

The Relationship of p53 Protein in Meningioma Grading and their Various Influencing Factors Amongst Neurosurgical Patients in Hospital Kuala Lumpur

R Kantha*, H M Saffari**, M Y Suryati***

*Department of Neurosciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, **Department of Neurosurgery Hospital Kuala Lumpur, ***Department of Pathology Hospital Kuala Lumpur

SUMMARY

Meningioma, is the second most frequent intracranial tumor in Malaysia and are classified according to the World Health Organization classification. The relationship of p53 protein in the determination of meningioma grading and their influencing factors were studied via immunohistochemistry studies on 77 intracranial meningiomas (67 benign, 10 atypical). The higher the p53 reaction was correlated to the poorer the histological grade (19.4% in benign and 90% in atypical meningioma) ($p < 0.001$). Other variables like age, sex, ethnicity, demographic location, surgical clearance, midline shift and contrast enhancement of CT Scan Brain and clinical features were found not to be significant.

KEY WORDS:

p53 Protein, Meningioma Grading

INTRODUCTION

Meningiomas are common neoplasm arising from arachnoid cap cells present in the meninges, thus the term meningioma. The term meningioma itself was coined by the father of modern neurosurgery, Harvey Cushing after observing the origin of meningioma from the meninges. Statistics from most neurosurgical series worldwide show that meningioma represent 13-19%¹⁻³ of central nervous system tumors however studies in Singapore have shown this figure to be as high as one third of all central nervous system tumors². Overall incidence of meningioma based, on population studies is 2.3/100,000⁴.

Incidence of meningioma increases with age and takes a slight dip after the age of eighty. The peak incidence in Caucasian patients are 70 years in females and 60 years in males respectively⁴. In contrast studies in Singapore showed the age to be 56 years in both male and female. Females have a higher prevalence for meningioma as opposed to males to a ratio of 2:1¹⁻³, which is in contrast to most other central nervous system tumors.

The etiology of meningioma is not clearly known however, trauma, viruses, radiation and other associations have been postulated. Some studies have shown some genetic aspects to meningioma and have localized chromosomal abnormality to the center of the long arm of chromosome 22. The loss of one

copy of chromosome 22 (monosomy) has been observed in up to 50% of patients with meningiomas.

p53 gene is located at the 13.1 short arm of chromosome 17 and composed of 11 exons. p53 protein, composed of 393 amino acids, is a nuclear phosphoprotein and involved in regulating proliferation, differentiation and apoptosis of cells. These functions are activated by sequence-specific binding of p53 protein with DNA. When p53 gene mutation occurs and the sequence of p53 protein changes, p53 protein loses its binding capacity with DNA and acts in a dominant negative fashion that blocks the p53 binding capacity.

The p53 tumor suppressor gene is the most commonly mutated gene in many tumor type, but the involvement of this gene in meningiomas is controversial. Some reports have shown high incidence of p53 over expression, particularly in atypical and malignant meningiomas³. The p53 tumor suppressor gene is known to be involved in several stages of cellular regulation and recently significant interest has been focused on the role of p53 in the control of apoptosis.

MATERIALS AND METHODS

All patients with histological confirmation of meningioma and underwent surgery in HKL from January 2002 and 2006 whom fulfilled the inclusion and exclusion criteria were included in this cross sectional study. A total number of 77 patients who fulfilled this criteria were studied where immunohistochemistry test was carried out to determine the expression of p53 protein in these samples.

Samples were obtained from the specimen bank of the Department of Pathology, Hospital Kuala Lumpur. Sections measuring 3-5 microns were obtained from the blocks and laid on poly-L-lysine slides and were kept in an oven for 20-30 minutes with the temperature of 60 degrees Celsius for the purpose of dewaxing and fixing. Water followed by xylene three times, Xylene 1, Xylene 2 and then Xylene 3 were used to dewax the slides. Absolute alcohol (100%) and then alcohol of 95% and 80% were used respectively to remove the xylene before being washed with saline.

The slides were then placed into a slide rack and put into a citrate buffer for pre treatment followed by placement into a

This article was accepted: 10 May 2007

Corresponding Author: Kantha Rasalingam, Department of Neurosciences, School of Medical Sciences, University Sains Malaysia, 16150 Kubang Kerian, Kelantan

microwave for 10 minutes with high intensity. They were removed from the microwave and cooled at room temperature for 20 minutes and washed with water. The slides were then placed onto a sequenza tray where by hydrogen peroxidase 3% was added and left to soak for 15 minutes and later washed three times.

The slides were then for immunohistochemistry. Monoclonal Mouse Anti-Human p53 Protein (Clone DO-7 by Dako Cytomation, Glostrup, Denmark, code.nr M7001) which was used in liquid form as cell culture supernatant dialyzed against 0.05 mol/l Tris/HCl, pH 7.2 and contained 15 mol/L NaN₃⁴. This was diluted with antibody diluents to 1:100. Tris Buffer Solution was added again to remove the antibodies that were not concern and this procedure was repeated thrice.

A second antibody, Dako Envision HRP Rabbit Mouse was applied for 30 minutes using a process called Dako Cytomation. It was washed with Tris Buffer Solution thrice. The slides were then removed and chromogen was added for 5 minutes to give color to the slides which were washed with water and dipped into hemotoxylin stain for 30 seconds.

Alcohol for decolourization and water was run for bluing process. Dehydration using alcohol starting with alcohol 80% to alcohol 95% and then finally alcohol 100% was done, there after was dipped into Xylene three times for clearing and was then ready for mounting onto the slide and labeled^{3,4}. The expressions of p53 protein were compared in two groups, benign meningioma and atypical meningioma. Other parameters such as clinical presentation, imaging findings, operative clearance and demographic studies were also performed. Chi square test was used to determine the significance of the various parameters.

RESULTS

There were 141 patients admitted and operated with the diagnosis of meningioma between January 2002 and January 2006, of whom only 77 patients fulfilled the criteria of this study. The reaction of p53 protein was graded as -ve for no reaction, 1+ve for mild reaction, 2+ for moderate reaction and +3 for severe reaction for Monoclonal Mouse Anti-Human

p53 protein (Clone DO-7)(3). In our study, 80% of the patients with atypical meningioma showed a reaction of 2+ and above as opposed to 0% who had 2+ and above in the benign group. One hundred percent of patients with benign meningiomas had reaction of -ve and 1+ of p53 protein compared to 20% in the atypical group. We analyzed the significance of grading of meningiomas with immunohistochemistry through correlation analyses and found a fair, significant and positive relation with increasing grades of meningioma. ($r=0.592$, $p<0.001$)(Table I) Other variables such as sex ($p=0.765$), ethnic groups ($p=0.960$), age ($p=0.337$), demographic location ($p=0.727$), headache ($p=0.644$), seizures ($p=0.924$), vomiting (0.555), paresis (0.153) and visual disturbance(0.918), location of tumor (0.591), contrast enhancement (0.514) and midline shift (0.799) and surgical clearance using Simpson's grading (0.909) were also analyzed for the differences in the proportions of variables using Chi Square test. We however did not find any significant difference between the benign and atypical meningioma groups with regards to the proportion of the variables. (Table II)

To find out the association between individual, independent variables and the dependent variable, we used univariate logistic regression. In the univariate analysis, apart from paresis ($p=0.096$) and immunohistochemistry ($p=0.001$), other variables did not show any significant association with the dependent variable (grades of meningiomas). These two variables (paresis and immunohistochemistry), qualified for multivariate analysis (selection criteria for multivariate was, $p<0.20$). In the multivariate analyses, only p53 immunohistochemistry ($p=0.001$) showed statistical significance to the grades of meningiomas as opposed to the relationship paresis ($p=0.130$) which was statistically insignificant to the grades of meningiomas.

DISCUSSION

The main objectives of this study was to see the relationship between p53 protein and the grading with the and prognosis of meningiomas. The expressions of p53 protein in meningiomas were categorized by immunohistochemistry methods graded as negative (-ve), 1+ for mild expression, 2+

Table I: Univariate Analysis of Reaction Between p53 Protein Reaction Based on Immunohistochemistry and Grades of Meningiomas

p53 reaction	Benign n(%)	Atypical n(%)	ODDS RATIO	p value
No (-ve)	54(80.6%)	1(10%)	1	-
Yes (1+,2+,3+)	13(19.4%)	9(90%)	37.365	0.001

Table II: p Value for Difference in Proportions of Variables in the Two Meningioma Groups

Variables	Benign Meningioma n(%)	Atypical Meningioma n(%)	p value
Headache	50(74.6%)	7 (70%)	0.644
Seizures	15(22.4%)	1 (10%)	0.924
Vomiting	17(25.4%)	1 (10%)	0.555
Paresis	27(40.3%)	1 (10%)	0.153
Visual Disturbance	12(17.9%)	2 (20%)	0.918
Contrast Enhancement	60(89.6%)	10(100%)	0.514
Midline Shift	39(58.2%)	6 (60%)	0.799

for moderate expression and 3+ as severe expression. Our study concluded that the over expression of p53 was found mainly in the atypical meningioma group with 5(50%) of patients having grade 2+, 3(30%) having grade 3+ and only 1(10%) having grade -ve and 1+ respectively. In the benign group however, majority of our patients, 54(80.6%) did not show any expression of p53 protein and 11(19.4%) did show mild expression (19.4%). This study concluded that there was a direct relationship between the expressions of p53 protein with higher grades of meningiomas with higher expression of p53 protein in atypical meningiomas as compared with benign meningioma. This has been found to be statistically significant in our study. ($p < 0.001$) It can be concurred with studies done by Cho *et al*² and Dass *et al*³ that there is a strong relationship between p53 protein and the grades of meningiomas, with the benign meningioma showing low or no expression of p53 protein and the higher grade of atypical meningiomas showing high grades of expression of p53 proteins^{5,8,10,11}.

The study also noted that like other studies worldwide that meningiomas of both grades were more prevalent in the female species than the male species. We also found that meningiomas presented in the later years of life with a peak around the ages of 55 to 65 years. The pattern of distribution based on ethnicity followed the population curve with Malays showing the most no of cases followed by Chinese and Indians. Analysis studied of the clinical features of these patients found that the most common presenting symptom was that of headaches, followed with limb weakness, vomiting, seizures and finally visual disturbances. Demographic studies revealed that our patients operated for meningioma came from all over the country especially from around the Klang Valley.

None or only a few of these patients came from states that have a neurosurgical center. Based on imaging, we found that the convexity was the most common location for meningiomas, followed by the parasagittal and sphenoid wing regions. In the spine, most of the cases were found mainly in the thoracic region.

It was concluded that most meningiomas enhanced on contrast administration and a significant no showed a midline shift on CT Scan Brain. Based on the surgical outcome, a high grade of clearance was achieved i.e., Simpson's 1 clearance (61%) for most of our patients and only a handful were left with clearance of Simpson's 4 (3%) and Simpson's 5 (0%) clearances.

REFERENCES

1. Das A, Tan WL, Smith DR. p53 point mutation is rare in meningiomas from Singaporean patients. *Asian J Surg*. 2005; 28(1): 7-10.
2. Dass A. Meningiomas in Singapore: Demographic and biological characteristics. *Journal of Neuro-Oncology* April 2000; 47(2): 153.
3. Cho H, Ha SY, Park SH, Park K, Chae YS. Role of p53 gene mutation in tumor aggressiveness of intracranial meningiomas. *J Korean Med Sci*. 1999; 14(2): 199-205.
4. Aguiar PH, Agner C, Simm R, Freitas AB, Tsanacis AM, Plese P. p53 protein expression in meningioma- a clinicopathologic study of 55 patients.
5. Rohringer M, Sutherland GR, Louw DF, Sima AA. Incidence and clinicopathological features of meningioma. *J Neurosurg*. 1989; 71 (5 Pt 1): 665-72.
6. Maier H, Ofner D, Hittmair A, Kitz K, Budka H. Classic, atypical, and anaplastic meningioma: three histopathological subtypes of clinical relevance. *J Neurosurg*. 1992; 77(4): 616-23.
7. Hwang SL, Howng SL. An analysis of brain tumors in south Taiwan. *Gaoxiong Yi Xue Ke Xue Za Zhi*. 1992; 8(12): 656-64.
8. Lamberts SW, Tanghe HL, Avezaat CJ, Braakman R, Wijngaarde R, Koper JW, *et al*. Mifepristone (RU 486) treatment of meningiomas. *J Neurol Neurosurg Psychiatry*. 1992; 55(6): 486-90.
9. Fuentes S, Chinot O, Dufour H, Paz-Paredes A, Metellus P, Barrie-Attarian M, *et al*. [Hydroxyurea treatment for unresectable meningioma]. *Neurochirurgie*. 2004; 50(4): 461-7.
10. Carella RJ, Ransohoff J, Newall J. Role of radiation therapy in the management of meningioma. *Neurosurgery*. 1982; 10(3): 332-9.
11. Al-Mefty O, Kadri PA, Pravdenkova S, Sawyer JR, Stangeby C, Husain M. Malignant progression in meningioma: documentation of a series and analysis of cytogenetic findings. *J Neurosurg*. 2004; 101(2): 210-8.
12. Lopez-Gines C, Gil-Benso R, Collado-Diaz M, Gregori-Romero M, Roldan P, Barbera J, *et al*. [Meningioma: a model of cytogenetic evolution in tumoral initiation and progresion]. *Neurocirugia (Astur)*. 2003; 14(6): 517-25.
13. Rieske P, Zakrzewska M, Biernat W, Bartkowiak J, Zimmermann A, Liberski PP. Atypical molecular background of glioblastoma and meningioma developed in a patient with Li-Fraumeni syndrome. *J Neurooncol*. 2005; 71(1): 27-30.
14. Ohkoudo M, Sawa H, Hara M, Saruta K, Aiso T, Ohki R, *et al*. Expression of p53, MDM2 protein and Ki-67 antigen in recurrent meningiomas. *J Neurooncol*. 1998; 38(1): 41-9.