (IHC) was carried out according to [22], and My BioSource protocol instructions.

Step-1: De-waxing and rehydration the slides were placed in an oven at (62°C) to remove the excess wax from the slices. The tissue was subsequently de-waxed by immersing slides in xylene (3-5 minutes) and rehydrated by soaking in decreasing ethanol concentrations (90 percent ethanol for 5 minutes, 70 percent ethanol for 5-3 minutes, 50 percent ethanol for 5 minutes, succeeded by a 5-minute rush in distilled water (DW).

Step 2: Endogenous Hydrogen peroxidase and protein blocking the slides were treated for 10 minutes and then for 5 minutes with a ready-tousle hydrogen peroxide solution to inhibit endogenous peroxidase. Following that, a protein block was applied to the tissue slices to block the non-specific protein and incubated for 10 minutes before being rinsed twice in PBS for 5 minutes

Step-3: Incubation of Primary Antibody The primary antibody Nestin (ab 176571) was applied to the section and left for 1 hour for the above-mentioned markers after being diluted 1:100 (10 µl from Ab+990 µl PBS). The slides were then washed three times with PBS for ten minutes before being incubated. The primary antibody was extracted, and the slides were washed in PBS for 5 minutes for all antibodies.

Step-4: Incubation of Secondary Antibody After washing the slides to remove the main antibodies. After 20 minutes of incubation at 25°C, the second antibodies were applied to the slides, which were then rinsed four times with PBS. The sections were then treated with Goat Anti-rabbit Horseradish Peroxidase (HRP)-Conjugate for 10-20 minutes before being rinsed with PBS twice for 5 minutes each.

Step-5: Visualizing by the Diaminobenzidine (DAB) Color metric reaction detection using peroxidase substrate was accomplished. The DAB reagents from the DAB kit were made immediately before use by mixing (1:50), one drop of chromogen for every 49 drops of particular chromogen diluent (DAB substrate). The slides were then incubated for 5-10 minutes, or until the brown hue appeared, before being washed twice in PBS for five minutes each.

Step-6: Counterstaining, dehydration, clearing, and mounting of slides were accomplished by counterstaining for 10 seconds in Hematoxylin to stain nuclei, followed by a one-minute wash with running water. The slides were dehydrated by dripping in increasing ethanol concentrations (30% ethanol for 30 secs, 95% ethanol for 30 secs, and 100% ethanol for 30 secs) and then cleaned by soaking in xylene (2 min). The super mount was added at that time. Then, place a cover slide over the slides and gently press to remove any bubbles; leave the slides overnight before examining.

The cytoplasm was the dominant location of the stain, with the intensity of the stain and the different intensity percentages of positive tumor cells being assessed blindly by examining histopathology.

The intensity of the stain was measured numerically by a two-headed microscope at 400x magnification, with the lowest score being negative, the highest being light, the second being moderate, and the third being intense.

Scoring system

Nestin expression appeared as brownish cytoplasmic. This study utilized only cytoplasmic staining, and two investigators used a two-headed microscope at 400x magnification to estimate the intensity of stain and the different intensity percentages of positive tumor cells without any prior

knowledge Numerical score is applied to NESTIN scoring as a scale from 0–3, with 0 = negative, 1 = light, 2 = moderate, and 3 = intense, and it is calculated by intensity percentages of positive tumor cells to the surface area covered [23] the score for the severity of the nesting reaction was determined by histopathology.

Statistical analyses

To detect the different effects in the groups (patient and control) a program of statistical packages of social science SPSS (2019) was used. For comparing the means significantly LSD_ least significant difference and T-test were used while the Chi-square test was used for the comparison of percentage (0.05 and 0.01) probability

$$\chi^2 = \Sigma \frac{(O - E)^2}{E}$$

χ^2: Chi-square, Σ: Summation, O: Observed No., E: Expected No. [24].

Results

Immunohistochemistry expression of NESTIN in control and patient groups with gender

Table (1) and figure (1)showed a high significant P≤0.01 between male patients and control with a mean (3.29 ± 0.56, 1.00±0.00, respectively) at p-value (0.0001) so in females (3.96±0.13, 1.00±0.00, respectively), The result showed non-significant P≤0.01 between male and female in patient at p-value (0.277) with mean (3.29±0.56, 3.69±0.13, respectively).

Distribution of sample study according to NESTIN Score of patients/Gender

Table (2) and figure (2) revealed a high significant p ≤ 0.01 in the proportion of distribution of patient samples according to the degree of scores in both males and females, the highest percentage was for males at scores 2,3, which was (42.86), and for females the highest percentage also in scores 2,3 which was (30.77, 69.23, respectively). We note from the same table that at score 0 is no interaction of Nestin in both sexes. Figure (3, 4, 5) which shows the immunological reaction of Nestin, which was mild in score (1), moderate in score (2), and strong in score (3), the cytoplasm appears brown depending on the degree of the disease.

Table (1): IHC expression of NESTIN in control and patient groups with gender.

Gender	Mean ±SE		P-value
	Control	Patients	
Male	1.00 ±0.00	3.29 ±0.56	0.0001 **
Female	1.00 ±0.00	3.69 ±0.13	0.0001 **
P-value	1.00 NS	0.277 NS	---
** (P≤0.01), NS: Non-Significant.			

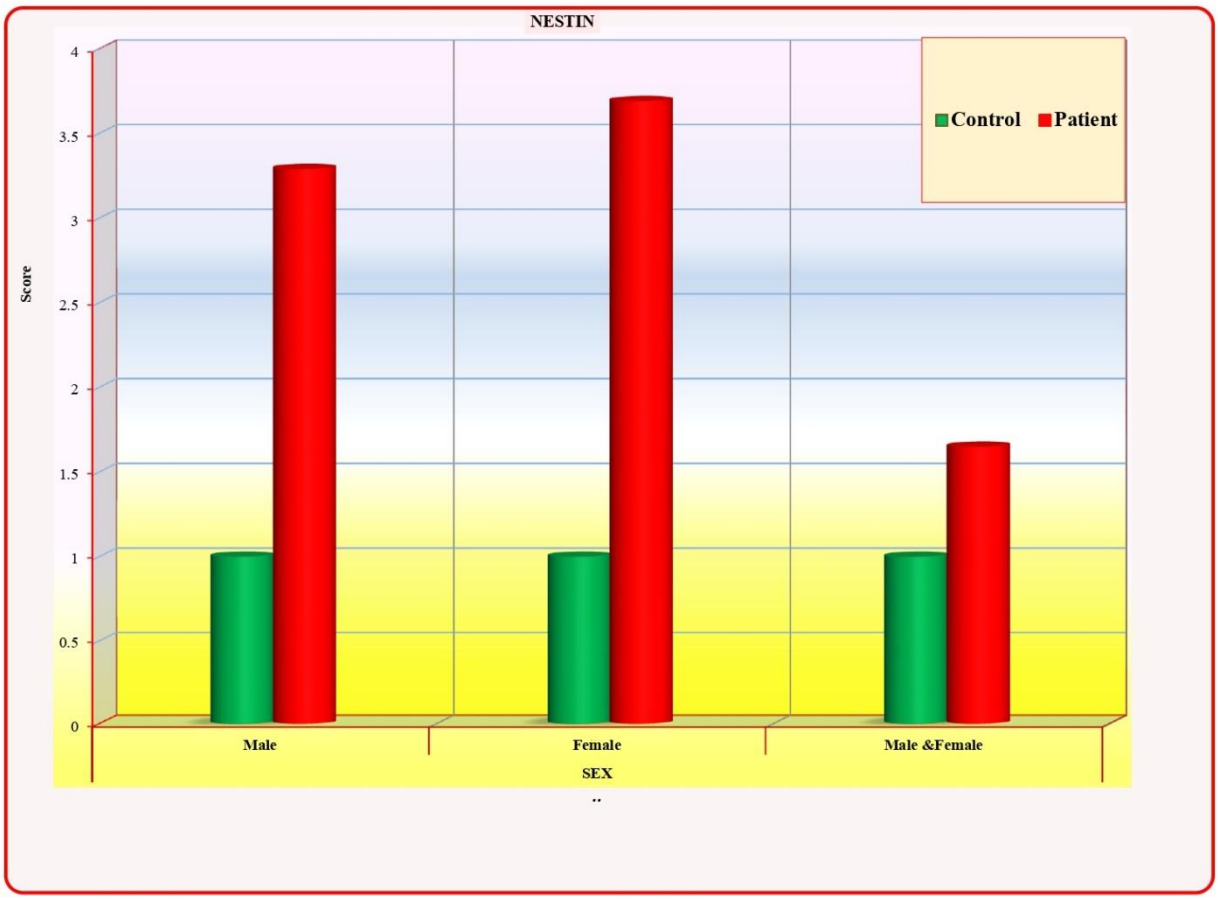

Figure (1): IHC expression of NESTIN in control and patient groups with gender.

Table (2): Distribution of sample study according to NESTIN Score of patients / Gender.

NISTIN Score	**Male Total no =21**		**Female Total no =13**	
	No	%	No	%
0	0	0.00	0	0.00
1	3	14.29	0	0.00
2	9	42.86	4	30.77
3	9	42.86	9	69.23
P-value	--	0.0037 **	--	0.0061 **
** ($P \leq 0.01$).				

Figure (2): Expression score of Nestin in high-grade Glioma according to male and female.

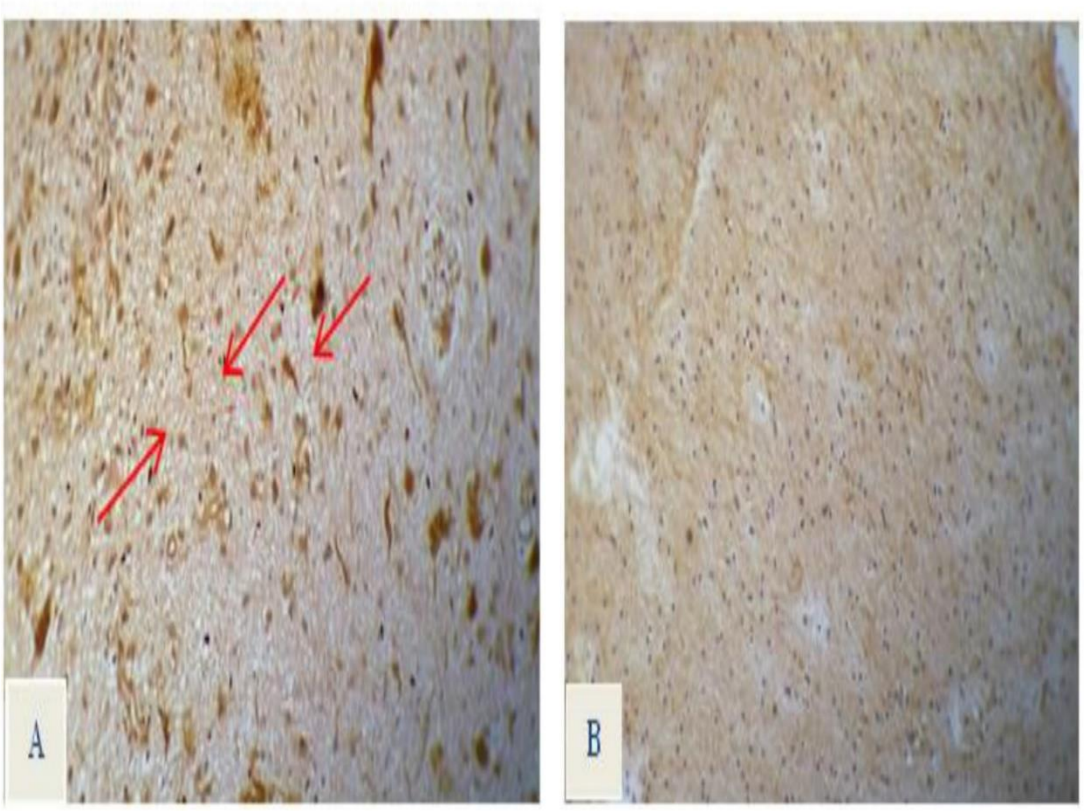

Figure (3): A and B: cross-section of the brain of Glioma (grade IV) patient with Nestin by IHC, showing brown cytoplasm stain in mild intensity (10X), score 1, red arrows.

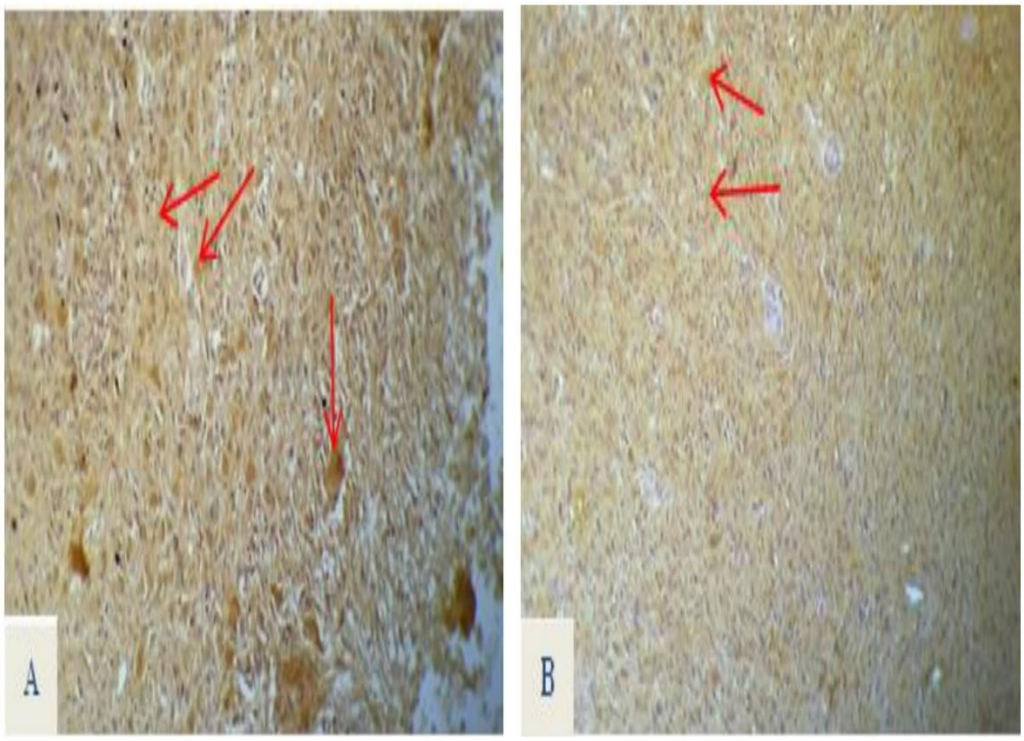

Figure (4): A and B: cross-section in the brain of Glioma (grade IV) patient with nestin by IHC, showing brown cytoplasm stain in moderate intensity (10X), score2, red arrows.

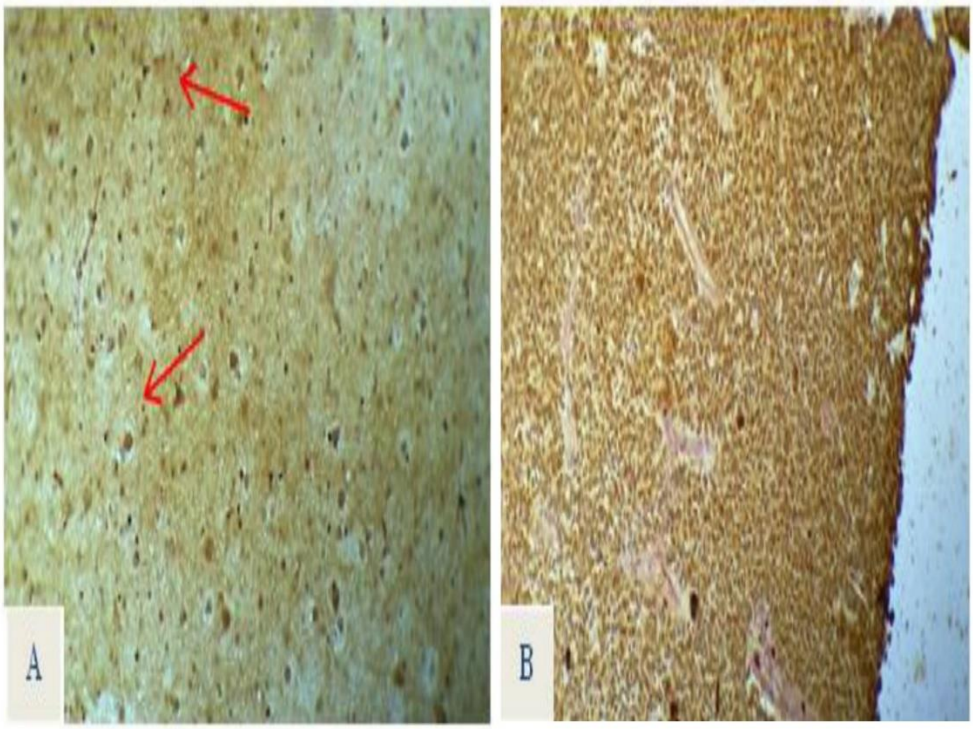

Figure (5): A and B: cross-section of the brain of Glioma (grade IV) patient with Nestin by IHC, showing brown cytoplasm stain in strong intensity (10X), score3, red arrows.

Discussion

It is evident from the data that brain tissue unaffected by glioma expresses less Nestin, which is congruent with [25] investigation in which he found that normal brain tissues have incredibly low levels of Nestin expression. As seen in Figures (1, 2, 3) which depict the immunological reaction of Nestin, which was mild in score (1), moderate in score (2), and strong in score (3), the expression of Nestin will be high due to its interaction with the antigen in patient samples. The cytoplasm appears brown depending on the severity of the disease. Some research has indicated that a higher grade and a worsening prognosis in Glioma are linked to Nestin expression. In 2007 [26], In agreement with the current study's findings, it was demonstrated that Nestin expression is a robust predictor of the prognosis for high-grade gliomas. It was expressed in 95.8% of 87 patients, indicating a large increase in low-grade tumors. Given the strong positive association between nestin expression and a poor prognosis, nestin has been validated as a useful marker for the diagnosis of high-grade Glioma. This has been proven by [27]; also, it was found by [28] Our recent investigation has shown the correlation between higher-grade gliomas and greater Nestin expression. Moreover, the discovery of [29] Our findings are in line with research that consistently revealed high-intensity Nestin expression in high-grade gliomas, making it a useful diagnostic marker. One theory explaining the association between nestin and high-grade glioma is that nestin is normally expressed in neural stem cells (NSCs), and progenitor cells may contain neural stem cell-like cells that can promote tumor growth, invasion, and treatment resistance, on the other side [30] declared that in 88 cases examined by immunohistochemical method, nestin was not a predictor of glioblastoma multiform; however, this is not in agreement with our findings. The table displays that the average value for females (3.69 ± 0.13) was greater than the average value for males (3.29 ± 0.56). Our study's findings were in agreement with the findings of [31] which demonstrates significant sex-based differences in clinical and radiological tumor parameters of Glioblastoma, also the result of the statistical analysis of our study is consistent with [25] which found that, like in our study, there are non-significant differences ($p \leq 0.01$ between males and females, p-value = 0.277) between the expression levels of Nestin in males and females (46.67 ± 28.13, 26.70 ± 24.53, respectively). However, the statistical analysis of the result revealed no difference between males and females regarding this aspect. Lastly, our research revealed no connection between patient sex and Nestin expression, which is consistent with the [28] study, while [32] discovered variations in the incidence and prognosis of gliomas in males and females, which may be tangentially related to variations in biological markers such as Nestin. Additionally, the investigation of [33] showed the impact of sex by examining gender-specific changes in glioma risk factors and survival, and by speculating on possible differences in tumor biology that may affect markers like Nestin.

In light of the paucity of direct data about the association between gender and Nestin expression in Gliomas, more research that is tailored to examining possible gender-based correlations or differences must be carried out. In light of this, while the literature currently in publication emphasizes the importance of Nestin expression in Glioma prognosis and clinic pathological features, more research focusing on gender-specific analyses is necessary to clarify how Nestin expression may differ between male and female Glioma patients. This could lead to more individualized treatment approaches [34], which agrees with what our study has shown, given that nestin is a well-known marker of neural stem and progenitor cells in the developing nervous system, its substantial correlation with high-grade glioma is likely caused by its expression in neural stem cells (NSCs) and its role in the aggressive character of these tumors. In gliomas, especially high-grade ones (glioblastoma multiforme, GBM), there's proof that stem-like cells

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in the tumor express nestin. These cells may have a role in the development, spread, and resistance to treatment of tumors [35].

Increased expression of Nestin is associated with poor prognostic parameters, tumor progression, and an increase in grade in Glioma. Nestin is a useful marker for identifying cancer stem cells (CSC) in high-grade Glioma, which are resistant to chemotherapy and radiation and can predict patient outcomes [36], their results are in agreement with those formerly stated by [24] found in their study that Nestin was expressed in 95.8% of 87 patients which was significantly higher in high- than in low-grade tumors.

[37] who demonstrated a correlation between Nestin and tumor grade in 72 cases, [38] tested 33 glioblastomas from 65 different brain tumor cases and they were found positive for Nestin, our study also found a link between Nestin and higher tumor rates.

In contrast; a prior study by [39] suggested that the use of a rabbit polyclonal anti-human nestin antibody in their study, rather than our more specific monoclonal antibody, may have contributed to their findings. showed that Nestin expression was observed in both low- and high-grade Glioma and did not relate with the grade of tumor in 16 cases of brain tumors. This is not consistent with the finding of our study, which showed that Nestin expression is related to high degrees of the disease [40], However, not in line with our research.

Conclusion

Nestin expression appears in high-grade Glioma, as its expression is associated with high grades of Glioma and may be a potential indicator of aggressiveness. From the findings of the research, it became clear that there is no significant relationship between Nestin expression and gender, Nestin is a useful marker for the diagnosis of high-grade gliomas, and Increased Nestin expression is correlated with tumor progression, and increasing grade.

Declaration of Conflicting Interests

The authors declare that they have no possible conflicts of interest.

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Ethical approval

The project received approval from the local ethical commission at the University of Baghdad, Baghdad, Iraq.

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References

1. Cizkova, D., Zurmanova, J. M., Gerykova, L., Kouvelas, A., Heles, M., Elsnicova, B., ... & Mokry, J. (2024). Nestin expression in intact and hypertrophic myocardium of spontaneously hypertensive rats during aging. Journal of Muscle Research and Cell Motility, 45(2), 41-51.
2. Mohsin RN, Mohamad BJ. Investigation of CD73 expression in Iraqi patient women with breast tumors. J Popul Therap Clin Pharmacol. 2023; 30(3): 240-257. https://doi.org/10.47750/jptcp.2023.30.03.026.
3. Yan X, Ma L, Yi D, Yoon JG, Diercks A, Foltz G, Tian Q. A CD133-related gene expression signature identifies an aggressive glioblastoma subtype with excessive mutations. Proc Nat Acad Sci. 2011; 2011; 108(4): 1591-1596. https://doi.org/10.1073/pnas.1018696108.
4. Alkhafaji KR, Al-Khateeb HM, Saadoon ZZ. Virulence estimation by calculation of relative expression of NESTIN in different grades of astrocytoma among Iraqi patients. J Fac Med. 2020; 61(3,4). https://doi.org/10.32007/jfacmedbagdad.613,41701.

5. Abdel Al Shakour, M., Elzayat, E., Mahmoud, K., Nassar, M., Abdel-Hamid, A. H. Biomolecular evaluation of apoptosis, cell cycle, oxidative stress, and limiting enzymes of the glycolytic pathway in hepatocellular carcinoma cell line HepG2 treated with crude snake venom with or without sorafenib. *Journal of Bioscience and Applied Research*, 2023; 9(3): 115-137. doi: 10.21608/jbaar.2023.315676

6. Abd-El-Raouf R, Ouf SA, Haggag MG, El-Yasergy KF, Zakaria MM. Mesenchymal and stemness transdifferentiation via *in-vitro* infection of T24 cell line with *Klebsiella pneumoniae*. Baghdad Sci J. 2023; 20(3): 0797-0797. https://doi.org/10.21123/bsj.2022.6826.

7. Kum Özşengezer, S., Altun, Z. S., Sanlav, G., Baran, B., Kızmazoğlu, D., Aktaş, S., ... & Olgun, N. (2024). Investigation of YAP-1, OTX-2, and nestin protein expressions in neuroblastoma: a preliminary study. Annals of Clinical and Translational Neurology.

8. Alabassi HM. Assessment of ZYXIN and E-cadherin tumour marker in Iraqi patients with glioma lesion of the brain. Biochem Cell Arch. 2019; 19(2): 4379-4383. https://doi.org/10.35124/bca.2019.19.2.4379.

9. Özcan H, Emiroğlu BG, Sabuncuoğlu H, Özdoğan S, Soyer A, Saygı T. A comparative study for glioma classification using deep convolutional neural networks. Math Biosci Eng. 2021; 18(2):1550-1572. https://doi.org/10.3934/mbe.2021080.

10. Wang, G., Wang, W., Zhang, Y., Gou, X., Zhang, Q., Huang, Y., ... & Li, Y. (2024). Ethanol changes Nestin-promoter induced neural stem cells to disturb newborn dendritic spine remodeling in the hippocampus of mice. Neural Regeneration Research, 19(2), 416-424.

11. Molinaro AM, Taylor JW, Wiencke JK, Wrensch MR. Genetic and molecular epidemiology of adult diffuse glioma. Nat Rev Neurol. 2019; 15(7): 405-417. https://doi.org/10.1038/s41582-019-0220-2.

12. Hasan, A. F., Jasim, N. A., Abid, A. T., & Tousson, E. (2024). Role of Salvia hispanica seeds extract on Ehrlich ascites model induced liver damage in female mice. Journal of Bioscience and Applied Research, 10(2), 161-169.

13. Thakkar JP, Dolecek TA, Horbinski C, Ostrom QT, Lightner DD, Barnholtz-Sloan JS, Villano JL. Epidemiologic and molecular prognostic review of glioblastoma. Cancer Epidemiol Biomark Prev. 2014; 23(10): 1985-1996. https://doi.org/10.1158/1055-9965.epi-14-0275.

14. Park D, Xiang AP, Mao FF, Zhang L, Di CG, Liu XM, Shao Y, Ma BF, Lee JH, Ha KS, Walton N, Lahn BT. Nestin is required for the proper self-renewal of neural stem cells. Stem cells. 2010; 28(12): 2162-2171. https://doi.org/10.1002/stem.541.

15. Dusart P, Fagerberg L, Perisic L, Civelek M, Struck E, Hedin U, Uhlén M, Trégouët DA, Renné T, Odeberg J, Butler LM. A systems-approach reveals human nestin is an endothelial-enriched, angiogenesis-independent intermediate filament protein. Sci Rep. 2018; 8(1): 14668. https://doi.org/10.1038/s41598-018-32859-4.

16. Abdel-Aal, R., Hussein, O., Elsaady, R., Abdelzaher, L. Celecoxib effect on rivastigmine anti-Alzheimer activity against aluminum chloride-induced neurobehavioral deficits as a rat model of Alzheimer's disease; novel perspectives for an old drug. *Journal of Medical and Life Science*, 2021; 3(4): 44-82. doi: 10.21608/jmals.2021.210630

17. Nowak A, Grzegrzółka J, Kmiecik A, Piotrowska A, Matkowski R, Dzięgiel P. Role of nestin expression in angiogenesis and breast cancer progression. Int J Oncol. 2018; 52(2): 527-535. https://doi.org/10.3892/ijo.2017.4223.

18. Matsuda Y, Ishiwata T, Yoshimura H, Hagio M, Arai T. Inhibition of nestin suppresses stem cell phenotype of glioblastomas through the alteration of post-translational modification of heat shock protein HSPA8/HSC71. Cancer Lett. 2015; 357(2): 602-611. https://doi.org/10.1016/j.canlet.2014.12.030.
19. Hasan, A. F., Alankooshi, A. A., Modher, M. N., El-Naggar, S. A., El-Wahsh, H. M., El-Bagoury, A. E., ... & Kabil, D. I. (2024). Artemisia Annua Extract Ameliorates Hepato-Renal Dysfunctions in Obese Rats. Opera Medica et Physiologica, 11(2), 47-65.
20. Hasan, A.F., Hameed, H.M., Hadid, M.A., and Tousson, E. (2024). Impact of Chia (Salvia hispanica) Seeds Extract on Ehrlich Ascites Model Induced Kidney Toxicity in Female Mice. Asian Journal of Dairy and Food Research. DOI: 10.18805/ ajdfr.DRF-397.
21. Kim SW, Roh J, Park CS. Immunohistochemistry for pathologists: protocols, pitfalls, and tips. J Pathol Translat Med. 2016; 50(6): 411-418. https://doi.org/10.4132%2Fjptm.2016.08.08.
22. O'Hurley G, Sjöstedt E, Rahman A, Li B, Kampf C, Pontén F, Gallagher WM, Lindskog C. Garbage in, garbage out: a critical evaluation of strategies used for validation of immunohistochemical biomarkers. Molec Oncol. 2014; 8(4): 783–798. https://doi.org/10.1016%2Fj.molonc.2014.03.008.
23. Yu X, Jiang Y, Wei W, Cong P, Ding Y, Xiang L, Wu K. Androgen receptor signaling regulates growth of glioblastoma multiforme in men. Tumor Biol. 2015; 36(2): 967-972. https://doi.org/10.1007/s13277-014-2709-z.
24. Fang BJ, Geng FY, Lu FQ, Wang YH, Zhang LQ, Meng FG. Expression and clinical significance of nestin in astrocytic tumors. J Buon. 2016; 21(1): 191-8.
25. George D, Mallery P. IBM SPSS statistics 26 step by step: A simple guide and reference. Routledge; 2019:402pp. https://doi.org/10.4324/9780429056765.
26. Amirinejad, M., Eftekhar-Vaghefi, S. H., Nematollahi Mahani, S. N., Salari, M., Yahyapour, R., & Ahmadi-Zeidabadi, M. (2024). Exposure to Low-Frequency Radiation Changes the Expression of Nestin, VEGF, BCRP and Apoptosis Markers During Glioma Treatment Strategy: An In Vitro Study. Current Radiopharmaceuticals, 17(1), 55-67.
27. Arai H, Ikota H, Sugawara KI, Nobusawa S, Hirato J, Nakazato Y. Nestin expression in brain tumors: its utility for pathological diagnosis and correlation with the prognosis of high-grade gliomas. Brain Tumor Pathol. 2012; 29(3): 160-167. https://doi.org/10.1007/s10014-012-0081-5.
28. Lv D, Lu L, Hu Z, Fei Z, Liu M, Wei L, Xu J. Nestin expression is associated with poor clinicopathological features and prognosis in glioma patients: an association study and meta-analysis. Molec Neurobiol. 2017; 54(1): 727-735. https://doi.org/10.1007/s12035-016-9689-5.
29. Woo CG. Clinicopathological Significance of Nestin Expression as a Diagnostic and Prognostic Marker in Brain Gliomas, Independent of *IDH* Mutation, Res Square. 2021: 1-10. https://doi.org/10.21203/rs.3.rs-994741/v1.
30. Kim KJ, Lee KH, Kim HS, Moon KS, Jung TY, Jung S, Lee MC. The presence of stem cell marker-expressing cells is not prognostically significant in glioblastomas. Neuropathology. 2011; 31(5): 494-502. https://doi.org/10.1111/j.1440-1789.2010.01194.x.
31. Gongala S, Garcia JA, Korakavi N, Patil N, Akbari H, Sloan A, Barnholtz-Sloan JS, Sun J, Griffith B, Poisson LM, Booth TC, Jain R, Mohan S, Nasralla MP, Bakas S, Tippareddy C, Puig J, Palmer JD, Shi W, Colen RR, Sotiras A, Ahn SS, Park YW, Davatzikos C, Badve C.

Sex-specific Differences in IDH1-Wildtype Glioblastoma patients in the ReSPOND Consortium. Am J Neuroradiol. 2024: ajnr.A8319. https://doi.org/10.3174/ajnr.a8319.

32. Gousias K, Markou M, Voulgaris S, Goussia A, Voulgari P, Bai M, Alamanos Y. Descriptive epidemiology of cerebral gliomas in northwest Greece and study of potential predisposing factors, 2005–2007. Neuroepidemiology. 2009; 33(2): 89-95. https://doi.org/10.1159/000222090.
33. Schwartzbaum JA, Fisher JL, Aldape KD, Wrensch M. Epidemiology and molecular pathology of glioma. Nat Clin Pract Neurol. 2006; 2(9): 494-503. https://doi.org/10.1038/ncpneuro0289.
34. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. Acta Neuropathologica. 2016; 131(6): 803-820. https://doi.org/10.1007/s00401-016-1545-1.
35. Fan Z, Liu Y, Li S, Liu X, Jiang T, Wang Y, Wang L. Association of tumor growth rates with molecular biomarker status: a longitudinal study of high-grade glioma. Aging (Albany NY). 2020; 12(9): 7908-7926. https://doi.org/10.18632/aging.103110.
36. Abdelkareem RM, Elnashar AT, Fadle KN, Muhammad, EM. Immunohistochemical expression of nestin as cancer stem cell marker in gliomas. J Neurosci Neurol Disord. 2019; 3(2): 162-166. https://doi.org/10.29328/journal.jnnd.1001027.
37. Ma YH, Mentlein R, Knerlich F, Kruse ML, Mehdorn HM, Held-Feindt J. Expression of stem cell markers in human astrocytomas of different WHO grades. J Neuro-oncol. 2008; 86(1): 31-45. https://doi.org/10.1007/s11060-007-9439-7.
38. Kitai R, Horita R, Sato K, Yoshida K, Arishima H, Higashino Y, Hashimoto N, Takeuchi H, Kubota T, Kikuta K. Nestin expression in astrocytic tumors delineates tumor infiltration. Brain Tumor Pathol. 2010; 27: 17-21. https://doi.org/10.1007/s10014-009-0261-0.
39. Rani SB, Mahadevan A, Anilkumar SR, Raju TR, Shankar SK. Expression of nestin-a stem cell associated intermediate filament in human CNS tumours. Indian J Med Res. 2006; 124(3): 269-280.
40.Chinnaiyan P, Cerna D, Burgan WE, Beam K, Williams ES, Camphausen K, Tofilon PJ. Postradiation sensitization of the histone deacetylase inhibitor valproic acid. Clin Cancer Res. 2008; 14(17): 5410-5415. https://doi.org/10.1158%2F1078-0432.CCR-08-0643.