Original Article

Association of Loss of Heterozygosity and *PTEN* Gene Abnormalities with Paraclinical, Clinical Modalities and Survival Time of Glioma Patients in Malaysia

Jafri Malin Abdullah, Ahmad Farizan, Ku Asmarina, Norafiza Zainuddin, Mazira Mohamad Ghazali, Hasnan Jaafar,[1] Mohd Nizam Isa[2] and Nyi Nyi Naing,[3] Departments of Neurosciences and [1]Pathology, and [3]Unit of Biostatistics and Research Methodology, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, and [2]Faculty of Medicine, University Technology MARA, Selangor, Malaysia.

BACKGROUND: The pattern of allelic loss of heterozygosity (LOH) and *PTEN* mutations appear to be associated with the progression of gliomas leading to a decrement in the survival rate of patients. This present study was carried out to determine the LOH and *PTEN* mutational status in glioma patients and its association with patients' survival.

METHODS: Thirty-seven Malaysian glioma patients of the Malay race were subject to *PTEN* mutational analysis and the presence of LOH using the cold single-strand conformation polymorphism method, and their clinical and paraclinical response were correlated.

RESULTS: Among analysed glioma patients, seven (21.6%) cases with *PTEN* mutations were detected and 12 (32.4%) of 37 patients showed presence of LOH. Univariate analysis showed that tumour grade, vascularization, *PTEN* mutation, LOH and combination of both *PTEN* mutation and LOH were significantly associated with glioma patients' survival. Multivariate analysis revealed that no factors contributed to survival time.

CONCLUSION: The results show that *PTEN* mutation and LOH are quite frequent in Malaysian glioma patients. However, they have no impact on the survival outcome of patients. [*Asian J Surg* 2006;29(4): 274–82]

Key Words: gliomas, LOH, Malaysian, prognosis, *PTEN* mutations, survival

Introduction

Malignant gliomas are the most common primary tumours of the brain.[1] The reported number of glioma cases in Malaysia are on the rise and has been increasing steadily over the years.[2] Over the decades, brain tumours, in particular glioblastoma multiforme (GBM), have retained their dismal prognosis despite advances in neurosurgical techniques, radiation and drug therapies. In particular, malignant GBM have defied all current therapeutic modalities. Gene therapy offers the potential to augment current neurosurgical, radiation and drug treatments with little increase in morbidity.[3]

The current interest in molecular genetics is to study the relationship between genetic alterations and how they contribute to the presenting illness, clinical and paraclinical findings as well as therapy and outcome.

Genetic alterations, which were continuously reported at high frequency in a variety of human tumours, including gliomas are loss of heterozygosity (LOH) at chromosome 10q23 and *PTEN* mutation. Loss of functional *PTEN* from tumour cells causes accumulation of critical second messenger lipids, leading to decreased apoptosis and/or increased mitogen signalling.[5]

Frequent allelic losses were reported in malignant astrocytoma, glioblastoma, anaplastic oligodendroglioma and ependymoma cases. In malignant astrocytoma, regular allelic losses were defined in chromosomes 10, 13q, 17p and 22q, suggesting the presence of tumour suppressor genes on these chromosomes.[6] They have also reported LOH cases on chromosome 17p in anaplastic oligodendroglioma samples. LOH on chromosomes 10 and 13q was found in most anaplastic ependymoma cases. While in glioblastomas, with partial loss of chromosome 10, at least three common deletions were found which are 10q14-pter, 10q23-24 and 10q25-qter, suggestive of multiple tumour suppressor genes.[6,7] Forty to sixty percent of reported deletions in glioblastoma cases occur on chromosome 9p21.[8]

Several studies have shown that LOH as well as *PTEN* mutation are correlated with poor prognosis in glioma patients.[5,9,10] They suggested that by losing function of those regions, the survival rates of the patients decreased.

The aim of this prospective study was to determine the relationship of *PTEN* mutation and LOH in gliomas of a group of Malaysian patients in relation to their clinical, radiological and pathological documentation response to therapy, as well as the survival time of the patients.

Patients and methods

Thirty-seven patients were studied over a period of 36 months. Parameters such as age, sex, classification of the tumours and presenting complaints such as headache, hemiplegia, epilepsy as well as radiological images, i.e. site, size, consistency of tumour, midline shift and vascularity, were compared with their management, *PTEN* mutation, LOH status, relapse rate and outcome. Social groups were divided into economic upper class (> US$250), middle class (US$100–250) and poor (< US$100).

All patients underwent magnetic resonance imaging (MRI) GE 1 Tesla (GE Healthcare, Chalfont St Giles, Bucks, UK) prior to surgery and received gadolinium following certain protocols. The location of tumours was recorded according to the respective lobes and sites.

Size of tumours was measured using the standard software provided by the GE MRI machine. The consistencies of tumours were reported by two blinded radiologists as defined by a Japanese study.[11] Calcification was detected by computed tomography (CT) scan of the brain with a Hounsfield value of between 100 and 300 H.U.[12] Haemorrhage in the tumour was defined as any hyperdensity (Hounsfield between 75 and 80 H.U.) measured using the classical protocol. Midline shift was defined as any deviation of the midline, taking the pineal gland as the centre.[13]

Vascularity was defined as any tumour blush seen on MRI[14] as seen by two blinded neuroradiologists. All patients underwent total removal of tumours confirmed by repeat CT scan with contrast within 24 hours of operation. If the tumour was still present, a reoperation was done to remove all tumour, confirmed again by CT scan of the brain with contrast. Treatment modalities were chemotherapy, radiotherapy and immunotherapy as requested and agreed by both radiation therapist/oncologist and patient. Recurrence on CT/MRI was defined as any disease or lesion returning, or showing a tendency to return from time to time, within the study period. Relapse of signs and symptoms were defined as the reoccurrence of symptoms similar to the previous complaint or signs seen again on physical examination without the knowledge of the radiological results.

PTEN mutations and LOH status were evaluated via various methods mentioned below. Outcome was defined as alive in good condition with a Karnofsky score of > 70, alive in poor condition, being unable to care for oneself completely with a score < 70 and dead.[15]

All patients were operated on and had a Karnofsky score > 70 prior to their inclusion in this study. All histopathological examinations were reported according to the World Health Organization (WHO) classification[16] and seen by at least three histopathologists and discussed in our neuropathology conference, and all *PTEN* and LOH specimens were analysed twice to rule out false or negative results.

Tumour specimens and DNA extraction
Thirty-seven specimens (human brain tumour tissues) from individual patients referred to Universiti Sains

Malaysia Hospital, Kelantan, Malaysia were collected. The tumours were classified according to WHO classification as eight astrocytoma (grade I), seven astrocytoma (grade II), nine anaplastic astrocytoma (grade III), seven GBM (grade IV), one oligodendroglioma, one ependymoma, one pleomorphic xanthoastrocytoma, four anaplastic oligodendroglioma and two anaplastic ependymoma. Genomic DNA was extracted from human tumour tissues using commercial extraction kits (QIAGEN Inc., Valencia, CA, USA). Normal samples were extracted from blood using commercial extraction kit (QIAGEN Inc.).

Polymerase chain reaction (PCR)–LOH analysis using microsatellite markers

LOH was detected in DNA samples using highly polymorphic microsatellite markers on chromosomes 10q, D10S532 (10q11), D10S541 (10q23) and D10S216 (10q25); chromosomes 9p21, D9S165 (9p21) and D17S1176 and chromosomes 13q12, D13S289 and D13S171 (Genome Data Base). Nonradioactive PCR was performed in a reaction mixture of 50 μL. Thirty cycles of PCR were performed using Gradient Mastercycler (Eppendorf). PCR products were loaded onto a denaturing polyacrylamide gel and electrophoresed by using a sequencing gel apparatus. The gel was then visualized by silver staining. All samples in which two distinct alleles of similar intensity were present in the normal DNA were considered to be informative. LOH was scored as positive when there was a clear reduction of signal intensity (or more than 50%) detected in one of the alleles of the tumour DNA compared with the paired normal DNA.[17]

PCR–single-strand conformation polymorphism (SSCP) analysis of PTEN gene

Prescreening for mutations on the *PTEN* gene was carried out by PCR–SSCP analysis of exons 5, 6 and 8 using previously described primers.[18] All PCR were performed with a Gradient Mastercycler (Eppendorf) in 50 μL volume assays, with 6% dimethyl sulfoxide for the primer sets of exon 5. After an initial denaturation at 94°C for 3 minutes, 30 cycles of denaturation (94°C for 1 minute), annealing (55°C for the primer set of exon 5 and 58°C for the primer set of exons 6 and 8) and extension (72°C for 1 minute) were performed. The final extension was performed for 10 minutes.

For PCR–SSCP analysis, 10 μL of heat-denatured samples of exon 6 were loaded onto a mutation detection enhancement gel (FMC Corp., Rockland, ME, USA) containing 10% glycerol, while samples of exons 5 and 8 were analysed using an 8% polyacrylamide gel containing 10% glycerol. Gel electrophoresis was performed using sequencing gel apparatus (dCode™ BIORAD; Bio-Rad Laboratories, Hercules, CA, USA) at 10–15 W for 16 hours. The temperature was maintained at 22°C using the apparatus. The gel was then visualized by silver staining (modification of Echt et al) after completion of electrophoresis. Samples that showed mobility shifts or aberrant band patterns compared to normal DNA were further analysed by the direct DNA sequencing (BioSynTech Sdn Bhd, Selangor, Malaysia).

Survival analysis for survival time

The comparison of survival time between groups was done by using log rank test. Univariate Cox proportional hazards regression was applied to determine potential prognostic factors of survival time. Level of significance was set at 0.25 and variables that were less than 0.25 were included in the multivariate analysis.

Multivariate Cox proportion regression was used to identify prognostic factors of survival time of brain tumour patients. Backward stepwise Cox regression was used. Likelihood ratio (LR) test was applied to determine significance of the model. The strength of association was determined by hazards ratio with its 95% confidence interval (CI). Log minus log plot and partial residual were applied to check the proportional hazards assumption of the final model. The results were presented by crude and adjusted hazards ratio, 95% CI, LR statistic and *p* value.

Results

Patient characteristics

There were 37 patients admitted and followed up for 3 years. The majority of patients were young (73% < 40 years old), with males being slightly more prevalent than females. All the patients were Malays with no family history of tumour, and the majority were from low social economic groups.

Among the four types of tumour, WHO grade III gliomas were the most common (54.1%) and grade IV gliomas were the least common (10.8%); 81% of patients complained of headache, 48.6% had hemiplegia and 29.7% had epilepsy.

Based on MRI or CT, 10.8% of patients had tumour located in the left temporal lobe, 32.4% in the left frontal and parietal lobe, 2.7% in the right temporal lobe, 13.5%

in the right frontal and parietal lobe, and the rest were located in other sites including cerebellum, fossa, midbrain, spine and medulla.

More than half (78.4%) of the tumours were > 2 cm in size, and the remainder were ≤ 2 cm in size. Angiovascularity was present in 62.2% of cases. Two modalities were instituted for patients after surgery: radiotherapy (86.5%) and chemotherapy (18.9%).

Follow-up was carried out after surgery; 59.5% of patients were reported to be alive at more than 2 years after surgery, while the remainder had died within 2 years of surgery.

Identification of PTEN gene mutation

Of 37 gliomas analysed, seven (21.6%) showed mutations in the *PTEN* gene. Of the seven detected mutations, two were found in exon 5 (28.6%), three were found in exon 6 (42.9%) and the other two (28.6%) were found in both exons 5 and 6 (Table 1). Samples that showed presence of mobility shifts were sent for DNA sequencing and the results revealed the presence of single base substitution in all cases. Three of the seven cases (42.9%) caused nonsense mutations and the other four cases (57.1%) caused missense mutations. Among the base substitutions, two cases were transition (G:C to A:C), three cases caused stop codon to occur (C:A to T:A) and the other two cases showed transition and transversion (G:T to C:T and C:G to T:G).

Identification of LOH status

Out of 37 samples analysed, 12 (32.4%) showed LOH in glioma cases. Of these 12 cases, one (8.3%) showed LOH in chromosome 9p, four (33.3%) in chromosome 10q, two (16.7%) in chromosome 13q, one (8.3%) in chromosome 17p, one (8.3%) in chromosomes 9p and 17q, one (8.3%) in chromosomes 10q and 9p, one (8.3%) in chromosomes 10q and 17p, and one (8.3%) in chromosomes 10q, 9p and 17p (Table 1).

Descriptions of patients with PTEN

Seven patients were found to have *PTEN* mutations in gliomas. Six patients were between the age of 1 and 40 years, and five of them were male. *PTEN* mutations were detected in one patient with WHO grade II (14.3%), three patients with grade III (42.9%) and three patients with grade IV (42.9%). Three (42.9%) of the patients had tumours located on the left side of the brain.

Four (57.1%) patients had epilepsy, four (57.1%) had hemiplegia and all seven (100%) experienced headache. Besides that, angiovascularity was present in all seven patients.

Only four (57.1%) of the patients received chemotherapy and the rest were not treated by either chemotherapy or radiotherapy. Six out of seven patients were reported to be alive for more than 2 years.

Descriptions of patients with LOH

Out of 12 reported LOH cases, seven (58.3%) were younger than 40 years old and eight (66.7%) were male. Out of 12 detected LOH, two were gliomas with WHO grade II (16.7%), six (50%) were grade III and four (33.3%) were tumours with grade IV. Eight (66.7%) of the patients had tumours located on the left side of the brain.

Four out of 12 patients (33.3%) were reported to have epilepsy, eight patients (66.7%) with hemiplegia and 11 (91.7%) of them experienced headache. Angiovascularity was present in 11 patients (91.7%). Seven of the patients (58.3%) received chemotherapy alone, two patients (16.7%) received combination of both chemotherapy and radiotherapy and three patients (25%) received neither chemotherapy nor radiotherapy.

Statistical analysis for survival time

For survival analysis, univariate Cox regression analysis revealed that histopathological grade (HR = 9.01; 95% CI = 1.17, 69.12; $p = 0.004$), vascular (HR = 8.83; 95% CI = 1.15, 67.72; $p = 0.005$), *PTEN* (HR = 4.09; 95% CI = 1.42, 11.78; $p = 0.012$), LOH (HR = 2.23; 95% CI = 1.11, 9.42; $p = 0.031$) and combined presence of *PTEN* and LOH (HR = 7.11; 95% CI = 1.86, 27.18; $p = 0.038$) were significant prognostic factors for the survival of patients with brain tumour (Table 2). However, multivariate Cox proportional hazards model indicate that no single variable was the prognostic factor for survival (Table 3).

Discussion

Previous studies have reported that LOH at chromosome 10q23 and mutations of the tumour suppressor gene, *PTEN*, occur at high frequency in a wide variety of human tumours including gliomas.[19,20]

The most frequently documented regions of allelic losses include 9p21, 10q23-25 and 17p13.[21] In glioblastoma, LOH at 10q23 is detected in almost 70% of

Table 1. Distribution of loss of heterozygosity and *PTEN* mutation cases in 37 low- and high-grade gliomas according to age, gender and histological findings

No.	Histological subtypes (WHO grade)	LOH				*PTEN* mutations	
		LOH 10q	LOH 9p	LOH 17p	LOH 13q	Exon 5	Exon 6
1	A (I)						
2	A (I)						
3	A (I)						
4	A (I)						
5	A (I)						
6	A (I)		No LOH			No mutations	
7	A (I)						
8	A (II)						
9	A (II)						
10	A (II)						
11	A (II)						
12	A (II)						
13	O (II)						
14	E (II)						
15	AA (III)	LOH	–	–	–	No mutations	
16	AA (III)	–	–	–	–	No mutations	
17	AA (III)	–	–	–	–	No mutations	
18	AA (III)	–	–	–	–	No mutations	Codon 173; C$\underline{G}$C→C$\underline{A}$C; Arg→His
19	AA (III)	–	–	–	–	No mutations	
20	AA (III)	LOH	LOH	–	–	No mutations	Codon 173; C$\underline{G}$C→C$\underline{A}$C; Arg→His
21	AA (III)	–	–	–	–	No mutations	
22	AA (III)	–	–	–	–	Codon 130; $\underline{C}$GA→$\underline{T}$GA; Arg→stop	No mutations
23	AA (III)	–	–	–	–	No mutations	
24	PXA (III)	LOH	LOH	LOH	–	Codon 130; $\underline{C}$GA→$\underline{T}$GA; Arg→stop	No mutations
25	AO (III)	–	LOH	LOH	–	No mutations	
26	AO (III)	–	–	–	LOH	No mutations	
27	AO (III)	–	–	–	–	No mutations	
28	AO (III)	–	LOH	–	–	No mutations	
29	AE (III)	–	–	–	LOH	No mutations	Codon 171; $\underline{C}$AG→$\underline{T}$AG; Gln→stop

(continued)

Table 1. (*continued*)

No.	Histological subtypes (WHO grade)	LOH				*PTEN* mutations	
		LOH 10q	LOH 9p	LOH 17p	LOH 13q	Exon 5	Exon 6
30	AE (III)	–	–	–	–	No mutations	No mutations
31	GBM (IV)	LOH	–	–	–	No mutations	No mutations
32	GBM (IV)	LOH	–	LOH	–	Codon 105; T<u>G</u>T→T<u>C</u>T; Cys→Ser	Codon 173; <u>C</u>GC→<u>T</u>GC; Arg→Cys
33	GBM (IV)	–	–	LOH	–	No mutations	No mutations
34	GBM (IV)	–	–	–	–	No mutations	No mutations
35	GBM (IV)	LOH	–	–	–	Codon 124; T<u>G</u>T→T<u>C</u>T; Cys→Ser	Codon 173; <u>C</u>GC→<u>T</u>GC; Arg→Cys
36	GBM (IV)	LOH	–	–	–	No mutations	No mutations
37	GBM (IV)	–	–	–	–	No mutations	No mutations

LOH = loss of heterozygosity; A = astrocytoma; O = oligodendroglioma; E = ependymoma; AA = anaplastic astrocytoma; PXA = pleomorphic xanthoastrocytoma with anaplastic features; AO = anaplastic oligodendroglioma; AE = anaplastic ependymoma; GBM = glioblastoma multiforme.

the cases but they are rarely seen in low-grade glial tumours.[22,23]

In gliomas, *PTEN* gene abnormalities are preferentially found in high-grade gliomas, particularly in GBM.[23–26] Deletions of the *PTEN* region at 10q23 are also reported as predominant findings in glioblastoma.[12,19,27] *PTEN* alterations are detectable in a low fraction (< 10%) of anaplastic astrocytomas and anaplastic oligodendrogliomas and, when present, indicate a poor prognosis.[28]

Our results revealed that *PTEN* mutations and LOH are frequent in gliomas, as reported in previous studies.[22–26] The nonsense and missense mutations found in glioma cases propose that truncated protein might be produced due to the mutations, which could lead to brain tumorigenesis. A study by Stahl has shown that loss of *PTEN* normal growth regulation can significantly lead to tumour development.[5] They investigated the development of malignant melanoma and discovered that loss of *PTEN* reduced apoptosis and promoted cell survival, thereby favouring melanoma tumour formation.

Studying the association between biological and clinical behaviours of gliomas may lead to improvement in predicting the clinical outcome of patients. *PTEN* gene and LOH status of tumour patients may be potential markers in molecular clinical research. This study was designed to determine the prognostic significance of *PTEN* gene mutations and LOH status in glioma patients and to correlate them with certain parameters. In this study, we analysed the mutational status of *PTEN* and LOH using molecular techniques.

Balesaria et al found that there was a correlation between any chromosome 10 loss and poorer performance status at presentation ($x^2 p = 0.005$) and with increasing age at diagnosis but not with tumour grade.[9] Thus, they suggested that LOH on chromosome 10 is an independent, adverse prognostic variable in high-grade glioma.

Similarly, Bower et al's study demonstrated that LOH on chromosome 10 is associated with reduced overall survival.[10] Their study also revealed significant findings between increasing age and poorer performance status at presentation.

With regard to patient survival, univariate analysis from our study shows that five parameters were associated with the survival rate of glioma patients, i.e. tumour grade, vascularization, *PTEN* mutation, LOH and combination of both *PTEN* mutation and LOH. However, multivariate analysis revealed that no factors contributed to

Table 2. Univariate Cox regression analysis of prognostic factors of brain tumour

Variable	Hazards ratio (95% CI)	LR test statistic	p*
Age	1.54 (0.52, 4.61)	0.57	0.450
Sex		0.19	0.661
Male	1		
Female	1.27 (0.44, 3.66)		
HPE grade		8.18	0.004
Lower grade	1		
Higher grade	9.01 (1.17, 69.12)		
Epilepsy		0.73	0.392
No	1		
Yes	0.59 (0.16, 2.11)		
Hemiplegia		1.13	0.288
No	1		
Yes	1.79 (0.60, 5.34)		
Headache		0.25	0.619
No	1		
Yes	1.44 (0.32, 6.43)		
Site		3.46	0.178
Others	1		
Nondominant	1.67 (0.28, 9.99)		
Dominant	3.14 (0.85, 11.64)		
Size		0.47	0.492
≤ 2 cm	1		
> 2 cm	1.84 (0.37, 7.33)		
Vascular		8.03	0.005
No	1		
Yes	8.83 (1.15–67.72)		
Chemotherapy		0.25	0.619
No	1		
Yes	1.44 (0.32–6.43)		
Radiotherapy		0.49	0.482
No	1		
Yes	0.52 (0.07–3.99)		
PTEN		6.36	0.012
No	1		
Yes	4.09 (1.42–11.78)		
LOH		4.63	0.031
No	1		
Yes	2.23 (1.11–9.42)		
Combined		8.44	0.038
LOH(−) *PTEN*(−)	1		
LOH(−) *PTEN*(+)	3.72 (0.68–20.43)		
LOH(+) *PTEN*(−)	2.62 (0.59–11.77)		
LOH(+) *PTEN*(+)	7.11 (1.86–27.18)		

*p value obtained from likelihood ratio test. CI = confidence interval; LR = likelihood ratio; LOH = loss of heterozygosity.

Table 3. Multivariate Cox regression analysis* of prognostic factors of brain tumour

Variable	Hazards ratio (95% CI)	Wald statistic	p
PTEN		3.19	0.074
No	1		
Yes	2.66 (0.91–7.80)		
HPE grade		3.14	0.077
Lower grade	1		
Higher grade	6.56 (0.82–52.68)		

*Likelihood ratio statistic applied for the model = 1.37; p = 0.503. CI = confidence interval.

the survival outcome of the glioma patients. There is a possibility of finding a significant association if the sample size is larger than the sample in this study. *PTEN* mutations were only found in seven of 37 cases (18.9%) and grades III and IV were found in 23 cases (62.2%). If the mutations were found in a higher proportion of grades III and IV tumours, there would have been a possibility of obtaining significant association between tumour grade and mutation.

Previous research have also shown that there are significant occurrences of gliomas with the presence of gene mutation in the *p53* gene. The *p53* gene mutations were reported to be significantly associated with the grade, side and consistency of tumour.[2] However, similar molecular analysis of the *p16* mutation revealed neither point mutation nor homozygous deletion, suggesting that this gene could not be involved and is not a common inactivation mechanism in the pathogenesis of gliomas in Malaysian patients.[4]

Our results showed that presence of *PTEN* mutations and LOH did not play any prognostic role in Malaysian patients. This may indicate that without *PTEN* mutations and LOH, patients do not necessarily have a good prognosis. From the results obtained, we conclude that other genetic markers may play important roles in glioma prognosis. Patients with *PTEN* mutations and presence of LOH may need to be analysed for other markers before receiving appropriate management of their tumours.

Acknowledgements

References

1. Kanzawa T, Ito H, Kondo Y, et al. Current and future gene therapy for malignant gliomas. *J Biomed Biotechnol* 2003;3:25–34.
2. Yusoff AA, Abdullah J, Abdullah MR, et al. Clinicopathological study: association of p53 tumor suppressor gene with paraclinical and clinical modalities of gliomas patients in Malaysia. *Acta Neurochir (Wien)* 2004;146:595–601.
3. Lam PYP, Breakefield XO. Potential of gene therapy for brain tumors. *Hum Mol Genet* 2001;10:777–87.
4. Abdullah JM, Zainuddin N, Sulong S, et al. Molecular genetic analysis of phosphatase and tensin homolog and *p16* tumor suppressor genes in patients with malignant glioma. *Neurosurg Focus* 2003;14:Article 6.
5. Stahl JM. Loss of *PTEN* promotes tumor development in malignant melanoma. *Cancer Res* 2003;63:12881–90.
6. Lee S, Kim J, Rhee C, et al. Loss of heterozygosity on chromosome 10, 13q 9 (Rb), 17p and *p53 gene* mutations in human brain gliomas. *J Korean Med Sci* 1995;10:442–8.
7. Fujisawa H, Reis RM, Nakamura M, et al. Loss of heterozygosity on chromosome 10 is more extensive primary (de novo) than in secondary glioblastoma. *Lab Invest* 2000;80:65–72.
8. Cheng Y, Ng H, Ding M, et al. Molecular analysis of microdissected de neve glioblastoma and paired astrocytic tumors. *J Neuropathol Exp Neurol* 1999;58:120–8.
9. Balesaria S, Brock C, Bower M, et al. Loss of chromosome 10 is an independent prognostic factor in high-grade gliomas. *Br J Cancer* 1998;81:1371–7.
10. Bower M, Brock C, Balesaria S, et al. Allelotype analysis for loss of chromosome 10 in high grade gliomas. European Society for Medical Oncology. (Abstract number: 581)
11. Okumura A, Takenaka K, Nishimura Y, et al. The characterization of human brain tumor using magnetization transfer technique in magnetic resonance imaging. *Neurol Res* 1999;21:250–4.
12. Lote K, Egeland T, Hoger B, et al. Prognostic significance of CT contrast enhancement within histological subgroups of intracranial glioma. *J Neurooncol* 1998;40:161–70.
13. Ropper AH. Lateral displacement of the brain and level of consciousness in patients with acute hemispheral mass. *N Engl J Med* 1986;314:953–8.
14. Yusoff MR, Abdullah J, Isa MN. Brain tumours in rural North East Malaysia. *J Islamic Acad Sci* 1998;11:121–9.
15. Karnofsky DA, Burchenal JH. In: Macleod CM, ed. *Evaluation of Chemotherapy Agents*. New York: Columbia University Press, 1949:191–205.
16. Kleihues P, Burger PC, Scheithauer BW. *Histological Typing of Tumours of the Central Nervous System*. Berlin: Springer-Verlag, 1993.
17. Scarisbrick JJ, Woolford AJ, Russell-Jones R, et al. Loss of heterozygosity on 10q and microsatellite instability in advanced stages of

primary cutaneous T-cell lymphoma and possible association with homozygous deletion of *PTEN*. *Blood* 2000;95:2937–42.

18. Sakai A, Thieblemont C, Wellmann A, et al. *PTEN gene* alterations in lymphoid neoplasm. *Blood* 1998;92:3410–5.

19. Li J, Yen C, Liaw D, et al. *PTEN*, a putative protein tyrosine phosphatase gene mutated in human brain, breast and prostate cancer. *Science* 1997;275:1943–7.

20. Steck PA, Pershouse MA, Jasser SA, et al. Identification of a candidate tumor suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat Genet* 1997;15: 356–62.

21. Watling CJ, van Meyel DJ, Ramsay DA, et al. Loss of heterozygosity analysis of chromosome 9, 10, and 17 in gliomas in families. *Can J Neurol Sci* 1995;22:17–21.

22. Bigner SH, Marker J, Burger PC, et al. Specific chromosomal abnormalities in malignant human gliomas. *Cancer Res* 1998;48:405–11.

23. Rasheed BKA, Stenzel TT, McLendon RE, et al. *PTEN gene* mutations are seen in high grade, but not in low-grade gliomas. *Cancer Res* 1996;57:4187–96.

24. Davies MP, Gibss FE, Halliwell N, et al. Mutation in the *PTEN/*MMAC1 gene in archival low grade and high grade gliomas. *Br J Cancer* 1999;79:1542–8.

25. Hill JR, Kuriyama N, Kuriyama H, et al. Molecular genetics of brain tumor. *Arch Neurol* 1999;56:439–42.

26. Maier D, Zhang Z, Taylor E, et al. Somatic deletion mapping on chromosome 10 and sequence analysis of *PTEN*/MMAC1 point to the 10q25-26 region as the primary target in low grade and high-grade gliomas. *Oncogene* 1998;16:3331–5.

27. Wang SI, Puc J, Li J, et al. Somatic mutations of *PTEN* in glioblastoma multiforme. *Cancer Res* 1997;57:4183–6.

28. Knobbe CB, Merlo A, Reinfenberger G. *PTEN* signaling in gliomas. *Neuro-oncol* 2002;4:196–211.