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Characteristics of Adult Glioblastoma in Kuwait

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Abstract

Introduction: Classification of Glioblastoma (GBM) into primary (p-GBM) and secondary (s-GBM) is based on IDH1 status. This study aims at evaluating selected biomarkers and clinical characteristics of adult GBM in Kuwait and establishing guidelines for case selection for DNA sequencing.

Methods: Archived blocks of 100 GBM initially diagnosed in the Histopathology Unit, Al-Sabah Hospital between 2009 and 2016 in patients aged ≥ 20 years were retrieved and used for Immunohistochemistry (IHC) for wild-type and mutant IDH1, p53 and EGFR. Patients' characteristics, presenting symptoms and duration, number of lesions, and tumor anatomic location and extent were extracted from the surgical records.

Results: S-GBM comprises 10%, spares patients aged ≥ 60 years, has a mean age of 40.9 years, significant association with young age (<50 years) and an exclusive lobar distribution with a predilection for frontal lobe (60%). P-GBM has a mean age of 50.7 years and a more varied anatomic distribution. In 2%, IHC is negative for both wild-type and mutant IDH1. About 38% and 34% are p53+ and EGFR+. Significantly, 80% of s-GBM and 33% of p-GBM are p53+. EGFR+ is observed in 20% s-GBM and below the age of 35 years. EGFR+ s-GBM is located only in the frontal lobe.

EGFR-/p53+, EGFR+-/p53-, EGFR+/p53+, and EGFR-/p53- phenotypes form 27%, 23%, 11% and 39% of all GBM respectively.

Symptoms lasted for <3 months in 83%. Only 1% of these have s-GBM. About 13% have multiple lesions.

Conclusion: Factors precluding DNA sequencing include patient's age (≥ 60 years), multiple lesions, short duration of symptoms and EGFR+/p53- phenotype. IHC for wild-type IDH1 is unnecessary for GBM investigation.

Keywords: Glioblastoma; IDH1; p53; EGFR; Kuwait

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Introduction

Glioblastoma (GBM) is the most common primary malignant brain tumor worldwide. Biologically, it is a highly aggressive astrocyte tumor with generally poor prognosis of about 3% 10-year- survival. Detection of genetic alterations with significant pathogenetic role is useful in its diagnosis and at times predicting prognosis.

Isocitrate Dehydrogenase 1 (IDH-1) is a cytoplasmic enzyme involved in oxidative decarboxylation of isocitrate. Its production is regulated by IDH-1 gene located at 2q34 [1]. Heterozygous missense mutations of nucleotides 395 and 394 at exon 4 of this gene have been detected in some GBM. About 90% of the mutations occurs at the nucleotide 395 and involves substitution of guanine with adenine. Consequently, arginine is replaced with histidine at position 132 of the amino acid chain (p.132R>H). Infrequent mutations include c.395G>T (p.132R>L), c.394C>T (p.132R>C), c.394C>G (p.132R>G) and c.394C>A (p.132R>S) [2].

Until recently, DNA sequencing has been used in detecting these mutations. The discovery of antibody specific for p.132R>H (Dianova Clone H09) has facilitated the use of Immunohistochemistry (IHC) in the diagnosis of mutant IDH-1. This is cheaper and faster than DNA sequencing. Despite its high specificity and sensitivity for the detection of c.395G>A mutation, a negative result does not necessarily indicate absence of mutation for two reasons. Firstly, it does not detect rare mutations because of lack of significant cross-reactivity with the protein products of these mutations. Secondly, Ikota et al. [3] have suggested that the expression of both mutant and wild type IDH1 on IHC may

be evenly suppressed by unknown internal mechanism [3]. Such false negative IHC may be detected by simultaneous or subsequent use of anti-IDH-1 wild type (wt-IDH-1) antibody. Since the frequency of the suppression phenomenon in GBM has not been determined, the cost effectiveness of IHC for anti-wt-IDH-1 in the routine investigation of GBM remains unknown.

DNA sequencing currently serves as a confirmatory procedure for IHC negative cases but must be used judiciously in order to ensure maximum benefits at minimum costs.

The 2016 World Health Organization (WHO) classification of tumors of the central nervous system has not only classified GBM into primary and secondary based on IDH-1 status but also suggested guideline for selection of cases for DNA sequencing [4].

Secondary GBM (s-GBM) has IDH-1 mutation and constitutes about 5% to 13% of all GBM. Hospital-based studies tended to yield a higher proportion than population-based studies [5-8]. Besides, it affects predominantly young adults with a mean age of between 32 to 48 years [9-13]. Significantly, these reports emanated from studies done in countries with a relatively high percentage of population above 50 years. The relative frequency of GBM in these countries increases with age. Thus, about 19% and 30% of adult GBM in US occur before the ages of 50 years and after 70 years respectively [14]. These observations raise a fundamental question as to whether countries with a relatively higher percentage of people younger than 50 years will have a higher proportion of s-GBM.

Kuwait is a small Middle Eastern country with only about 12% of its 2016 estimated population aged ≥ 50 years [15]. This is a remarkable contrast with US with about 34.1% of the estimated 2016 population in similar age group [16].

The major objective of this study is to evaluate the genetic and salient clinical characteristics of adult GBM in Kuwait and suggest guidelines for selection of cases for DNA sequencing.

Materials and Methods

One hundred (100) consecutive cases of GBM newly diagnosed in Pathology Department of Al-Sabah Hospital, Kuwait in patients aged ≥ 20 years between 2009 and 2016 were culled from the departmental records. Retrieved archived blocks were used for Immunohistochemistry (IHC) using anti-IDH1 mutant anti-body (Dianova, Hamburg, Germany; clone H09, mouse monoclonal), anti-wild type IDH-1 anti-body (RC041RTU7, Medaysis, CA, USA, Polyclonal rabbit), anti-p53 anti-body (AM239-5M, Biogenex, USA Clone D07, monoclonal, mouse) and anti-EGFR anti-body (Dako, CA, USA, clone H11, monoclonal mouse).

A moderate to strong nuclear expression in ≥ 10% of cells was considered positive for p53, while membranous and cytoplasmic staining was interpreted as positive for EGFR.

Patients' characteristics, presenting symptoms and their duration, number of lesions at presentation, and tumor location were extracted from the Records Departments of Ibn Sina Hospital. SPSS version 24 was used for statistical analysis.

Statistical Package for the Social Sciences (SPSS version 23, IBM, Chicago II, USA) has been used for all statistical analysis.

Results

The sample size comprises 73 men and 27 women aged between 23 and 80 years with a median age of 51.5 years. About 20% are aged ≥ 60 years, (Figure 1).

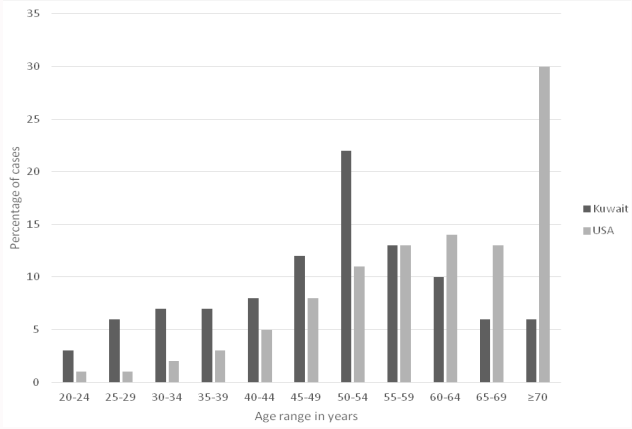

Figure 1: Age distribution of glioblastoma patients in the study and USA [16].

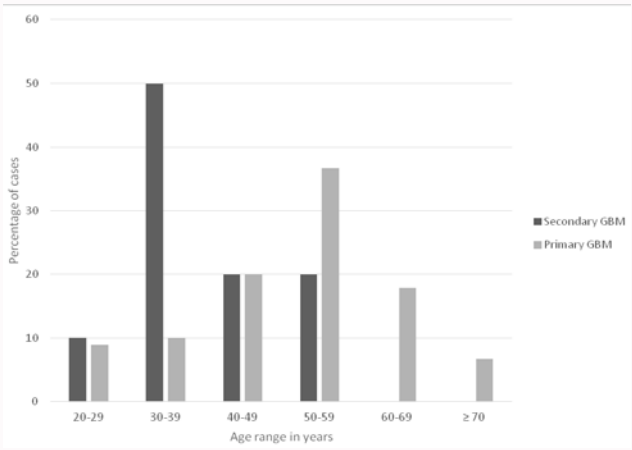

Figure 2: Age distribution of primary and secondary adult Glioblastoma.

Mutant IDH-1 (mut-IDH1)

Mutant-IDH-1 (c.395G>A) has been detected in 10 patients- 6 men and 4 women aged 29-59 years with a mean age of 40.9 years. Although 80% are <50 years, they constitute only 18.6% of all tumors in this age group (Figure 2). There is a significant association (p=0.04) between mut-IDH-1 expression and age groups <50 versus ≥ 50 years. However, linear relationship with individual age groups is not detected by scatter plot. All mutant-IDH-1 positive cases are classified as s-GBM.

IDH-1 mutation is absent in 90 patients made up of 67 men and 23 women aged 23 to 80 years with a mean age of 50.7 years (Figure 2). In general, they form 81.4% of all GBM seen before the age of 50 years. The male/female ratio is 2.9. All are classified as p-GBM.

The difference in mean ages of patients with s-GBM and p-GBM is statistically significant (p=0.02).

IDH1 wild type

IDH-1 wild type (wt-IDH-1) is expressed in 88 cases. Ten negative tumors are positive for mut-IDH1, while 2 are negative for both mut-IDH-1 and wt-IDH-1.

p53 expression

Over-expression of p53 has been detected in 38 patients (38%),

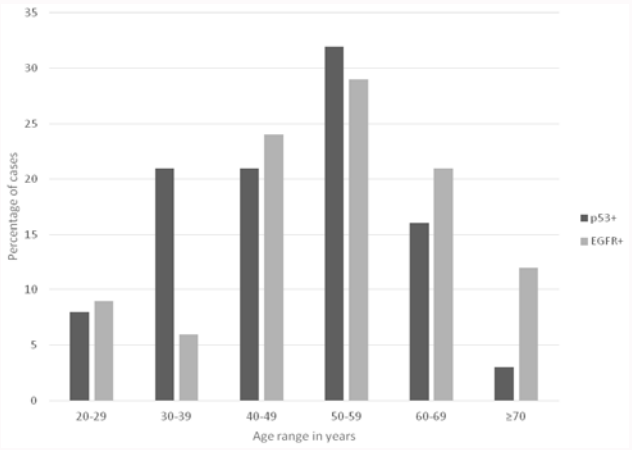

Figure 3: Age distribution of p53+ and EGFR+ adult Glioblastoma.

aged 23 to 70 years, with a mean age of 47.76 years. It is observed in all age groups (Figure 3). The mean age of patients with p53 negative GBM is 50.87 years. About 80% of s-GBM and 33% of p-GBM express p53. The difference is statistically significant (p=0.004).

Epidermal growth factor receptor (EGFR) expression

EGFR expression is detected in 34 patients (34%), aged 24 to 80 years, with a mean age of 53.21 years. The mean age of EGFR+ cases is significantly (p=0.047) greater than that of EGFR- cases (47.88 years). It is observed in all age groups. Four (12%) are younger than 35 years (Figure 3). About 20% of s-GBM and 36% of p-GBM are positive for EGFR. Overall, p-GBM constitutes about 94% of all EGFR+GBM.

P53+/EGFR-

Twenty-seven patients (27%), with a mean age of 48.48 years, have GBM with p53+/EGFR- phenotype. They comprise 21 p-GBM and 6 s-GBM. Generally, 60% of s-GBM and 23.33% of p-GBM have this phenotype. The difference in proportion is statistically significant (p=0.01).

EGFR+/p53-

This phenotype is seen in 23 patients (23%) with a mean age of 56.65 years. All have p-GBM. About 73.91% are older than 60 years.

EGFR+/p53+ (double positive)

Eleven (11%) GBM patients with a mean age of 46 years have concurrent expression of p53 and EGFR. Three are younger than 35 years. The GBM is primary in 9 cases and secondary in 2 cases. The p-GBM patients are predominantly male (89%).

EGFR-/p53- (double negative)

GBM in 39 patients (39%) with a mean age of 47.46 years are negative for both EGFR and p53. They consist of 37 p-GBM and 2 s-GBM.

Number of lesions

Eighty-seven (87) patients have solitary and 13 multiple lesions.

Multiple lesions (Multifocal GBM): Multifocal GBM constitutes 13% of all GBM cases. It has been observed in 11 men and 2 women with a mean age of 50.85 years. There is no relationship with age. It is encountered exclusively in p-GBM. Four tumors (36%) are positive for both EGFR and p53, while 5 (46%) are negative for both.

Solitary lesions (87%): These are present in 62 men and 25

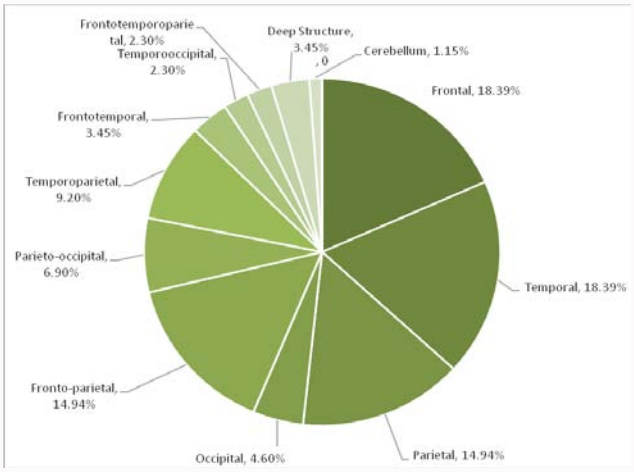

Figure 4a: Anatomic distribution of solitary glioblastoma in adults: All glioblastoma.

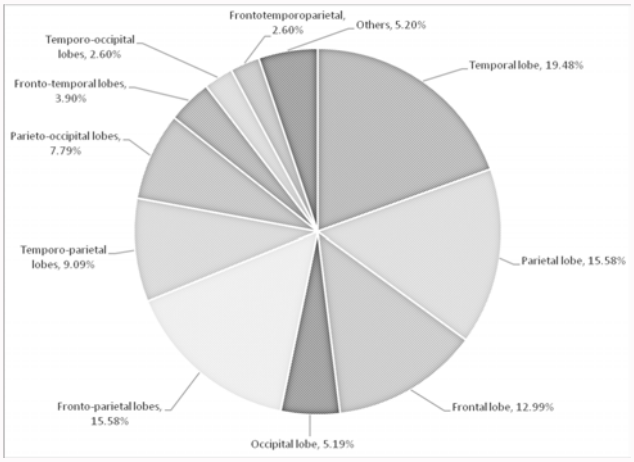

Figure 4b: Primary glioblastoma.

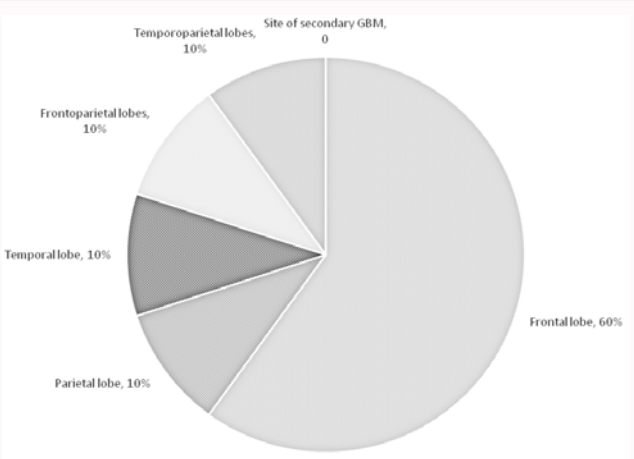

Figure 4c: Secondary Glioblastoma.

women. The GBM is primary in 77 (88.5%) and secondary in 10 (11.5%).

Site of solitary lesions: The lesions are located as follows: Lobar distribution- 83 (95.40%), thalamus 2 (2.30%), splenium 1 (1.15%) and cerebellum 1 (1.15%). The lobar distribution involved the right

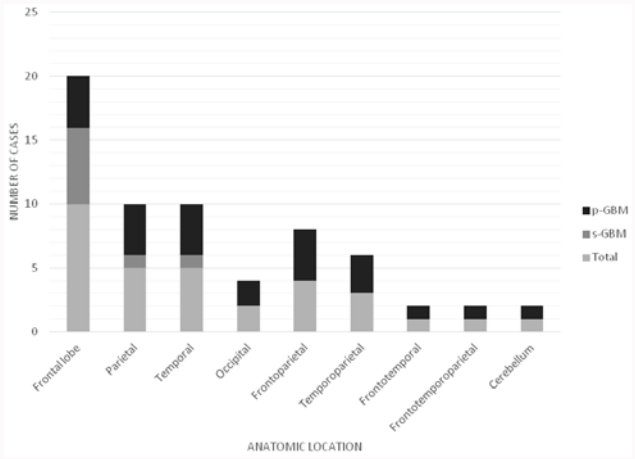

Figure 5: Anatomic location of p53 positive solitary glioblastoma in adults.

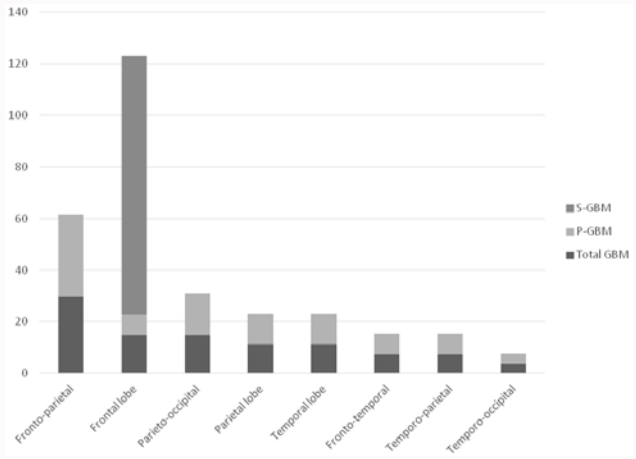

Figure 6: Anatomic location of EGFR positive glioblastoma in adults.

hemisphere in 47 cases (56.63%) and left in 36 cases (43.37%). Frontal and temporal lobes (18%, each) are the most common location of glioblastoma as a group. However, there is a substantial difference in the anatomic distribution of primary and secondary GBM.

Secondary GBM is exclusively lobar in distribution but spares the occipital lobe. About 80% s-GBM lesions are limited to one lobe while 20% overlap two adjacent lobes. Most (60%) are in the frontal lobe.

Unlike s-GBM, primary GBM has a more varied distribution; affects the occipital lobe, deep cerebral structures and cerebellum. About 56% are confined to 1 lobe while 44% overlap adjacent lobes. In 2 cases, the GBM overlaps 3 lobes. The temporal lobe (20%) is the most common site of non-overlapping lesions of p-GBM, while frontoparietal (16%) is the most common for overlapping lesions (Figure 4).

Thirty-two solitary lesions express p53. About 71% have a monolobar distribution with majority (31%) domiciled in the frontal lobe. Secondary GBM that express p53 is located only in the frontal, parietal and temporal lobes. It constitutes 60% of p53+ frontal lobe GBM. None of the overlapping s-GBM expresses p53 (Figure 5).

Twenty-seven solitary lesions express EGFR. Most (63%) occupy more than one lobe with fronto-parietal region as the most common (Figure 6). There are 25 p-GBM and 2 s-GBM. EGFR+ s-GBM is located only in the frontal lobe where it forms 50% of the tumors.

There is a significant difference in the proportion of p53+ and EGFR+ tumors confined to one lobe (p=0.0096)

Presenting Symptoms

These are available for 94 patients. They include Stroke-Like Syndrome (SLS), symptoms of Intracranial Hypertension (ICH) with and without focal neurologic deficit and seizures. Rarely, memory disturbance, behavioral abnormality, psychiatric disorder and urine incontinence are part of the presenting syndrome. Twenty-six patients (27.7%), have history of seizures either as the sole presenting syndrome (3 patients) or in addition to other symptoms. About 52% of patients with seizures are aged ≥ 50 years.

Duration of Symptoms

Data on this are available for 92 patients. Out of these, 76 (82.6%) have symptoms for <3 months, while 16 (17.4%) have longer duration of symptoms. Seventy-five (98.68%) patients with short duration of symptoms have p-GBM. The biopsy of the rare (1.32%) s-GBM in this group has histological features of a WHO grade II astrocytoma in some areas. Of the 16 patients with long duration of symptoms, 7 (43.75%) have p-GBM and 9 (56.25%) s-GBM.

Information on duration of symptoms is available for 24/26 patients who presented with a history of seizures. Long duration has been observed in about 11 patients (45.8%).

Discussion

The results of this study suggest that Kuwait has a significantly smaller proportion (12%) of elderly patients with GBM than the US (43%) [14]. This has management and survival implications and calls for further investigation to determine whether GBM patients in Kuwait have better overall survival than USA.

Furthermore, they indicate that population structure has no effect on the proportion of patients with s-GBM. The relative frequency of 10% and mean age of 40.9 years are consistent with reports of other hospital-based studies done in countries with a relatively higher percentage of elderly in their population.

As in other countries, s-GBM in Kuwait is a disease of predominantly young adults, with 80% occurring before the age of 50 years.

The 2016 WHO classification of tumors of the central nervous system has recommended that DNA sequencing should not be done for GBM in patients aged ≥ 55 years. This will exclude about 70% of GBM in US and underlies the 43% savings in cost and 53% reduction in turn-around time reported by DeWitt et al [17].

The absence of s-GBM in patients aged ≥ 60 years suggests that 60 years should be the cut-off age in Kuwait for DNA sequencing. Since this will exclude only about 20% of GBM patients, there is a need to establish other exclusion criteria to ensure maximum benefit at minimum costs.

EGFR+GBM display some peculiarities in this series. Its occurrence in patients aged <35 years and in 20% of s-GBM are considered exceptional. Significantly, EGFR+ s-GBM is located only in the frontal lobe. This raises the probability that anatomic location can be used as a criterion for selection of cases for DNA sequencing.

Furthermore, the results indicate that multiple lesions and

EGFR+/p53- phenotype are exclusive features of p-GBM. Multiplicity of lesions is considered a poor prognostic factor [18,19]. DNA sequencing is not recommended for GBM with these features.

About 83% of GBM patients have symptoms for less than 3 months. Significantly, mutant IDH-1 has been detected in about 1% of tumors in this group by IHC. Ohno et al. [20] reported a frequency of about 4.7%. Statistically, there is no significant difference between the two observations. Both confirm the notion that s-GBM may rarely have a rapid evolution. The extremely low frequency of the most common IDH1 mutation (c.395G>A) indicates that occurrence of rare variants of IDH1 mutation in this group in Kuwait, on the balance of probabilities, is highly unlikely. Therefore, it is proposed that all IHC mut-IDH1 negative GBM in patients with <3 months' duration of symptoms should be regarded as p-GBM without DNA sequencing. Probably, this proposal may require validation by DNA sequencing of a larger sample size than the one used for the study.

There is a relatively high frequency (56.25%) of mut-IDH1 positive GBM in patients with ≥ 3 months' duration of symptoms. This raises a reasonable suspicion that rare variants of IDH1 mutation may be detected in this group. DNA sequencing is recommended routinely for IHC negative mut-IDH1 GBM in patients with duration of symptoms ≥ 3 months.

Based on the above observations, the suggested guidelines for exclusion of IHC mutant IDH1 negative cases for DNA sequencing should include age ≥ 60 years, presentation with multiple lesions, short duration of symptoms and EGFR+/p53- phenotype. Consequently, about 15% of GBM in Kuwait may require DNA sequencing. These guidelines may be of interest to practitioners in developing countries who are encumbered by twin problem of limited or no access to facilities for DNA sequencing and financial constraints.

Mutually exclusive results for mutant and wild-type-IDH-1 have been observed in 98% of all GBM. The non-expression of both mutant and wild type IDH-1 in about 2% of GBM is probably caused by an unknown internal suppression factor as suggested by Ikota et al [3]. The ambiguity in both cases has been resolved by DNA sequencing which confirmed absence of mutation. Both cases are classified as p-GBM. More importantly, it suggests that the suppression phenomenon is rare and does not support simultaneous or subsequent IHC for wild-type IDH-1 in routine investigation of GBM.

The absence of significant difference in the frequency of seizures in patients with short and long duration of symptoms suggests that seizures do not necessarily lead to early diagnosis and treatment. It is necessary to explore the relationship between duration of symptoms in patients with seizure and survival.

In concordance with other reports, s-GBM has a predilection for the frontal lobe. The predominantly frontal lobe location most likely contributes to the purported better prognosis of s-GBM as tumors in non-eloquent brain are amenable to wide or total resection.

Conclusion

A few inferences can be made from the results of this study with respect to Kuwait. Firstly, the population structure has no influence on the relative frequency of s-GBM. Secondly, s-GBM apparently does not occur after the 6th decade of life. Thirdly, factors that preclude DNA sequencing include patient's age (≥ 60 years), duration of symptoms <3 months, multiple lesions and EGFR+/p53- phenotype.

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Author Contributions

Anwar Ghanim Al-Banaw: Obtained fund for the research, participated in design of the study, supervised the immunohistochemistry and evaluation of slides; was involved in statistical analysis, interpretation of data and production of manuscript. He has read and approved the final version.

Sarah Talib Hussain: Wrote the research proposal for grant approval, carried out the immunohistochemistry, collected clinical data; participated in slide evaluation and interpretation, statistical analysis, literature search and production of the manuscript. She has read and approved the final version of the manuscript.

Kenneth Chukwuka Katchy: Originated the concept; culled cases from Al-Sabah Pathology records and retrieved archived blocks, participated in the design of the study, slide evaluation and interpretation; literature search; interpretation of data, statistical analysis, and production of the manuscript. He has read and approved the final version.

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