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SIGNIFICANCE OF *BRAF*^{V600E} MUTATION IN INTRA-AXIAL BRAIN TUMOR IN MALAYSIAN PATIENTS: CASE SERIES AND LITERATURE REVIEW

A.A. Mohamed Yusoff, S.M. Abd Radzak, S.Z.N. Mohd Khair, J.M. Abdullah*
Department of Neurosciences, School of Medical Sciences, University Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

***Background*: To date, *BRAF* mutations in brain tumor patients have not been characterized in the Malaysian population. Based on the numerous reported studies, there are main mutations that exist in *BRAF* gene in various types of cancers. A missense mutation in codon 600 of the *BRAF* nuclear oncogene (*BRAF*^{V600E}) is the most prevalent hotspot point mutation that has been identified in multiple human malignancies. *Aim*: We here aimed to find out the frequency of *BRAF*^{V600E} mutation in a series of Malaysian patients with brain tumors and if any association exists between *BRAF*^{V600E} mutation and clinicopathological features of patients. *Material and Methods*: Fresh frozen tumor tissue samples from 50 Malaysian brain tumor patients were analyzed for *BRAF*^{V600E} mutational status, and its correlation with clinicopathological features (including age, gender, and tumor localization such as intra-axial: within the brain substance or extra-axial: outside the brain substance) was examined. *Results*: The overall *BRAF*^{V600E} mutation frequency was determined to be 22% (in 11 of 50 patients). *BRAF*^{V600E} was significantly correlated with the tumor location group, which shows *BRAF*^{V600E} was more frequent in the intra-axial tumor than the extra-axial tumor group. In this study, we also observed that male patients were slightly more susceptible to *BRAF*^{V600E} mutation, and this mutation was predominant in patients of the age group < 40 years. However, these parameters did not translate to statistical significance. *Conclusion*: The data demonstrate that *BRAF*^{V600E} mutation is observed significantly more often in intra-axial brain tumor patients, which can serve as baseline information for further research on genetic alteration that occurs during brain tumor progression in the Malaysian population.**
***Key Words*: brain tumor, intra-axial tumor, *BRAF*^{V600E} mutation, Malaysia.**

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The primary brain and other intracranial central nervous system (CNS) tumors are a heterogeneous group of neoplasms. The epidemiology review report of brain cancer in the world determined that the incidence of CNS cancer is increasing [1, 2]. Its incidence and mortality rate has been reported to be 3.4 and 2.5 per 100,000 people in the world [2]. In Malaysia, the incidence trends of CNS tumors also have been increasing by years, and it is estimated to be 9.8% in 2020 [3]. Despite the great progress that has been performed in understanding biology of these tumors and modern advances in treatments comprising in neurosurgical and radiotherapy techniques management, the 5-year survival rate for primary malignant CNS tumors is one of the worst among all human cancers.

Primary brain tumors comprise many diverse tumor types originating from the brain parenchyma or meninges, which fall under the WHO classification scheme of tumors [4]. They can be divided into two subcategories based on whether the site of the origin (the localization of the tumor developed) is intra-axial (intraparenchymal) or extra-axial (outside the brain substance). Intra-axial tumors are defined as the lesions that develop within the brain parenchyma. The most common intra-axial tumor types are gliomas. Extra-axial tumors are lesions, which originate outside the brain substance commonly arise from the skull, meninges, or tissues other than the brain parenchyma. The most prevalent extra-axial tumor types are meningiomas, craniopharyngiomas, and schwannomas.

The progression of brain tumors has been implicated with multiple oncogenic events, involving several genetic and epigenetic defects in signaling pathways with a diversity of altered genes. The v-RAF murine sarcoma viral oncogene homolog B1 (*BRAF*) gene is involved in brain tumorigenesis since a few researchers for the first time have discovered the mutations of the *BRAF* gene in CNS tumors [5–7]. The human *BRAF* gene is located on 7q34, and its mRNA spans 2478-bp. It is 190-Kbp long consisting of 18 exons, which encodes the cytosolic 766 amino acids of serine-threonine protein kinase of the RAF family. The BRAF protein plays roles in modulating the RAS/RAF/MEK (mitogen-activated protein kinase)/ERK (extracellular signal-regulated kinase) signaling pathway (also known as the MAPK/ERK kinase pathway), which is responsible for a wide range of cellular functions, including cellular proliferation, survival, differentiation, migration, angiogenesis, and apoptosis [8]. Modulation of MAPK/ERK kinase pathway is crucial for the stability between extracellular signaling and gene transcription. Thus, mutations in *BRAF* trigger inappropriate activation of this pathway that ultimately can result in uncontrolled cell proliferation and tumorigenesis [8].

Following the pioneering work by the Davies's team regarding *BRAF* mutations in human cancer [5],

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*Correspondence: E-mail: azizmdy@yahoo.com
Abbreviations used: A II – astrocytoma WHO grade II; AA III – anaplastic astrocytoma WHO grade III; CNS – central nervous system; GG – ganglioglioma; PA – pilocytic astrocytoma WHO grade I; PCR – polymerase chain reaction; PXA III – anaplastic pleomorphic xanthoastrocytoma WHO grade III; RFLP – restriction fragment length polymorphism.

a substantial number of studies have discovered *BRAF* mutations in a variety of human solid cancer including melanomas [9–12], papillary thyroid carcinomas [13–16], colorectal cancers [17–19], prostate cancers [20–22], ovarian cancers [23–25], and brain tumors [6, 7, 26]. As stated in the database by Catalogue of Somatic Mutations in Cancer, *BRAF* mutations are harbored mostly in about 55% of papillary thyroid carcinomas, 45% of the melanomas, 13% of colorectal cancers and 8% of brain tumors (www.sanger.ac.uk/genetics/CGP/cosmic). The majority of studies noted that most *BRAF* mutations occurred within the kinase activation domain region that is restricted to exon 11 or 15. A missense mutation in exon 15 at nucleotide position 1799 of the *BRAF* gene accounts for over 90% of the mutations identified in cancer studies [27]. This point mutation (T to A transversion) turns the amino acid substitution at codon 600 from valine into glutamic acid (referred to as $BRAF^{V600E}$: nucleotide 1799 T > A; codon GTG > GAG). This mutation leads to an alteration in the conformation of the active kinase site that results in the high-level constitutive activation of the ERK pathway, contributing to tumorigenesis [28]. In addition to $BRAF^{V600E}$ mutation, another predominant defect in the *BRAF* gene is a chromosomal rearrangement, which is involved in a tandem duplication of 7q34 that transformed into a fusion between the previously undefined gene *KIAA1549* and the *BRAF* genes [29]. This novel *KIAA1549-BRAF* oncogenic fusion seems to be present in most low-grade glioma, particularly, the majority of pilocytic astrocytomas (PA) [29, 30] and predicts the best clinical outcome among pediatric low-grade astrocytomas [31].

The role of $BRAF^{V600E}$ mutation as a potential therapeutic target and biomarker of the progression of brain tumors has attracted the attention of the researchers in cancer field. Nevertheless, the data on this mutation in the Asian population, specifically from Southeast Asians are insufficient. To the best of our knowledge, no data are reporting the frequency of $BRAF^{V600E}$ mutation in the Malaysian population with brain tumors. In the present study, we aimed to evaluate the frequency of $BRAF^{V600E}$ mutation in a series of Malaysian patients with brain tumors and to assess the association between this mutation and clinic-pathological characteristics.

MATERIALS AND METHODS

Case series. The investigation was performed using freshly frozen brain tumor specimens obtained from 50 Malaysian patients who underwent the neurosurgical operation at the Department of Neurosciences, Universiti Sains Malaysia, Health Campus, in 2016–2019. Patients who received radiotherapy and chemotherapy were excluded from this study. A least two consultant neuropathologists confirmed the histopathological diagnosis and grading of tumor tissue samples according to the WHO classification of brain tumors. This study was conducted in line with the Declaration of Helsinki, with all patients provided written informed consent and was approved by the Research Ethics Committee of Universiti Sains Malaysia (IRB Reg. № 00004494). The control group represented normal human brain tissues collected from 20 archival paraffin-embedded autopsy tissues of motor vehicle fatal accident victims. A summary of patients' and tumor characteristics is presented in Table 1.

Genomic DNA extraction and quantification. Total genomic DNA was extracted from tumor tissues as per the manufacturer's instructions using the QIAamp DNA mini kit (QIAGEN, Hilden, Germany). The genomic DNA concentration was quantified using NanoDrop ND1000 spectrophotometry (Thermo Scientific, Waltham, MA, USA). All qualified DNAs were stored at –80 °C until analysis.

Polymerase chain reaction (PCR) amplification of BRAF codon V600E. A fragment in exon 15 of the *BRAF* gene spanning codon 600, was PCR-amplified using the specific primer pair. PCR primer sequences were as follows: Forward primer: GCTTGCTCTGATAGGAAAATGAG; Reverse primer: GTAACTCAGCAGCATCTCAGG. The expected amplicon length is 237 bp. The reaction mixture (20 µL) of the PCR contained: 100 ng of total DNA, 1.5 mM HF buffer, 0.5 µM of each primer, 200 µM of dNTPs, and 0.02 U/µl of Phusion high-fidelity DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA). The PCR cycles were performed using the following conditions: 30 cycles of 98 °C for 10 s, 58 °C for 30 s, 72 °C for 30 s, and final elongation for 2 min.

Restriction fragment length polymorphism (RFLP) analysis. Hotspot codon V600E mutation of the *BRAF* gene was analyzed by RFLP. TspRI restriction endonuclease (New England Biolabs, Ipswich, MA, USA) digestion was performed for all 237-bp *BRAF* PCR products based on the manufacturer's instructions. Digestions were carried out in a total volume of 15 µL. The reaction mixture consisted of 3 µL of PCR product, 1 µL of restriction buffer (10X), 0.5 µL of restriction enzyme (10 U/µL) and the volume was adjusted with sterile distilled water. Then the digestion reaction was incubated for 20 min at 65 °C. The restriction fragments were analyzed on 4% MetaPhor™

Table 1. Demographic and clinic-pathological characteristics of patients

Patients' parameters	Number (n)	Percentage
Gender		
Male	27	54
Female	23	46
Age (years)		
< 40	28	56
⩾ 40	22	44
Mean	36.44	
Range	5–73 years	
Tumor type (grade)		
Intra-axial tumors	22	44
Pilocytic astrocytoma I	4	8
Astrocytoma II	5	10
Anaplastic astrocytoma III	2	4
Anaplastic pleomorphic xantho-astrocytoma III	3	6
Glioblastoma multiforme IV	8	16
Extra-axial tumors	28	56
Meningioma	21	42
Craniopharyngioma	5	10
Schwannoma	2	4

agarose gel in TBE buffer, stained with SYBR safe, and visualized under ultraviolet light. Wild-type *BRAF* showed in three bands of 117 bp, 87 bp, and 33 bp, whereas *BRAF*^{V600E} mutation resulted in four bands of 204 bp, 117 bp, 87 bp, and 33 bp.

Sanger DNA sequencing. Samples that displayed the mutation pattern in the RFLP assay were finally validated by Sanger DNA sequencing. PCR products for suspected samples were purified using a QIAquick PCR purification kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocols and sequenced by the same primers as that described in the PCR amplification steps. Sequencing was carried out using a Big Dye Terminator cycle sequencing kit (Applied Biosystems Inc., USA) on an ABI Prism 3700 DNA Analyzer automated sequencer (Applied Biosystems Inc., USA).

Statistical analysis. Statistical analysis was performed using GraphPad Prism software version 8.4.1 (Graphpad Software Inc., San Diego, CA, USA). The association between BRAF^{V600E} status and clinicpathological features was analyzed using Fisher's exact tests. The difference was considered statistically significant when the *p*-value was smaller than 0.05.

RESULTS

Detection of BRAF^{V600E} in brain tumors. A fragment of the *BRAF* gene, spanning the nucleotide 1799T>A mutation hotspot at codon 600, was amplified by PCR using the specific primers. A PCR amplicon of the expected size, 237 bp was observed in agarose gel electrophoresis (Fig. 1).

The RFLP assay was applied to use the TspRI digestion for the *BRAF* PCR amplicons encompassing 1799T>A in different fragments. In the RFLP fragment patterns, wild type yielded there fragments 117 bp, 87 bp, and 33 bp, while, the heterozygote *BRAF*^{V600E} mutant yielded four fragments of 204 bp, 117 bp, 87 bp, and 33 bp (Fig. 2).

In the case of *BRAF*^{V600E} detection in the RFLP assay, it was further validated by DNA sequencing. A comparison of results obtained using RFLP assay and DNA sequencing showed a consistent finding (Fig. 3). This *BRAF* mutation T to A transversion at nucleotide position 1799, converts the amino acid change from valine codon (GTG) into a glutamic acid codon (GAG) at amino acid position 600 (V600E) (see Fig. 3).

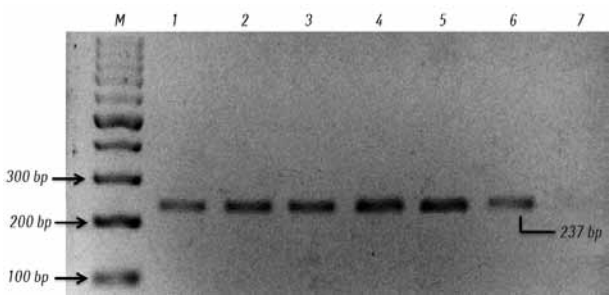

Fig. 1. Agarose gel electrophoresis of *BRAF* PCR products. Amplification products of the desired size of 237-bp were observed in 2% agarose gel. Lane M, 100-bp DNA marker; Lanes 1–6, tumor samples; Lane 7, negative control

Frequency of BRAF^{V600E} mutation and its clinicopathological association. Overall, 11 cases (22%) with *BRAF*^{V600E} mutation were identified by PCR-RFLP and then confirmed by Sanger sequencing. The *BRAF*^{V600E} mutation was detected in 2 (40%) of 5 As II, 1 (50%) of 2 AAs III, 2 (66.7%) of 3 PXAs III, 3 (37.5%) of 8 GBMs, 2 (9.5%) of 21 meningiomas and 1 (20%)

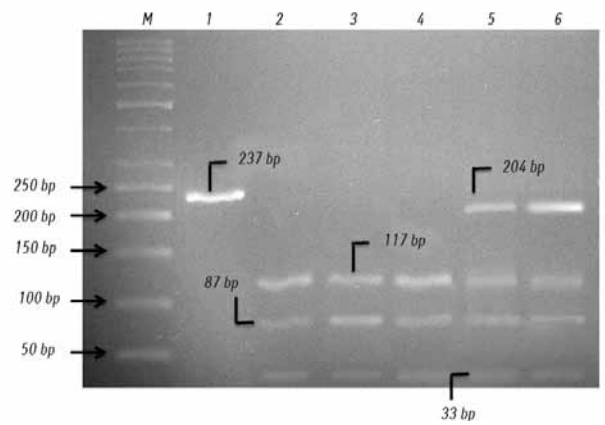

Fig. 2. PCR-RFLP assay of *BRAF*^{V600E} obtained by 4% MetaPhor™ agarose gel electrophoresis. Lane M, 50-bp DNA ladder; Lane 1, undigested product (237-bp); Lanes 2, normal control; Lane 3–6, tumor samples. Three bands at 117 bp, 87 bp, and 33 bp indicate a wild-type. Four bands at 204 bp, 117 bp, 87 bp, and 33 bp indicate a heterozygous mutation

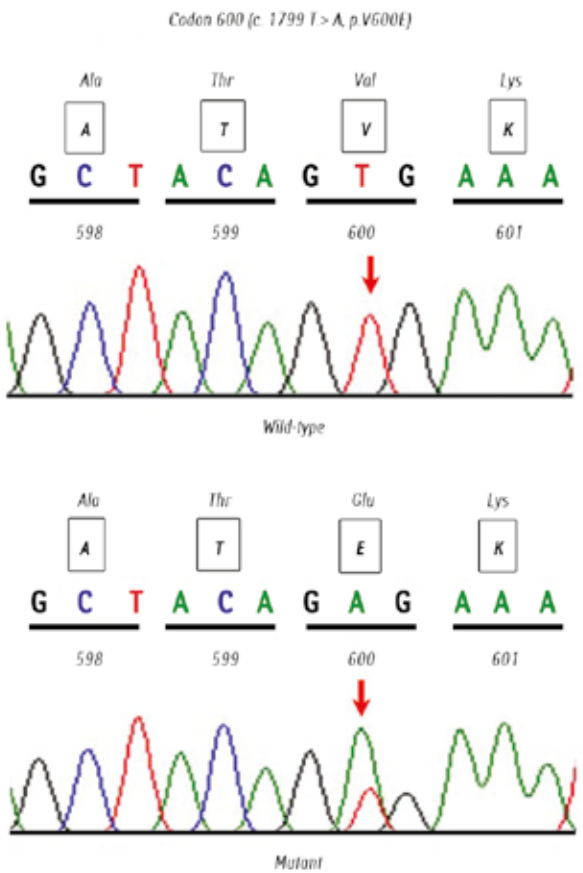

Fig. 3. Representative Sanger sequencing chromatogram demonstrating validation of *BRAF*^{V600E} that shows a heterozygous mutation of T to A transversion at nucleotide position 1799 (codon 600)

Table 2. Association between *BRAF*^{V600E} status with clinicopathological parameters

Patients' parameters (n = 50)	*BRAF*^{V600E} mutation (n = 11 (22%))	*BRAF*^{V600E} wild-type (n = 39 (78%))	*p*-value
Gender			1.000
Male	6 (22.2%)	21 (77.8%)	
Female	5 (21.7%)	18 (78.3%)	
Age (years)			0.7344
< 40	7 (25.0%)	21 (75.0%)	
⩾ 40	4 (18.2%)	18 (81.8%)	
Tumor type (grade)			0.0419*
Intra-axial tumors	8 (36.4%)	14 (63.6%)	
Pilocytic astrocytoma I	0 (0)	4 (100%)	
Astrocytoma II	2 (40%)	3 (60%)	
Anaplastic astrocytoma III	1 (50%)	1 (50%)	
Anaplastic pleomorphic xanthoastrocytoma III	2 (66.7%)	1 (33.3%)	
Glioblastoma multiforme IV	3 (37.5%)	5 (62.5%)	
Extra-axial tumors	3 (10.7%)	25 (89.3%)	
Meningioma	2 (9.5%)	19 (90.5%)	
Craniopharyngioma	1 (20%)	4 (80%)	
Schwannoma	0 (0)	2 (100%)	

Note: *$p < 0.05$.

of 5 craniopharyngiomas. No *BRAF*^{V600E} mutation was found in any cases of PA or schwannoma.

The clinicopathological features of the patients with and without *BRAF*^{V600E} mutation are summarized in Table 2. We examined the relation between *BRAF*^{V600E} mutation status and various clinico-pathological features, including age at diagnosis, types of tumor, and gender. Regarding the tumor types, we distributed the patients into two groups based on the tumor localization — intra-axial and extra-axial.

In this study, we found that *BRAF*^{V600E} mutation was significantly associated with intra-axial tumor patients ($p = 0.0419$). We revealed that *BRAF*^{V600E} mutation was more common in the intra-axial tumor (36.4%) than the extra-axial tumor group (10.7%)

DISCUSSION

BRAF activating mutation (*BRAF*^{V600E}) with the substitution of valine to glutamic acid at amino acid 600, is a well-established molecular biomarker worldwide and has been identified in various cancers with variable frequency. After the initial discovery of *BRAF*^{V600E} mutation in brain tumors (glioma cell lines) [5], many attempts were carried out to define the possible role of this mutation and to associate it with clinico-pathological characteristics/features of brain tumor cases. In one of the earliest studies, this hotspot mutation was identified in two cases of glioblastoma as well as in one gliosarcoma case [6]. Basto *et al*. [7] reported 6% of *BRAF*^{V600E} mutation in 34 glioblastomas. Since then, various groups of scientists have started research with more comprehensive mutational analysis to detect *BRAF* gene mutations in brain tumors. There are only few studies having been performed with large numbers of primary brain tumor patients in determining the prevalence of *BRAF*^{V600E} mutation. So far, two large case series of primary brain tumors have been conducted, mainly by Schindler *et al*. [32] who screened 1,320 CNS tumors and revealed 7% (96/1320) cases of mutation, and in the study of Behling *et al*. [33] on 969 analyzed tumor cases, only 1% (7/784) of the tumors had a *BRAF*^{V600E} mutation.

The *BRAF*^{V600E} mutation has been reported in different types of adult and pediatric primary brain tumors with diverse rates, including PA (< 18% cases) [32, 34–44], pleomorphic xanthoastrocytomas (PXA; 25–78% cases) [32, 35, 37, 38, 41–52], gangliogliomas (GG; 18–88% cases) [32, 35, 37, 38, 40–43, 45, 53–61], astrocytomas WHO grade II (A II; 3–44% cases) [33, 34, 43, 56, 57, 62–64], anaplastic astrocytomas WHO grade III (AA III; 3–33% cases) [32, 37, 62, 64, 65], glioblastoma WHO grade IV (GBM; 2–38%) [6, 7, 32, 37, 42, 46, 57, 62, 64–68], epithelioid glioblastoma (E-GBM; 17–93% cases) [33, 51, 68–73], desmoplastic infantile astrocytoma/ganglioglioma (DIA/DIG; 6–75%) [45, 54, 74–76], dysembryoplastic neuroepithelial tumor (DNET; 5–97%) [38, 42, 48, 54, 56, 60], craniopharyngiomas (CP; 15–94% cases) [77–80], and only a small number reported cases in astroblastoma [81] and meningiomas [33, 82]. The *BRAF*^{V600E} mutation also has been characterized by Chatterjee *et al*. [83] as a common event in the non-infantile variant of DIA/DIG. Published previous reports of *BRAF*^{V600E} mutation in primary brain tumor types are summarized in Supplementary Table S1 online.*

In our study, 22% (11 out of 50 patients) of the patients were found to harbor a *BRAF*^{V600E} mutation. Our research is one of the first efforts to study the frequency of *BRAF*^{V600E} mutation in Malaysian patients and subsequently to correlate it with clinicopathological parameters of brain tumor cases. There is a lack of data from Malaysia regarding *BRAF* mutation status, and when literature searches were performed via Pubmed, Google Scholar, and other relevant resources/databases, no information or published data exist concerning *BRAF*^{V600E} mutation in brain tumors in Malaysia to date. For the past few years, scientists from Asian countries have paid intensive attention to the *BRAF*^{V600E} mutation in different kinds of primary brain tumors. A study performed in China revealed that 67.9% of PXA cases had the *BRAF*^{V600E} mutation [52], while another research in India reported a 30% mutation frequency in GG (I) [59]. In a distinct study also conducted in China stated the rate of *BRAF*^{V600E} muta-

*The Supplementary Material for this article can be found online at: https://www.researchgate.net/ publication/351989923_Supplementary_Table_1_S1_Previously_reported_BRAF_V600E_mutation_in_CNS_tumors_summary_literature_reports_AuthorsReferences_Tumor_Types_BRAFV600E_mutation_of_cases

tion was as high as 44.4% in brainstem GG (II) [58]. The frequency of *BRAF*^{V600E} mutation observed in our series is close to the one reported by Mung *et al*. [37] in a series of 223 CNS tumors from a Korean population, determining the frequency at 16.1% of *BRAF*^{V600E} mutation. The low frequency of BRAF^{V600E} mutation in brain tumor cases has also been identified in Asian countries, including Japan (5.1%) and China (5.9%) patients, in two previous studies by Hatae *et al*. [42] and Chan *et al*. [64], respectively.

Indeed, the role of *BRAF* gene has been intensively studied predominantly in the western countries in order to clarify the adverse effect of *BRAF* mutations in the western population, in particularly in the USA. Schiffman *et al*. [62] studied the *BRAF*^{V600E} mutation in 41 pediatric gliomas and identified 17.1% (7/41) of mutation in overall cases. Moreover, other studies from the United States by Behling *et al*. [33] and Ballester *et al*. [57] reported the lower frequency of *BRAF*^{V600E} mutation in primary brain tumors with a rate of 1% (7/784) and 3.2% (12/381), respectively. The other studies, outside Asian, such as Canada, Portugal, and Germany, revealed a frequency of *BRAF*^{V600E} mutation in pediatric LLG at 17% [43], pediatric gliomas at 16% [44], and CNS tumors at 7% [32], respectively.

Some reports showed a negative finding of *BRAF*^{V600E} mutation in brain tumor patients. Previous studies carried out by groups among a Greece population [84] and an Indian population [85] failed to show the *BRAF*^{V600E} mutation in all brain tumor samples studied. Some of the possibilities for the observed discrepancies between these various studies included etiological factors, as well as the various demographic features of the patient populations in different parts of the world, which could have influenced the genetic predisposition and consequently affected the variation of *BRAF*^{V600E} mutation rates.

In the present study, we found a high *BRAF*^{V600E} mutation frequency in APXA (66.7%) and AA III (50%), an average frequency ranges from 20% to 40% in CP, GBM, and A II, and a low frequency in meningiomas (9.5%). Our results were remarkably consistent across the studies described by Dias-Santagata *et al*. [46], Schindler *et al*. [32], Myung *et al*. [37], Chappé *et al*. [38], Ida *et al*. [47], Lohkamp *et al*. [50], Hatae *et al*. [42], Lassaletta *et al*. [43] and Ma *et al*. [52], where *BRAF*^{V600E} mutation was predominantly observed in PXA and APXA with a high-frequency range from 60% to 83%. In the present study, the *BRAF*^{V600E} mutation was found in 40% of A II cases which is in line with the previously published report of Lassaletta *et al*. [43]. Meanwhile, the *BRAF*^{V600E} mutation seems to occur far less frequently in GBM. However, it has been reported fairly common in GBM with the epithelioid variant [51, 72, 73]. Our findings were similar to those of Tosuner *et al*. [68], who also discovered the mutation frequency in 37.5% of GBMs.

We observed a lower mutation rate of *BRAF*^{V600E} in meningiomas, where only 2 cases were detected. *BRAF* mutations are believed to be extremely rare in meningiomas, and only a limited number of studies have been carried out so far. Schindler *et al*. [32] previously reported no existence of *BRAF*^{V600E} mutation in 71 analyzed meningiomas. Moreover, Behling *et al*. [33] yielded a lower rate of *BRAF*^{V600E} mutation with 0.85% in all meningiomas grade I to III. This is in agreement with the work of Pepe *et al*. [82], who found less frequent *BRAF* mutations (8.7%) in grade I meningiomas. We also found the *BRAF*^{V600E} mutation in 20% (1/5) of CP cases. Past researches suggested that 15–94% of patients with CP tumors harbor *BRAF*^{V600E} mutations with the most common in the papillary variant [77–80].

In the present study, no *BRAF*^{V600E} mutation was found in all 4 cases of PA. Similarly, Schiffman *et al*. [62] were also unable to uncover any PAs to be mutated with *BRAF*^{V600E}. In another study, Faulkner *et al*. [30] performed immunohistochemistry assay to detect BRAF^{V600E} mutation in 32 PA patients and found no mutation in the cohort. Formerly, the *BRAF*^{V600E} mutation rate has been described in a low percentage in PA [26]. Among those studies of *BRAF*^{V600E} mutation in PA, Myung *et al.* [37] and Bannykh *et al*. [39] had published the highest rate of *BRAF*^{V600E} mutation in PA with 15.6% and 17.6%, respectively.

Apart from PA, as consistent with the previous findings from Schindler *et al*. [32] and Behling *et al*. [33], we also did not identify any *BRAF*^{V600E} mutation in our schwannoma cases. We believed that *BRAF*^{V600E} mutation could be an extremely rare event that occurred in these kinds of tumors. However, in contrast to these findings, Serrano *et al*. [86] revealed that 3 out of 16 (18.7%) sporadic schwannoma patients had a *BRAF*^{V600E} mutation.

In the present study, we further divided our brain tumor patients into two subcategories according to the location of tumor, namely, brain parenchyma or meninges. We observed a significant association between *BRAF*^{V600E} mutation and the localization of the tumor category which means that *BRAF*^{V600E} mutation was discovered more often in intra-axial tumors compared to extra-axial tumors (p = 0.0419). There have not been many reports on the association of *BRAF* alterations with tumor location of brain tumors. In 2015, Faulkner *et al*. [30] revealed that according to tumor location, patients with PA in the midline outside of the cerebellum were significantly more likely to harbor a *KIAA1549-BRAF* 15-9 fusion.

The *BRAF*^{V600E} mutation is believed to be present in a significant subset of cases of primary brain tumors [87]. It prognostic relevance seems to depend on the histological type, age of diagnosis, and localization of tumor. A study by Tabouret *et al*. [49] determined that PXA with mutated BRAFV600E conferred a favorable outcome, while in two separate studies by Dahiya *et al*. [53] and Ho *et al*. [88] reported that the presence of BRAFV600E mutations was considered indicators of a poor prognostic factor among GG and diencephalic pediatric low-grade glioma patients, respectively. Our finding was in contrast to a previous study in which histologic grade of brain tumors was not correlated with the *BRAF*^{V600E} mutation [37]. Furthermore, in accordance with previous studies by Myung *et al*. [37] and Frazão

et al. [44], the present study showed no statistically significant association between *BRAF*^{V600E} mutation with respect to patients' age and gender.

We determined that *BRAF*^{V600E} mutation frequency in the age group < 40 years was insignificantly higher compared with the age group > 40 (p = 0.7344). *BRAF*^{V600E} mutation frequency in females was slightly lower than in male patients, although not statistically significant (p = 1.000). However, some studies have reported a significant correlation between age or/and gender with *BRAF*^{V600E} mutation. Behling *et al*. [33] found that patients with *BRAF*^{V600E} mutated astrocytic tumors were significantly younger (mean age 15.3 years) compared to wild-type cases (58.2 years). In a study of glioneuronal tumors, Zhang *et al*. [60] detected that the *BRAF*^{V600E} mutation in females (58%) was significantly higher than that in male patients (17.4%). In another study, Chen *et al*. [58] reported that the *BRAF*^{V600E} mutation was significantly more frequently observed in female patients with diffuse gliomas of the mean age of 33.5 years.

The reasons for these diversities are unclear. However, these discrepancies might be explained by the difference in sample sizes and genetic admixture of the population studied. Besides that, another explanation is due to the different research methods/assays used to detect *BRAF*^{V600E} mutation. A variety of research methodologies is available for mutation detection, and in the present study, PCR-RFLP assay was performed, followed by Sanger sequencing for the verification of a *BRAF*^{V600E} mutation. We observed that PCR-RFLP assay is a simple, rapid, and inexpensive screening tool for a point mutation in brain tumor patients, and it is possible to detect our patients harboring a hot spot *BRAF*^{V600E} mutation. We also determined that Sanger sequencing confirmed all of the positive and negative of the PCR-RFLP results. Our study is in agreement with the previously reported use of these combination techniques for the detection of *BRAF*^{V600E} mutation associated with cancer cases [9, 13, 17].

Identification of *BRAF*^{V600E} mutation in a considerable rate of our patients indicates that this gene may play a role in the incidence of brain tumors in the Malaysian population. Further research with larger sample sizes, and perhaps with additionally analyzing the entire *BRAF* coding regions in all the patients, is truly needed to understand the mechanisms involved in the mutation of the *BRAF* gene in the brain tumorigenesis, particularly in the Malaysian population. Interestingly, some studies have demonstrated that the presence of *BRAF*^{V600E} alone is not enough to trigger gliomagenesis. Combination with other genetic alterations (such as *ATRX* inactivation, *CDKN2A/B* homozygous deletion, and *TERT* promoter mutation) may necessarily be required in promoting tumor development [73, 89, 90]. For the future, additional molecular and genetic markers analysis is worth investigating.

The advancement in the study of the mutation might be the starting point for future possible *BRAF*^{V600E} targeted therapy, which stated by Smith-Cohn *et al*. [91] to be beneficial for a variety of cancers such as melanoma and anaplastic thyroid cancer. In the meantime, high-level evidence for targeted therapy efficacy in primary brain tumors is still limited, as such minimal clinical benefit was observed for targeting *BRAF*^{V600E} in anaplastic PXA and glioblastoma patients from the United States.

The specific genetic diagnosis of brain tumors for *BRAF* mutations or fusions is essential in the context of emerging targeted therapies [92, 93]. The BRAF mutation-specific small molecule inhibitors have been effectively tested in metastasized melanoma. Patients harboring *BRAF*^{V600E}-mutated malignant melanoma metastases have displayed significant improvement in overall survival after treating with BRAF specific inhibitors [92, 94]. These inhibitors can also be considered to treat different cancer types, particularly CNS tumors with BRAF alterations. However, some research indicates that the treatment appears to be less effective in the CNS due to the existence of physical obstacles imposed by the blood-brain barrier that prevents sufficient drug delivery to the brain [92].

So far, responsiveness to BRAF (vemurafenib, dabrafenib)/MEK inhibitors has been observed in CNS tumor patients in some studies and clinical trials [65, 95, 96]. In 2018, Kaley *et al.* [96] conducted the VE-BASKET study, a non-randomized open-label multicohort study for *BRAF*^{V600}-mutant glioma patients. They found that several *BRAF*^{V600E} mutant tumor cases, mainly in PXA, exhibited the best response to vemurafenib treatment. Several ongoing early-stage clinical trials are now focused on targeted-treatment for the recurrent *BRAF*^{V600E}-mutant gliomas in children and young adults and pediatric primary low-grade gliomas using vemurafenib (NCT0174189) and dabrafenib with trametinib (NCT01748149) [92, 93]. A phase II clinical trial is now also underways for recruiting adult craniopharyngioma patients with BRAF-mutated tumors for vemurafenib and cobimetinib treatment (NCT03224767) [92, 93].

Up to now, there is uncertainty regarding the relative effectiveness of *BRAF*^{V600E} mutation-specific antibodies in the CNS. Thus, several attempts have been made for the evaluation of ERK signaling pathway activity in recurrent tumor tissue and cerebrospinal fluid following dabrafenib and/or trametinib treatment [92].

To sum up, this study is the first to be conducted in Malaysia regarding *BRAF*^{V600E} mutation in primary brain tumors in the Malaysian population. Despite that, the number of patients in the present study was small, the data show a considerable *BRAF*^{V600E} mutation rate in primary brain tumor patients, most significantly observed in the intra-axial tumors group. This study will provide information about the frequency or prevalence of *BRAF* gene mutations, which specifically will serve as baseline data for further research on the possible involvement of *BRAF*^{V600E} mutation in the brain tumorigenesis events in the Malaysian population.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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ЗНАЧЕННЯ МУТАЦІЇ BRAF^{V600E} ПРИ ІНТРААКСІАЛЬНИХ ПУХЛИНАХ МОЗКУ У ПАЦІЄНТІВ МАЛАЙЗІЇ: СЕРІЯ ВИПАДКІВ І ОГЛЯД ЛІТЕРАТУРИ

А.А. Мохамед Юсофф *, С. Абд Радзак, С.З.Н. Мохд Хаир, Дж.М. Абдулла

Відділення неврології, Школа медичних наук, Університет Сайнс Малайзія, Медичний кампус, Кубанго Керіан 16150, Келантан, Малайзія

Стан питання: До сьогодні мутації у гені *BRAF* у пацієнтів з пухлинами головного мозку не були охарактеризовані серед населення Малайзії. У численних дослідженнях повідомляється про існування основних мутацій у гені *BRAF* при різних типах раку. Міссенс-мутація в кодоні 600 ядерного онкогена *BRAF* (*BRAF*^{V600E}) є найбільш поширеною мутацією в «гарячій точці», яка була ідентифікована у численних злоякісних новоутвореннях людини. ***Мета:*** з'ясувати частоту мутації *BRAF*^{V600E} у серії малазійських пацієнтів з пухлинами головного мозку і встановити, чи існує зв'язок між мутацією *BRAF*^{V600E} і клініко-патологічними особливостями пацієнтів. ***Матеріал та методи:*** Мутаційний статус *BRAF*^{V600E} було проаналізовано у свіжозаморожених зразках пухлинної тканини, отриманих від 50 малазійських пацієнтів з пухлинами головного мозку, та визначено його кореляцію з клініко-патологічними особливостями пацієнтів (включаючи вік, стать і локалізацію пухлини, наприклад, інтрааксіальна — всередині речовини мозку, або позааксіальна: поза речовиною мозку). ***Результати:*** Загальна частота мутації *BRAF*^{V600E} склала 22% (11 з 50 пацієнтів). Наявність мутації *BRAF*^{V600E} достовірно корелювала з локалізацією пухлини, зокрема показано, що *BRAF*^{V600E} частіше зустрічалась у групі інтрааксіальних пухлин, ніж у групі екстрааксіальних пухлин. У цьому дослідженні ми також виявили, що пацієнти чоловічої статі були дещо більш сприйнятливі до мутації *BRAF*^{V600E}, і ця мутація переважала у пацієнтів вікової групи <40 років. Однак ці параметри не мали статистичної значущості. ***Висновок:*** Згідно отриманих даних, мутація *BRAF*^{V600E} достовірно частіше спостерігається у пацієнтів з інтрааксіальною пухлиною головного мозку, що може бути основою для подальших досліджень генетичних змін, які відбуваються під час прогресування пухлинного процесу у головному мозку у малайзійській популяції.

Ключові слова: пухлини головного мозку, інтрааксіальні пухлини, мутація *BRAF*^{V600E}, Малайзія.