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Immunohistochemical Expression of IDH Mutation in Primary Brain Tumors : Ekspresi Imunohistokimia Mutasi IDH pada Tumor Otak Primer

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Abstract. Background: Brain tumors constitute 1–2% of adult malignancies, with glioblastoma and meningioma as the most frequent subtypes. Mutations in the isocitrate dehydrogenase (IDH) gene have emerged as clinically relevant diagnostic and prognostic markers. Specific Background: While IDH mutations are well documented in gliomas, their distribution across different brain tumor subtypes in Iraq remains insufficiently characterized. Knowledge Gap: Limited regional studies exist on IDH immunohistochemical (IHC) expression and its association with tumor grade and clinical parameters. Aim: To determine the frequency and clinicopathological correlation of IDH mutations in primary brain tumors. Methods: A total of 41 brain tumor specimens collected in Mosul (2019–2022) were examined histologically and immunohistochemically for IDH expression. Results: Glioblastoma was the most common tumor (26.8%), followed by meningioma (22%). IDH positivity was observed in 40% of tested cases, including glioblastoma, medulloblastoma, gliosarcoma, and atypical meningioma. Meningioma grade I and anaplastic astrocytoma showed no IDH expression. Novelty: This is among the first regional reports highlighting diverse IDH mutation patterns across tumor subtypes. Implications: Findings suggest that IDH assessment provides diagnostic value and supports its potential use in refining classification and management of brain tumors in Iraq.

Highlights:

1. IDH mutation detected in multiple brain tumor subtypes.
2. Glioblastoma is the most frequent primary brain tumor.
3. IDH testing supports diagnostic and prognostic evaluation.

Keywords: Glioblastoma, Brain Tumors, IDH Mutation, Immunohistochemistry, Clinicopathology

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Introduction

Brain tumors constitute 1%-2% of tumours in adults , the incidence does not vary markedly between regions or population (1). The predominant tumour types in adults are metastasis, glial neoplasms and meningioma (2). The worldwide incidence of brain tumours has been reported to be around 3.9 /one lac/year in males and 3.0 /one lac/year in females (1). The recent trends from developed countries show a decline in low grade tumours and an increase in higher grade tumours. Some studies have suggested an increased incidence of CNS neoplasms associated with certain occupations, dietary factors and exposure to chemicals, but most of these reports have not been confirmed and causative agents have not been identified(1,3).

The majority of primary tumours of CNS are meningiomas and gliomas; the most common WHO grade IV tumour being glioblastoma Not Otherwise Specified (NOS). Due to improved diagnostic and treatment modalities available; particularly in the developing countries, an increased incidence of glial tumours (by 1%-2% per year) has been observed (2).

Gliomas arise from the glial cells, and constitute about half of all primary intracranial tumors. Depending on the cell of origin, gliomas are mainly of three types: Astrocytoma, Oligodendroglioma (OLIG) and Ependymoma (EPEN). High grade gliomas are the most frequent and lethal tumors originating in the central nervous system. (4) .

The most frequent and biologically aggressive subtype is Glioblastoma (GBM). Historically, Glioblastoma(GBM) have been categorized into two groups – primary and secondary, on the basis of their clinical presentation (5) .

The Secondary Glioblastoma (sec-GBM) are said to be tumors that have clinical, radiologic or histopathologic evidence of malignant progression from a preexisting lower-grade tumor, whereas primary GBMs (prim-GBM) have no such history and present de novo as advanced cancers at the time of diagnosis (6) .

The histopathologic findings of primary and secondary GBMs are indistinguishable, and their prognosis does not appear to be significantly different after adjustment for age (7,8).

However, these variants of GBM differ significantly with respect to genetic alteration. EGFR amplification, MDM2 amplification and PTEN mutations are typical of

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primary GBM while TP53 mutations are more frequent in secondary GBM (9). Substantial research has been focused on identification of gene alterations in GBM that might help to stratify GBM patients depending on prognosis and response to therapy. the present study sought to evaluate the relative frequency of primary brain tumors, correlate it with type, grade and clinical parameter, and study the immunehistochemical (IHC) expression of IDH mutation in primary brain tumors.

Methods

Specimen and study design: Forty one cases of brain tumor specimen were included in this case series study, they were collected from private laboratories in Mosul city during the period from September 2019 to June 2022. Clinicopathological data, such as age, gender, grade and tumor location were obtained from the medical reports.

Inclusion criteria: Age, gender, histopathological diagnosis, grade, and tumor location.

Exclusion criteria: Metastatic tumor

Histopathological method: Each case was already fixed in 10% formalin, processed, embedded in paraffin wax, Serial sections on paraffin blocks is done with 3 microns thickness, then sections stained by hematoxylin and eosin (H&E) as follows:

Deparaffinising and rehydration: the slides were immersed alternately in the following solutions at room temperature for the indicated times; xylene 1 for 2 minutes, xylene 2 for 2 minutes, 100% ethanol for 2 minutes, 100% ethanol for 2 minutes, 95% ethanol for 2 minutes, water wash for 2 minutes.

Hematoxylin staining: the slides were immersed in hematoxylin for 3 minutes. Then wash with water for 1 minute. Differentiation in mild glacial acetic acid for 1 minute. Then wash in water for 1 minute.

Bluing 1 minute: Then wash in water for 1 minute. After that put in 95% ethanol for 1 minute.

Eosin staining: the slides immersed in eosin for 45 seconds.

Dehydration and clearing: all the slides were sequentially immersed in the following solutions, 95% ethanol for 1 minute, 100% ethanol for 1 minute, 100% ethanol for 1 minute, xylene1 for 2 minute, xylene2 for 2 minutes. Mounting: the slides mounted with DPX and covered with cover slips. Then careful and meticulous slides assessments were done, then IHC for IDH1/IDH2 were performed on 10 cases by the following method.

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Immunohistochemical method: Labelled polymer and enhanced polymer systems (PathnSitu) method according to PathnSitu recommendation was used to stain the tissue by IDH1 antibody. Four microns sections were obtained from formalin fixed-paraffin embedded tissue blocks and mounted on positively charged slides. Slide baking: the slides were placed in a hot air oven at 70 C° for 20 minutes.

Deparaffinising and rehydration: the slides were immersed sequentially in the Following solutions at room temperature for the indicated times, xylene 1 for 5 minutes, xylene 2 for 5 minutes, xylene 3 for 5 minutes, 100% ethanol for 3 minutes, 70% ethanol for 3 minutes, 50% ethanol for 3 minute, distilled water 1 for 2 minutes, distilled water 2 for 2 minutes. Antigen retrieval, slides are placed in a plastic jar filled with prepared (1 part of Tris EDTA buffer in 49 parts of distilled water) Tris EDTA retrieval buffer (pH 9).

Then the jars placed in steamer for heating, after that they kept cooling for 10 Minutes at room temperature, then the slides were removed from the antigen retrieval solution and placed in distilled water. PAPPEN was used to mark around the tissue section. PolyExcel hydrogen Peroxide: enough drops of Peroxidase block reagent were applied onto the tissue covering the whole section and incubated at room. Temperature for 5 minutes in humid chamber, after that the slides were tilted to drain off peroxide block.

Primary antibody (IDH1): Primary antibody was applied onto each section and incubated at room temperature for 30 minutes in humid chamber, and then slides were washed with a immunowash buffer in three sequential steps.

Immunowash for 1minute for 3 times, PolyExcel target binder: cover the tissue section with target binder and incubate for 10 minutes at room temperature. PolyExcel polyHRP: cover the section with polyExcel polyHRP and incubate for 10 minutes at room temperature in humid chamber. Then slides were washed with immunowash buffer in three sequential steps for 1 minute each. PolyExcel stunn DAB/Substrate-chromogen solution: prepared working solution (DAB buffer 1ml + DAB chromogen 1drop) was applied on each section covering the whole specimen and incubated in darkness at room temperature for10 minutes. Then slides were washed in three sequential steps using distilled water for 1minute. Immunowash 2 times for 1minute each. Hematoxylin counter stain solution were applied covering the whole section and incubated at room

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temperature for 2 minutes. After that, the slides were rinsed gently with tap water until the counter stain washes away. Dehydration the slides were immersed sequentially in the following solutions at room temperature for the indicated times. 70% ethanol for 2 minutes, 90% ethanol for 2 minutes, 100% ethanol for 2 minutes, 100% ethanol for 2 minutes, xylene 1 for 2 minutes, xylene 2 for 2 minutes, xylene 3 for 2 minutes. DPX mountant: the slides mounted with DPX mountant and covered with cover slips and left to dry.

Positive results show strong cytoplasmic staining that appeared only in the tumor cells. Determination of IDH1 positivity did by visual semi quantitative assessment of the proportion of the positively staining tumor cells. Cases of equal or more than 10% IDH1 expression considered as positive, while cases with less than 10% cells were negative.

Statistical Analysis: The statistical studies were performed using the statistical package for social sciences (SPSS) of version 26 program. The significant correlation was done by using Fishers exact test and chi square test. Value was considered statistically significant when P value is ≤ 0.05.

Results and Discussion

A. Results

Patients demography: A total of 41 cases of brain tumors were collected, theirage ranged from 4 to 72 years. The mean age at presentation were 36.61 ±9.81 years (95% CI for mean range from 29.68-42.21). The studied samples consist of 21 (51.2%) males and 20 (48.8%) females with male to female ratio of 1.05:1 (Figure 1).

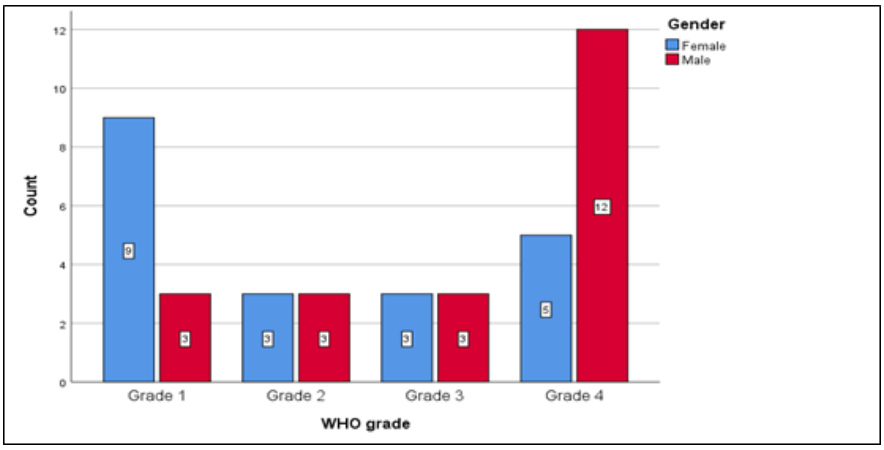

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Figure 1. Gender distribution of gliomas according to WHO grade.

Location, subtype and grade of brain tumor: The results reveal that 31 tumors (75.6%) were located supratententorial while the remaining 10 (24.4%) were located infratentorial, and the Supratentorial location is the most frequent occurrence in female gender (46.3%) than male (29.3), with a highly significant differences (p-value=0.005) (Figure 2 and Figure 3).

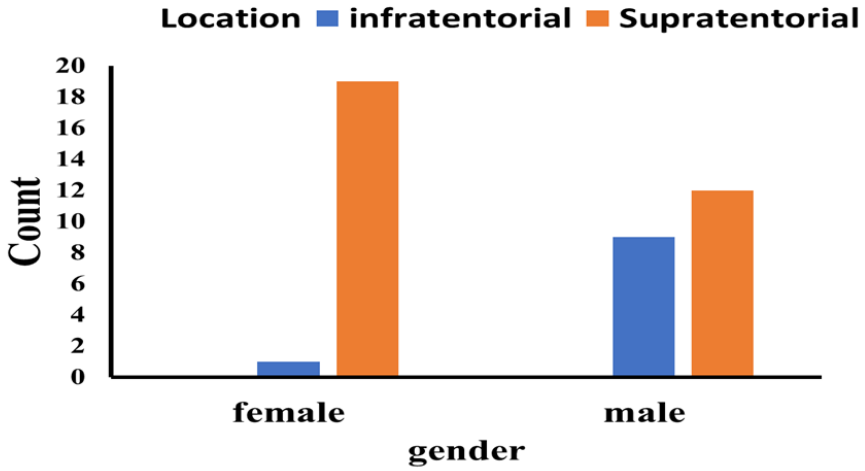

Figure 2. correlation of gender with Location of brain tumors.

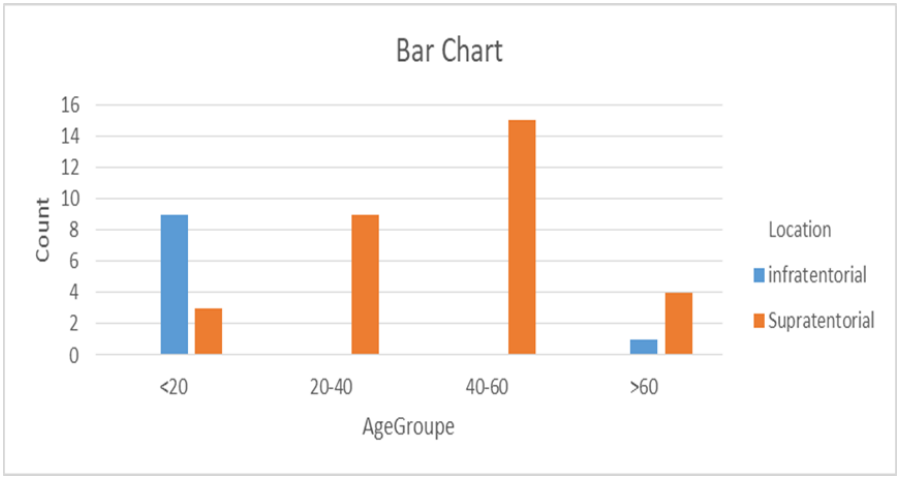

Figure 3. Location of brain tumors according to age group.

The supratentorial location is the most frequent occurrence in age group between 20 to 60 (58.6%), with a highly significance p-value (0.000). Regarding the histological diagnosis of brain tumors, results reveal that the predominant subtype was GBM which found in 11 (26.8%) of patient, while the least was in

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atypical Meningioma grade II and Craniopharyngioma which found in one (2.4%) patient each. The frequency of the rest of tumors with its frequencies is shown in table III.1. The tumor were graded according to WHO grade as follows: 12 (29.3%) as grade 1; 17 (41.5%) as grade 4; and 6 (14.6%) as both grade 2 & 3 (Table 1 and Figure 4).

Table 1. Histologic diagnosis of brain tumors

Subtype	Frequency	Percent %
GBM (grade IV)	11	26.8
Meningioma (grade I)	9	22
Medulloblastoma (grade IV)	5	12.2
Astrocytoma (grade II)	3	7.3
Oligodendroglioma	3	7.3
Anaplastic astrocytoma (grade III)	2	4.8
Anaplastic meningioma (grade III)	2	4.9
Atypical meningioma (grade II)	1	2.4
Craniopharyngioma	1	2.4
Total	41	100

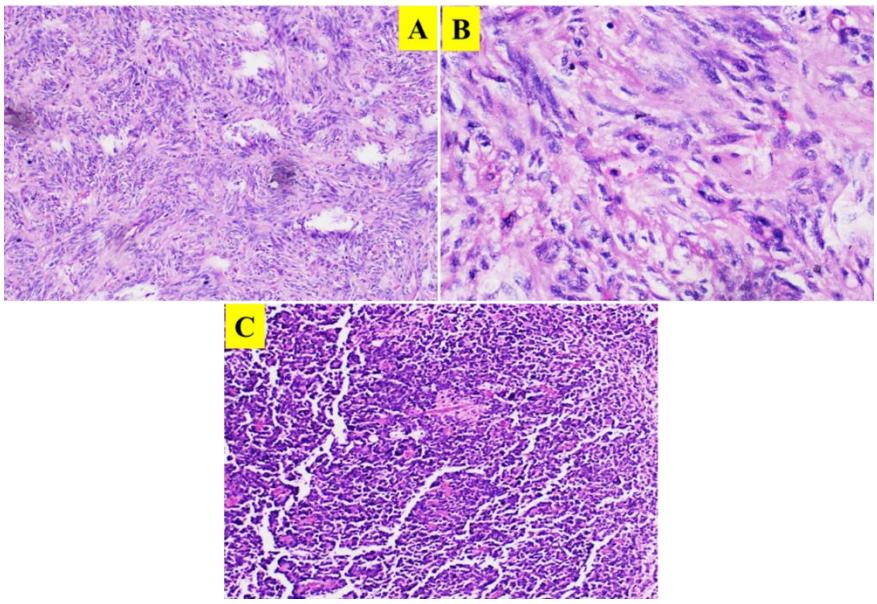

Figure 4. Meningioma fibroblastic type. (A) Cellular tumor withmeningothelial whorls, 100X (B) Syncytial cells with indistinct cell membranes and eosinophilic cytoplasm, 400X (C) Medulloblastoma with small blue round cell

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tumor, syncytial arrangement of densely packed undifferentiated cells and homer wright rosette, 100X. Hematoxyline and eosin stain.

The GBM grade IV (26.8%) and Meningioma grade I (19.5%) is the most frequent subtype of brain tumor, with a highly significance p-value (0.000). IDH Mutation: Positive cytoplasmic IDH staining was found in 4 (40%) of cases of brain tumors, this positive staining was appear only in tumor cells and not shown in normal brain tissue cases, and the result were as follow: 1 out of 4 (25%) of medulloblastoma, 1 out of 2 (50%) of GBM, 1 (100%) of atypical meningioma and 1 (100%) of gliosarcoma were positive for IDH mutation. While 1 (100%) of meningioma grade 1 and 1 (100%) of anaplastic astrocytoma were negative for IDH mutation (Table 2 and Figure 5).

Table 2. IDH status and the types of brain tumor,P-value >0.005

Types of brain tumor	IDH (+ve)	IDH (-ve)	Total
Medulloblastoma %	1 (25%)	3 (75%)	4 (100%)
Atypical meningioma %	1 (100%)	-	1 (100%)
Meningioma(grade 1) %	-	1 (100%)	1 (100%)
Anaplastic astrocytoma %	-	1 (100%)	1 (100%)
Gliosarcoma %	1 (100%)	-	1 (100%)
GBM %	1 (50%)	1 (50%)	2 (100%)

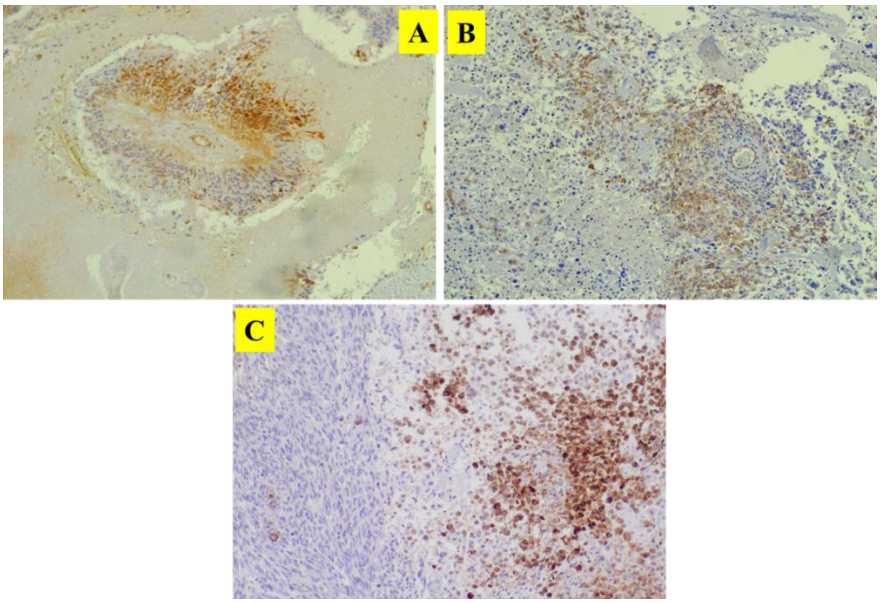

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Figure 5. IDH immunostain for the slides prepared. (A) Rosette in IDH mutant Medulloblastoma(400x). (B) GBM with diffuse cytoplasmic reactivity in tumor cells and necrotic area in the left side(400x). (C) Gliosarcoma with IDH positive glial cells(long arrow) and IDH negative sarcomatous component (short arrow).

B. Discussion

Brain tumors represent a highly heterogeneous group of neoplastic diseases with strong variation of incidence by age, and partially by gender (10). According to the current study which was done on 41 cases of brain tumor, the mean age at diagnosis of brain tumor was (36.61)years, compared with other studies done in Mosul city by Mohammed Sami Saeed &, United Arab Emirates by Sarah Khan et al. the mean age of diagnosis was (31.19) and (33.48)years respectively (11,12).

In this study the most common tumors found between the age of (0-12) years were Medulloblastoma, Ependymoma and Pilocytic astrocytoma and this compatible with research done by Ramandeep S. Arora et al (13). Regarding the gender distribution in this study, it was found that the brain tumors were slightly more common in males than in females with (51.2%) and (48.8%) respectively, this result is in line with Mohammed Sami Saeed study with 58(53.21%) were males and 51 (46.78%) were females and the male to female ratio was 1.13:1 (11) and Ramandeep S. Arora et al with males and females incidence rates were (9.96) and (8.52) per 100,000 person-years, respectively, giving a male-to-female ratio of (1.17:1) (13). In keeping with research done by Gi-Ming Wang et al (12) we found that the frequency of low grade gliomas were more common in females, while the high grade gliomas were more common in males.

In this study We found that Meningiomas showed a strong female preponderance and this compatible with Bernd Holleczek et al (13) whose took data of (992) patients who were diagnosed between 2000 and 2015 as Meningioma ; among these almost three out of four (72%) were women, resulting in a female:male ratio of 2.53. In this study we found that GBM was the predominant brain tumor subtype and constitute (26.8%) of cases, this result is compatible with study done between 1995 and 2017 by Hiba A Wanis et al, a total of(133 669) cases of adult primary tumor were registered in England, the Glioblastoma was the most frequent tumor subtype (31.8%) (14).

There is a significant differences in the distribution of the brain tumors according to the site, it has been found that the supratentorial brain tumors account for (75.6%) while the infratentorial tumors account for (24.4%), this is in line with study conducted by Olivia Desty Sabunga (15) and Omer NS et al (16) who said that this could be due to the supratentorial location containing most glial cells.

In this study IDH mutation was found in (25%) of medulloblastoma, (50%) of GBM, (100%) of atypical meningioma and gliosarcoma, compared with study done in

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Baghdad city by Zahraa Marwan et al (17) (87.5%) of secondary GBM was positive for IDH mutation with no mutation were found among medulloblastoma, meningioma and gliosarcoma. And another study done by Olivia Desty Sabunga (15) reveal (71.4 %) of GBM, (93.3%) of anaplastic astrocytoma and (45.5%) of diffuse astrocytoma were IDH mutant, and this is due to small sample size of our study.

Conclusions

The most frequent type of brain tumors was glioblastoma(GBM). GBM were more common in males while meningiomas were more common in females. IDH have a diagnostic role for detection of brain tumors. Further studies with larger number of patients to confirm the diagnostic significance of IDH mutation in the diagnosis of secondary gliomas.

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