RESEARCH ARTICLE

Association of The IDH1 C.395G>A (R132H) Mutation with Histological Type in Malay Brain Tumors

Abdul Aziz Mohamed Yusoff[1]*, Fatin Najwa Zulfakhar[2], Mohd Dasuki Sul'ain[2], Zamzuri Idris[1], Jafri Malin Abdullah[1,3]

Abstract

Background: Brain tumors, constituting one of the most deadly forms of cancer worldwide, result from the accumulation of multiple genetic and epigenetic alterations in genes and signaling pathways. Isocitrate dehydrogenase enzyme isoform 1 (IDH1) mutations are frequently identified in primary brain tumors and acute myeloid leukemia. Studies on IDH1 gene mutations have been extensively performed in various populations worldwide but not in Malaysia. This work was conducted to study the prevalence of IDH1 c.395G>A (R132H) hotspot mutations in a group of Malaysian patients with brain tumors in order to gain local data for the IDH1 mutation profile in our population. **Methods:** Mutation analysis of c.395G>A (R132H) of IDH1 was performed in 40 brain tumor specimens by the polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP) and then verified by direct sequencing. Associations between the IDH1 c.395G>A (R132H) mutation and clinicopathologic characteristics were also analyzed. **Results:** The IDH1 c.395G>A (R132H) mutation was detected in 14/40 patients (35%). A significant association was found with histological tumor types, but not with age, gender and race. **Conclusions:** IDH1 is frequently mutated and associated with histological subtypes in Malay brain tumors.

Keywords: Brain tumors- IDH1 mutation- Malaysia

Asian Pac J Cancer Prev, **17 (12)**, 5195-5201

Introduction

Worldwide, brain tumor is considered one of the most dramatic and rapidly fatal of all human cancers. About half of patients with brain tumors are still survive for at least a year after diagnosis. Brain tumor still ranked on the top as the biggest killer among cancers of children and young adults ages 20-39. The worldwide review of brain tumor by de Robles et al. (2014) using a random-effects model found that the overall incidence rate of all brain tumors to be 10.82 (95% CI: 8.63–13.56) per 100 000 person-years. In 2016, it is expected that nearly 23,770 new cases (13,350 males and 10,420 females) of brain and CNS tumors will be diagnosed and 16,050 (9,440 males and 6,610 females) will die due to this tumor in the United States (Siegel et al., 2016). In 2012, it was estimated that the age-standardised incidence rate of brain and CNS tumors in Malaysia to be 2.8 per 100,000 population/year with cumulative rate 0.3%. This rate was increasing faster until it became the third commonest pediatric cancer in Malaysia after leukemia and lymphoma (Lim et al., 2008; Goh et al., 2014).

Multiple oncogenic events have been recognized in the progression of brain tumor cells involving several defects in signaling pathways with a variety of mutated genes. Isocitrate dehydrogenase enzyme isoforms 1 (IDH1) has been shown to be associated with gliomagenesis since the first mutations of IDH1 were discovered in 2008 (Parsons et al., 2008). The IDH1 mRNA spans 2,402-bp and consists of 10 exons that encode the cytosolic 414 amino acids of isocitrate dehydrogenase enzyme. This enzyme catalyzes the conversion of isocitrate to α-ketoglutarate in mitochondrial kreb's cycle.

Mutations in IDH1 gene have been identified in a large proportion of World Health Organization (WHO) grade II astrocytoma (59- 88%) (Balss et al., 2008; Hartmann et al., 2009; Ichimura et al., 2009; Sanson et al., 2009; Watanabe et al., 2009; Yan et al., 2009; Ahmadi et al., 2012; Mukasa et al., 2012; Thon et al., 2012; Yao et al., 2013), grade III anaplastic astrocytoma (50- 78%), grade II and III oligodendrogliomas (49- 86%) (Balss et al., 2008; Hartmann et al., 2009; Ichimura et al., 2009; Sanson et al., 2009; Watanabe et al., 2009; Yan et al., 2009) and grade IV secondary glioblastomas (73- 90%) (Balss et al., 2008; Bleeker et al., 2009; Hartmann et al., 2009; Ichimura et al., 2009; Sanson et al., 2009; Watanabe et al., 2009; Yan et al., 2009; Polivka et al., 2014; Li et al., 2015). Moreover, IDH1 mutations have also been reported in patients with blood cancer, for instance, in acute myeloid leukemia and

*[1]Department of Neurosciences, School of Medical Sciences, [2]School of Health Sciences, [3]Center for Neuroscience Services and Research, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia. *For correspondence: azizmdy@yahoo.com*

myelodysplastic syndromes (Elsayed et al., 2014; Im et al., 2014; Jin et al., 2014; Molenaar et al., 2015; Platt et al., 2015)

Among the IDH1 mutations observed in cancer studies, over 90% are in codon 132 that cause a G to A transition at nucleotide position 395, resulting in the substitution of arginine for histidine (IDH1 R132H: nucleotide 395 G>A; codon CGT >CAT). This mutation at active site would lead to the conversion of α-ketoglutarate to the novel oncometabolite 2-hydroxyglutarate (2-HG), contributing to tumorigenesis.

IDH1 mutations have been proposed as predictive biomarkers which could be used in clinical practice to assess gliomas prognosis (Nobusawa et al., 2009; Sanson et al., 2009; Yan et al., 2012; Stupp et al., 2014). Studies have shown that IDH1 mutations are correlated with younger age, good prognosis, and sensitivity to treatment (Baldewpersad et al., 2013; Mondesir et al., 2016).

Since there is no study available from Malaysia, we sought to determine the frequency of IDH1 c.395G>A mutation in Malaysian brain tumor patients and to investigate its associations with other clinicopathological parameters.

Materials and Methods

Patients

Forty patients who were newly diagnosed with brain tumor and underwent surgery at the Department of Neurosciences, Hospital Universiti Sains Malaysia from April 2012 to February 2015 were recruited in this study. Patients who had received previous radiotherapy to the brain or chemotherapy for any reason were excluded. This study was approved by the Ethics Committee in Research of the institution. A pathologist reviewed and confirmed the brain tumor histological diagnosis of all cases based on World Health Organization (WHO) criteria. Fresh tumor samples from patients were snap-frozen in liquid nitrogen and immediately stored at -80 °C prior to DNA extraction. For comparative purposes, blood samples from 40 unrelated healthy controls free of brain tumors or other major of CNS tumors were also collected.

DNA extraction

Genomic DNA was extracted from patients' tumor tissue and blood samples obtained from healthy control using the QIAamp DNA mini kit (Qiagen, Germany) according to the manufacturer's instructions. The concentration, purity and quality of the extracted DNA were determined by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA) and 1% gel agarose electrophoresis. The extracted DNA was stored at −20°C until further use.

PCR-RFLP analysis of IDH1 c.395G>A (R132H) mutation

The IDH1 codon 132 at exon 4 was PCR-amplified using the specific mismatched primer pair as previously reported by Meyer et al. (2010). PCR primer sequences, 5'-TGG GTA AAA CCT ATC ATC ATC GAT-3' (forward) and 5'-TGT GTT GAG ATG GAC GCC TA-3' (reverse) were designed to amplify a 261-bp fragment that contains the R132H site of IDH1 (Figure 1). PCR was carried out in a total volume of 20µl, containing 100ng total DNA, 10mM of each primer, 200µM of dNTPs and 0.02U/µL of Phusion DNA polymerase (Thermo Fisher Scientific, USA). Thermocycling conditions were 98°C for 30s, followed by 35 cycles of 98°C for 30s, annealing for 15s at 55°C and extension at 72°C for 15s, and finally 72°C for 5min. Amplified PCR products were separated on 2% agarose gel, visualized with SYBR green stain.

The amplified PCR products were subjected to RFLP using the FastDigest PvuI (Thermo Fisher Scientific, USA) restriction enzyme for 30min at 37°C for enzyme digestion, and separated on 4% agarose gel stained with SYBR green. After completion digestion, the appearances of only one band upon electrophoretic separation which results 237-bp indicates that a wild type. Detection of a combination of two bands 261-bp and 237-bp reveals a mutation (Figure 2).

The samples that showed aberrant pattern in RFLP assay were reamplified from genomic DNA. Amplified products were purified using QIAquick PCR Purification kit (QIAGEN, Germany) and subjected to direct DNA sequencing analysis using the same primers as that used in the PCR reactions.

DNA sequencing analysis

Direct DNA sequencing of the purified PCR products was performed to confirm nucleotide alteration at position 395 (G-to-A substitution) in codon 132 using the same primers (including the forward and reverse primer) as that described in the PCR reactions. Sequencing was performed using a Big Dye Terminator cycle sequencing kit (Applied Biosystems, USA) on an ABI Prism 3700 DNA Analyser automated sequencer (Applied Biosystems, USA). The DNA sequence results were analyzed and aligned using the BioEdit Sequence Alignment Editor Version 7.0.5.3 (Ibis Bioscience, USA).

Statistical analysis

The statistical analysis was carried out using GraphPad Prism software version 5 (Graphpad Software, CA, USA). The descriptive statistics analysis was performed for general data presentation (median and range, mean and standard deviation and frequency (%)). The Chi-square test was used to analyze the association of IDH1 c.395G>A (R132H) mutation with the clinicopathological parameters (age, gender, race and histological subtypes) of the patients. Statistical significance was defined as $p<0.05$.

Results

Clinicopathological features

Among the 40 patients, 23 were male and 17 were female. The age ranged between 3 and 68 years old, with a median of 47.5 years (Table 1). Of all these patients, 2 patients diagnosed with pilocytic astrocytoma WHO grade I, 3 astrocytoma WHO grade II, 14 glioblastoma multiforme WHO grade IV, 1 ependymoma WHO grade II, 2 anaplastic ependymoma WHO grade III, 3 anaplastic oligodendroglioma WHO grade III, 11 meningioma

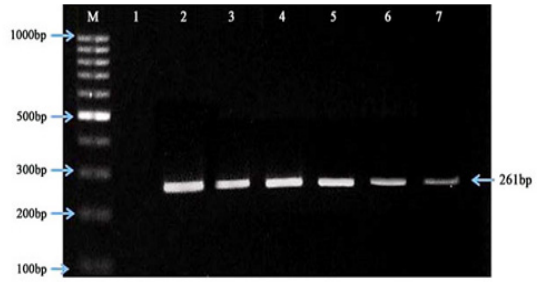

Figure 1. Agarose Gel Electrophoresis of IDH1 PCR Products. Amplification products of the expected size of 261-bp were detected in 2% agarose gels; Lane M, 100bp DNA marker; Lane 1 , Negative control; Lane 2-7, tumor samples

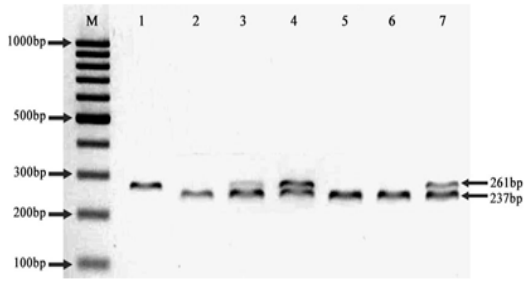

Figure 2. Detection of IDH1 c.395G>A Mutation by PCR-RFLP Analysis. Fragment of IDH1 Gene Digested with Pvu1 Enzyme. Lane M, 100bp DNA marker; Lane 1, undigest product; Lane 2, normal control; Lane 3-7, samples from patients; Patients 5 and 6 show only one band corresponding to wild type IDH1; Patients 3, 4 and 7, show mutant band carried by the patients; *one band at 237-bp indicates a wild type; *two bands at 261-bp and 237-bp indicate a mutation

WHO grade I, 2 medulloblastoma WHO grade IV, 2 schwannoma WHO grade I.

Amplification, PCR-RFLP screening and sequencing for IDH1 c.395G>A (R132H)

Figure 1 shows the resolution of the amplified fragment of the IDH1 gene on a 2% agarose gel.

Figure 1 shows the results of PCR amplification of IDH1 using the sample of brain tumor from patients. With the sequence-specific mismatched primer, fragment of the expected size was successfully amplified by PCR. The PCR-RFLP analysis for the IDH1 c.395G>A (R132H) mutation is presented in Figure 2. The expected RFLP patterns were successfully obtained after completion digestion and separation on agarose gel. To confirm the RFLP results, direct DNA sequencing was performed to the selected samples. DNA sequencing results were consistent with the pattern obtained by RFLP assay in all cases, where all patients with IDH1 mutation had the same replacement of nucleotide G to A (Figure 3). This G to A transition at nucleotide position 395, converts the amino acid change from arginine codon (CGT) into a histidine codon (CAT) at amino acid position 132 (R132H).

Frequency of IDH1 mutation and its association with clinico¯pathological variables

Among the 40 brain tumors tested, 14 cases (35%) were identified with IDH1 c.395G>A (R132H) mutation. Moreover, all of these mutations were heterozygous

Table 1. Relationship between the IDH1 c.395G>A (R132H) Mutation Status and Clinicopathological Variables

Parameter	Total no. of patients (%)	IDH1 c.395G>A (R132H) mutation status		p-value
		Mutation	No mutation	
Number of patients	40 (100%)	14 (35%)	26 (65%)	
Age (yr)				
Mean	41.7±18.6			
Range	3-68 years			
≤40	15 (37.5%)	5 (33.3%)	10 (66.7%)	0.864
>40	25(62.5%)	9 (36.0%)	16 (64.0%)	
Gender				
Male	23 (57.5%)	10 (43.5%)	13 (56.5%)	0.191
Female	17 (42.5%)	4 (23.5%)	13 (76.5%)	
Race				
Malay	38 (95.0%)	13 (34.2%)	25 (65.8%)	0.648
Chinese	2 (5.0%)	1 (50%)	1 (50%)	
Histological tumor types (grade)				
Pilocytic astrocytoma WHO grade I	2 (5.0%)	0 (0%)	2 (100%)	0.01
Astrocytoma WHO grade II	3 (7.5%)	1 (33.3%)	2 (66.7%)	
Glioblastoma multiforme WHO grade IV	14 (35.0%)	9 (64.3%)	5 (35.7%)	
Ependymoma WHO grade II	1 (2.5%)	0 (0%)	1 (100%)	
Anaplastic Ependymoma WHO grade III	2(5.0%)	2 (100%)	0 (0%)	
Anaplastic Oligodendroglioma WHO grade III	3 (7.5%)	2 (66.7%)	1 (33.3%)	
Meningioma WHO grade I	11 (27.5%)	0 (0%)	11 (100%)	
Medulloblastoma WHO grade IV	2(5.0%)	0 (0%)	2 (100%)	
Schwannoma WHO grade I	2(5.0%)	0 (0%)	2 (100%)	

Table 2. Frequency of IDH1 Mutations in Gliomas in Asian Patients

Asian country	IDH1 mutated	IDH1 mutated rate	Reference
I) East Asian			
China	75/203	36.90%	Qi et al., 2011
	19/118	16.10%	Yan et al., 2012
	52/97	53.60%	Zhou et al., 2012
	111/193	57.50%	Qi et al., 2014
	108/203	53.20%	Zhang et al., 2014
	309/417	74.00%	Li et al., 2015
	488/811	55.20%	Wang et al., 2016
Japan	39/125	31.20%	Sonoda et al., 2009
	73/250	29.20%	Mukasa et al., 2012
	10/128	7.80%	Ohno et al., 2016
Korea	Apr-25	16.00%	Kang et al., 2009
	72/134	53.70%	Myung et al., 2012
	Oct-42	23.80%	Ha et al., 2013
II) South Asian			
India	46/100	46.00%	Jha et al., 2011
	31/74	41.90%	Thota et al., 2012
	Jun-32	18.70%	Das et al., 2013
	28/50	56.00%	Agarwal et al., 2013
II) Southeast Asian			
Malaysia	14/40	35.00%	Present study

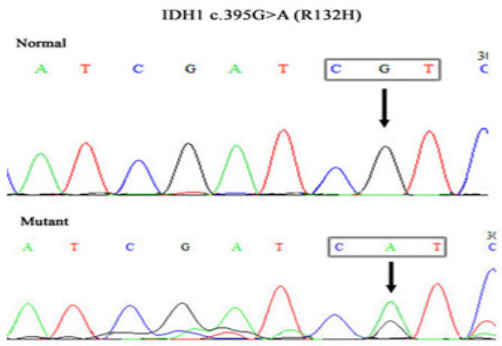

Figure 3. Sequence Results of IDH1 in the Brain Tumor Patient (Mutant) and a Control/Normal Individual. Electropherograms display a heterozygous mutation of G>A transition at nucleotide position 395; The square shows the nucleotides comprising a codon 132 of IDH1. This mutation results in amino acid exchange Arg → His (CGT → CAT)

mutations. None of the patient-matched blood samples and control group showed IDH1 c.395G>A mutation. Table 1 describes the association of IDH1 c.395G>A mutation status and its association with gender, age, race and histological tumor types of the patients.

No statistically significant relation between age of the patient and IDH1 c.395G>A mutation status was noted (p=0.864) even though IDH1 c.395G>A mutation was found to more apparent in the age group >40 (n = 9/25, 36.0%) compared with the age group ≤40 (n=5/15, 33.3%).

In our study, IDH1 c.395G>A mutation was more commonly seen in male. Out of the males included in the study, 43.5% (n=10/23) showed IDH1 c.395G>A mutation as compared to 23.5% (n=4/17) of females and the association was not statistically significant (p=0.191). A significant association was found between the IDH1 c.395G>A mutation with different histological tumor types (p=0.010). Glioblastoma multiforme WHO grade IV was identified to be the highest (n=9/14, 64.3%) compared with astrocytoma WHO grade II (n=1/14, 7.14%), anaplastic oligodendroglioma WHO grade III (n=2/14, 14.3%) and anaplastic ependymoma WHO grade III (n=2/14, 14.3%). Apart from above types, the other tumor types (pilocytic astrocytoma WHO grade I, ependymoma WHO grade II, meningioma WHO grade I, medulloblastoma WHO grade IV and schwannoma WHO grade I), did not reveal this mutation. Furthermore, we did not observe a significant relationship between IDH1 c.395G>A mutation with patient's race (p=0.648).

Discussion

Although the incidence of IDH1 gene mutations has been extensively studied worldwide in various ethnic groups, so far there was no information about the involvement or the role of the IDH1 gene in brain tumors among Malaysian patients. Also no data available even on any other types of cancer.

The majority of studies on IDH1 mutations have focused on western populations with gliomas and acute myeloid leukemia (Balss et al., 2008; Bleeker et al., 2009; Hartmann et al., 2009; Ichimura et al., 2009; Sanson et al., 2009; Watanabe et al., 2009; Ahmadi et al., 2012; Thon et al., 2012; Im et al., 2014; Polivka et al., 2014;

Molenaar et al., 2015; Platt et al., 2015). A large number of investigations on the role of the IDH1 gene have also been undertaken in Asian patients in order to clarify the contribution of IDH1 mutations in the Asian population. The investigations have been focused mainly in East and South Asian populations for instance Chinese (Zou et al., 2010; Qi et al., 2011; Lin et al., 2012; Yan et al., 2012; Zhou et al., 2012; Guan et al., 2013; Qi et al., 2014; Zhang et al., 2014; Li et al., 2015; Wang et al., 2016), Japanese (Mukasa et al., 2012; Sonoda et al., 2009; Ohno et al., 2016), Korean (Kang et al., 2009; Myung et al., 2012; Ha et al., 2013) and Indian (Jha et al., 2011; Thota et al., 2012; Agarwal et al., 2013; Das et al., 2013; Raveendran et al., 2015) patients but have not yet been carried out in Malaysian patients. The prevalence of IDH1 mutation in Asian patients with gliomas has been reported to be 7.8% to 74.0% (Table 2). In China, IDH1 mutations have been claimed to be present in about 16-74% of gliomas (Table 2), which is higher than what is reported in other Asian countries (Qi et al., 2011; Yan et al., 2012; Zhou et al., 2012; Qi et al., 2014; Zhang et al., 2014; Li et al., 2015; Wang et al., 2016).

The present study was the first in Malaysia to determine the genetic alteration aspect of IDH1 gene in brain tumors. In a total of 40 Malaysian patients with brain tumors was analyzed for mutations throughout the hotspot region, c.395G>A (R132H) of the IDH1 gene, using PCR-RFLP and then verified by direct sequencing. The c.395G>A point mutation, well-known to be a heterozygous missense mutation in IDH1 exon 4, resulting in a R132H amino acid substitution, is the most frequent and represents more than 90% of all the mutations identified in the IDH1 gene (Gupta et al., 2013).

In this study, the IDH1 c.395G>A (R132H) mutation was observed in 14 brain tumor patients with the rate of 35%. Our mutation rate more closely approximates those previously reported by Qi et al. (2011) in Chinese population (36.9%) and Sonada et al. (2009) in Japanese population (31.2%). Furthermore, our mutation rate is lower than other studies that reported by Li et al. (2015) (China; 74%), Myung et al. (2012) (Korea; 53.7%) and Agarwal et al., (2013) (india; 56%). Possible explanations for the difference in the mutation rate could be due to small sample size or differences in ethnicity. Malaysia is one of such multiethnic countries that located in the Southeast part of Asia, with about half the population is ethnic Malay. Most of the samples obtained in our study were from the Malay ethic group and only two from Chinese ethic group. There might be differences in genetic predisposition between Malay ethnicity and other Asian countries populations even though Southeast Asia seems to be more influenced by India and China background.

In the present study, IDH1 c.395G>A (R132H) mutation was found in a frequency as high as 64.3% in glioblastoma multiforme (IV) and also significantly associated between histologic types of the tumor ($p<0.05$). These findings were in agreement with a meta-analysis study by Zou et al., (2013) reported that IDH mutations were significantly correlated with glioma tumor grade and higher rate were found in GBM. However, we did not find a significant relationship between IDH1 c.395G>A (R132H) mutation and other clinicopathologic characteristics such as age, gender and race.

Several studies have shown an association between the IDH1 mutations and clinicopathologic and prognostic factors in brain tumors. Yan et al., (2012) ($p=0.002$), Li et al., (2015) ($p=0.041$), Ohno et al., (2016) ($p<0.001$), Ha et al., (2013) ($p=0.003$), Jha et al., (2011) ($p=0.001$), Thota et al., (2012) ($p<0.001$) and Das et al., (2013) ($p=0.047$) revealed the IDH1 mutation status was associated with younger age. However, Zhang et al., (2014) and Myung et al., (2012) reported that age was not associated with the IDH1 mutation.

In our study, the prevalence of mutation in patients aged >40 years was higher than in ≤40 years patients ($p=0.864$). However, there was no significant correlation in patients with old age. Most previous reports indicate that IDH1 mutations are found to be more evident in male gender (Yan et al., 2012; Agarwal et al., 2013; Qi et al., 2014; Zhang et al., 2014). We found a higher prevalence of mutation in the male gender in our study, although this is not statistically significant ($p=0.191$).

Studies have demonstrated that mutated IDH1 plays an important role in predicting of gliomas patient outcome (Yan et al., 2009; Ichimura et al., 2009; Sanson et al., 2009; Polivka et al., 2014; Ohno et al., 2016). In more recent meta-analysis study by Zou et al. (2013) and Chen et al. (2016) also confirmed the prognostic role of IDH1 mutations in gliomas. IDH1 mutations are a favorable prognostic indicator of improved overall survival time. Survival is significantly longer in glioma patients with IDH1 mutant than in those with wild-type IDH1 (Yan et al., 2009; Sanson et al., 2009; Myung et al., 2012; Yan et al., 2012; Yao et al., 2013; Polivka et al., 2014; Ohno et al., 2016). Furthermore, several studies have also revealed that IDH1 mutations predict good response to chemotherapy resulting in better survival for both low-grade (Houillier et al., 2010) and high-grade (SongTao et al., 2012; Ohno et al., 2016) gliomas cases. Our study had several limitations. This study was primarily limited due to the small sample size and the lack of follow-up period time as well as patient survival data. Therefore, our results could not be indicated that the presence of IDH1 c.395G>A (R132H) mutation was associated with worse or better prognosis. Larger patient group studies with the extended clinical follow-up and survival rate data are necessary for establishing the prognostic value of IDH1 mutation in brain tumor among Malaysian population. Further investigations of IDH gene mutation may be needed not only in the hotspot codon R132 of IDH1 but then should be expanded to another two IDH isoforms, IDH2 (hotspot codon R172) and IDH3, which both are mitochondrial isoforms of IDH.

In conclusion, we have identified 35% of IDH1 c.395G>A (R132H) mutation in Malaysian patients with the high grade of gliomas. Its frequency varies greatly among different ethnic groups. Based on our search to the previous published reports on IDH1 mutations, we are confident that this is the first investigation of IDH1 mutation in a Southeast Asia population involving Malaysian brain tumor patients. Although a relatively small number of patients were investigated, we believe that our finding is able to provide a new important

data on IDH1 gene mutations for Asian populations especially in the Southeast Asia region. Identification of IDH1 mutations can serve as a diagnostic indicator and prognostic marker in brain tumor of Malaysian patients.

Acknowledgments

We would like to thank the patients for their participation and cooperation in providing tissue samples and information. The financial assistance was supported by the Research University Grant (RUI) (1001/PPSP/812110) (Universiti Sains Malaysia); the Fundamental Research Grant Scheme (FRGS) (203/PPSP/6171161) (Ministry of Higher Education of Malaysia).

Statement conflict of Interest

All authors declare no conflicts of interest.

References

Agarwal S, Sharma MC, Jha P, et al (2013). Comparative study of IDH1 mutations in gliomas by immunohistochemistry and DNA sequencing. *Neuro Oncol*, **15**, 718-26.

Ahmadi R, Stockhammer F, Becker N, et al (2012). No prognostic value of IDH1 mutations in a series of 100 WHO grade II astrocytomas. *J Neurooncol*, **109**, 15-22.

Baldewpersad Tewarie NM, Burgers IA, Dawood Y, et al (2013). NADP+ -dependent IDH1 R132 mutation and its relevance for glioma patient survival. Med Hypotheses, 80, 728-31.

Balss J, Meyer J, Mueller W, et al (2008). Analysis of the IDH1 codon 132 mutation in brain tumors. *Acta Neuropathol*, **116**, 597–602.

Bleeker FE, Lamba S, Leenstra S, et al (2009). IDH1 mutations at residue p.R132 (IDH1(R132)) occur frequently in high-grade gliomas but not in other solid tumors. *Hum Mutat*, **30**, 7-11.

Chen JR, Yao Y, Xu HZ, et al (2016). Isocitrate dehydrogenase (IDH)1/2 mutations as prognostic markers in patients with glioblastomas. *Medicine (Baltimore)*, **95**, e2583.

Das BR, Tangri R, Ahmad F, et al (2013). Molecular investigation of isocitrate dehydrogenase gene (IDH) mutations in gliomas: first report of IDH2 mutations in Indian patients. *Asian Pac J Cancer Prev*, **14**, 7261-4.

de Robles P, Fiest KM, Frolkis AD, et al (2015). The worldwide incidence and prevalence of primary brain tumors: a systematic review and meta-analysis. *Neuro Oncol*, 17, 776-83.

Elsayed GM, Nassar HR, Zaher A, et al (2014). Prognostic value of IDH1 mutations identified with PCR-RFLP assay in acute myeloid leukemia patients. *J Egypt Natl Canc Inst*, **26**, 43-9.

Goh CH, Lu YY, Lau BL, et al (2014). Wong A. Brain and spinal tumour. *Med J Malaysia*, **69**, 261-7.

Guan L, Gao L, Wang L, et al (2013). The Frequency and clinical significance of IDH1 mutations in Chinese acute myeloid leukemia patients. *PLoS One*, **8**, e83334.

Gupta R, Flanagan S, Li CC, et al (2013). Expanding the spectrum of IDH1 mutations in gliomas. *Mod Pathol*, **26**, 619-25.

Ha SY, Kang SY, Do IG, et al (2013). Glioblastoma with oligodendroglial component represents a subgroup of glioblastoma with high prevalence of IDH1 mutation and association with younger age. *J Neurooncol*, **112**, 439-48.

Hartmann C, Meyer J, Balss J, et al (2009). Type and frequency of IDH1 and IDH2 mutations are related to astrocytic and oligodendroglial differentiation and age: a study of 1,010 diffuse gliomas. *Acta Neuropathol*, **118**, 469-74.

Houillier C, Wang X, Kaloshi G, et al (2010). IDH1 or IDH2 mutations predict longer survival and response to temozolomide in low-grade gliomas. *Neurology*, **75**, 1560-6.

Ichimura K, Pearson DM, Kocialkowski S, et al (2009). IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. *Neuro Oncol*, **11**, 341-7.

Im AP, Sehgal AR, Carroll MP, et al (2014). DNMT3A and IDH mutations in acute myeloid leukemia and other myeloid malignancies: associations with prognosis and potential treatment strategies. *Leukemia*, **28**, 1774-83.

Jha P, Suri V, Sharma V, et al (2011). IDH1 mutations in gliomas: first series from a tertiary care centre in India with comprehensive review of literature. *Exp Mol Pathol*, **91**, 385-93.

Jin J, Hu C, Yu M, et al (2014). Prognostic value of isocitrate dehydrogenase mutations in myelodysplastic syndromes: a retrospective cohort study and meta-analysis. *PLoS One*, **9**, e100206.

Kang MR, Kim MS, Oh JE, et al (2009). Mutational analysis of IDH1 codon 132 in glioblastomas and other common cancers. *Int J Cancer*, **125**, 353-5.

Li J, Zhang H, Wang L, et al (2015). Comparative study of IDH1 mutations in gliomas by high resolution melting analysis, immunohistochemistry and direct DNA sequencing. *Mol Med Rep*, **12**, 4376-81.

Li MY, Wang YY, Cai JQ, et al (2015). Isocitrate dehydrogenase 1 gene mutation is associated with prognosis in clinical low-grade gliomas. *PLoS One*, 10, e0130872.

Lim CC, Rampal S, Halimah Y (2008). Cancer incidence in Peninsular Malaysia, 2003-2005. Kuala Lumpur: National Cancer Registry; 2008.

Lin J, Yao DM, Qian J, et al (2012). IDH1 and IDH2 mutation analysis in Chinese patients with acute myeloid leukemia and myelodysplastic syndrome. *Ann Hematol*, **91**, 519-25.

Meyer J, Pusch S, Balss J, et al (2010). PCR- and restriction endonuclease-based detection of IDH1 mutations. *Brain Pathol*, **20**, 298-300.

Molenaar RJ, Thota S, Nagata Y, et al (2015). Clinical and biological implications of ancestral and non-ancestral IDH1 and IDH2 mutations in myeloid neoplasms. *Leukemia*, **29**, 2134-42.

Mondesir J, Willekens C, Touat M, et al (2016). IDH1 and IDH2 mutations as novel therapeutic targets: current perspectives. *J Blood Med*, **7**, 171-80.

Mukasa A, Takayanagi S, Saito K, et al (2012). Significance of IDH mutations varies with tumor histology, grade, and genetics in Japanese glioma patients. *Cancer Sci*, **103**, 587-92.

Myung JK, Cho HJ, Park CK, et al (2012). IDH1 mutation of gliomas with long-term survival analysis. *Oncol Rep*, **28**, 1639-44.

Nobusawa S, Watanabe T, Kleihues P, et al (2009). IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. *Clin Cancer Res*, **15**, 6002-7.

Ohno M, Narita Y, Miyakita Y, et al (2016). Glioblastomas with IDH1/2 mutations have a short clinical history and have a favorable clinical outcome. *Jpn J Clin Oncol*, **46**, 31-9.

Parsons DW, Jones S, Zhang X, et al (2008). An integrated genomic analysis of human glioblastoma multiforme. *Science*, **321**, 1807-12.

Platt MY, Fathi AT, Borger DR, et al (2015). Detection of dual IDH1 and IDH2 mutations by targeted next-generation sequencing in acute myeloid leukemia and myelodysplastic syndromes. *J Mol Diagn*, **17**, 661-8.

Polivka J, Polivka J Jr, Rohan V, et al (2014). Isocitrate dehydrogenase-1 mutations as prognostic biomarker in glioblastoma multiforme patients in West Bohemia. *Biomed*

Res Int, **2014**, 735659.
Qi S, Yu L, Li H, et al (2014). Isocitrate dehydrogenase mutation is associated with tumor location and magnetic resonance imaging characteristics in astrocytic neoplasms. *Oncol Lett*, **7**, 1895-1902.
Qi ST, Yu L, Lu YT, et al (2011). IDH mutations occur frequently in Chinese glioma patients and predict longer survival but not response to concomitant chemoradiotherapy in anaplastic gliomas. *Oncol Rep*, **26**, 1479-85.
Raveendran S, Sarojam S, Vijay S, et al (2015). Mutation analysis of IDH1/2 genes in unselected de novo acute myeloid leukaemia patients in India - identification of a Novel IDH2 mutation. *Asian Pac J Cancer Prev*, **16**, 4095-101.
Sanson M, Marie Y, Paris S, et al (2009). Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. *J Clin Oncol*, **27**, 4150-4.
Siegel RL, Miller KD, Jemal A, (2016). Cancer statistics, 2016. *CA Cancer J Clin*, **66**, 7-30.
SongTao Q, Lei Y, Si G, et al (2012). IDH mutations predict longer survival and response to temozolomide in secondary glioblastoma. *Cancer Sci*, **103**, 269-73.
Sonoda Y, Kumabe T, Nakamura T, et al (2009). Analysis of IDH1 and IDH2 mutations in Japanese glioma patients. *Cancer Sci*, **100**, 1996-8.
Stupp R, Brada M, van den Bent MJ, et al (2014). High-grade glioma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, **25**, 93-101.
Thon N, Eigenbrod S, Kreth S, et al (2012). IDH1 mutations in grade II astrocytomas are associated with unfavorable progression-free survival and prolonged postrecurrence survival. *Cancer*, **118**, 452-60.
Thota B, Shukla SK, Srividya MR, et al (2012). IDH1 mutations in diffusely infiltrating astrocytomas: grade specificity, association with protein expression, and clinical relevance. *Am J Clin Pathol*, **138**, 177-84.
Wang HY, Tang K, Liang TY, et al (2016). The comparison of clinical and biological characteristics between IDH1 and IDH2 mutations in gliomas. *J Exp Clin Cancer Res*, **35**, 86.
Watanabe T, Nobusawa S, Kleihues P, et al (2009). IDH1 mutations are early events in the development of astrocytomas and oligodendrogliomas. *Am J Pathol*, **174**, 1149-53.
Yan H, Parsons DW, Jin G, et al (2009). IDH1 and IDH2 mutations in gliomas. *N Engl J Med*, **360**, 765-73.
Yan W, Zhang W, You G, et al (2012). Correlation of IDH1 mutation with clinicopathologic factors and prognosis in primary glioblastoma: a report of 118 patients from China. *PLoS One*, **7**, e30339.
Yao Y, Chan AK, Qin ZY, et al (2013). Mutation analysis of IDH1 in paired gliomas revealed IDH1 mutation was not associated with malignant progression but predicted longer survival. *PLoS One*, **8**, e67421.
Zhang CB, Bao ZS, Wang HJ, et al (2014). Correlation of IDH1/2 mutation with clinicopathologic factors and prognosis in anaplastic gliomas: a report of 203 patients from China. *J Cancer Res Clin Oncol*, **140**, 45-51.
Zhou YX, Wang JX, Feng M, et al (2012). Analysis of isocitrate dehydrogenase 1 mutation in 97 patients with glioma. *J Mol Neurosci*, **47**, 442-7.
Zou P, Xu H, Chen P, et al (2013). IDH1/IDH2 mutations define the prognosis and molecular profiles of patients with gliomas: a meta-analysis. *PLoS One*, **8**, e68782.
Zou Y, Zeng Y, Zhang DF, et al (2010). IDH1 and IDH2 mutations are frequent in Chinese patients with acute myeloid leukemia but rare in other types of hematological disorders. *Biochem Biophys Res Commun*, **402**, 378-83.