CORRELATION BETWEEN MUTANT p53 PROTEIN EXPRESSION AND HISTOPATHOLOGICAL GRADING IN ASTROCYTOMA PATIENTS

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ABSTRACT

Mutation of p53 gene plays an important role in astrocytoma carcinogenesis. The mutation process results in p53 mutant which fails to stop the tumor's cellular proliferation or apoptotic process resulting new cells with persistent genetic mutation. p53 mutant expression detected by imunnohistochemistry staining shows the biological behavior of tumor cell. Some authors found that p53 protein expression relates to histopathological grading which affects the prognosis. However, some studies showed different results. These findings invite debatable subject around p53 mutant and histophatological grading. The objective of this study was to prove the correlation between p53 mutant protein expression with histophatologic grading in astrocytoma patients. This was an analytic cross-sectional study using imunohistochemical staining for p53 mutant expression, conducted from January to August 2005. Data were analyzed to find the relation between WHO histopathological grading, age, and sex, and p53 protein expression. The confidence interval was a = .05. The results found that 33 astrocytoma patients were operated, with age range from 4 to 62 years old, averagely 30.91 years old. Most patients aged between 31-40 years old., predominantly male (male: female = 60.6% : 39.4%). Histopathological diagnosis mostly was diffuse astrocytoma (WHO grade II) found in 48.5% cases, pilocytic astrocytoma (WHO grade III) 30.3%, and Globlastoma multiforme 6.1%. Mild expression of p53 was found in WHO grade I. Mild expression of p53 increased to 14 (42.4%) in WHO grade II and 2 patients had severe degree. Percentage of patients of WHO grade III showed severe grade of expression increasing to 6 patients (18.2%). In WHO grade IV group, there was 1 patient (3.0%) with severe expression and 1 patient with very severe expression. Statistical analysis using Spearman correlation test showed that there was a correlation between p53 mutant expression with increasing histopathological grading of astrocytoma, p=.001 (p<.0001) with correlation coefficient 0.736. In conclusion, there is positive correlation between p53 mutant protein expression and histopathological grading (p<.0001; correlation coefficient 0.736), as well as age (p<0.05; correlation coefficient 0.434)

Keywords: *Astrocytoma, p53 mutant expression, histopathological grading, immunohistochemistry*

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INTRODUCTION

Astrocytoma is an intracranial neoplasma originating from astrocyte neuroepithelial cells, constituting 60% from all intracranial primary tumors. Five to seven new cases were found in 100,000 populations annually, which incidence ratio in male and female of 1.8 : 1 (Hao Ding et al. 2000; Gregory & Peter 1990). In Dr Soetomo Hospital, Surabaya, between 2002 and 2004 there were 405 brain tumor patients, and 328 of which were subjected to operation. Pathological examination in 29% of these or 40 patients revealed astrocytoma (Anab & Wahyuhadi 2005). The management of astrocytoma is highly affected by acid gradation of histopathological grading. WHO divides into three gradings: Grade I (Pilocystic Astrocytoma), which is curable with radical operation; Grade II (Diffuse Astrocytoma) with survival expectancy more than 5 years; Grade III (Anaplastic astrocytoma), with survival expectancy of 2 years (50%), 5 years (2%) and increased 10% if the patient receives radiotherap; and Grade IV (Glioblastoma Multiforme) with survival expectancy of 9 months (50%), 1.5 years(10%) (Flowers 2000; Greenberg et al. 1999). The tumor can undertake "upgrading" (to become more malignant). The problem is the time and the causes of the change remain unknown clearly. A theory has been developed that the emergence of the tumor results from environmental effect and the presence of predisposing genetic abnormality. Imbalance between oncogene and gene suppressor play an important role. Gene suppressor that has been found to have a major role is p53 (Litofsky et al. 1997; Nayak et al. 2004). Some studies on the effect of p53 mutant on astrocytome progressiveness remain controversial.

Litofsky et al., Weixin et al., and Put Ti Noi et al. suggest that there is a positive correlation between excessive expression of p53 mutant an increased astrocytoma gradation. However, Nayak et al. and Cunningham et al. found that there was no correlation between p53 mutant expression and astrocytoma gradation. Since, in addition to these findings, p53 mutant can be examined immunohistochemically in Surabaya, the authors attempted to find whether in Surabaya, particularly in Dr Soetomo Hospital, a correlation exists between p53 mutant expression and astrocytoma gradation. Such study had never been conducted previously. The finding of such correlation can be used as diagnostic tool and for determining therapy modality.

MATERIALS AND METHODS

Observation was conducted at once on variables whose events had already occurred. This study used analytic observational design. Population comprised all paraffin blocs preserved at the Department of Anatomic Pathology, which were clinically and histopathologically showed the appearance of astrocytoma, from January 2002 to August 2005. Samples consisted of paraffin blocs from all operated patients in Dr Soetomo Hospital, from January 2002 to August 2005, who were clinically and histopathologically showed astrocytoma and selected according to WHO histopathological grading. Samples size was 33 samples. The inclusion criteria comprised primary tumor, well-preserved astrocytoma paraffin bloc dating from January 2002 to August 2005, the paraffin blocs are eligible for immunohistochemical examination and can be well-read, and the patients received no previous radiation therapy or chemotherapy. Histopathological diagnosis was the diagnosis obtained from examination of astrocytoma preparations with Hematoxilin Eosin staining.

Histopathological grading was based on the change of cellular shape and characteristics and tissue architecture examinaed from HE stained preparations with magnification of 100 and 400 times using light microscope. The evaluation was classified into: 1. Pilocytic Astrocytomas (WHO grade I). Histopathological examination revealed no anaplastic cells, mild cellularity, absence of mitosis, minimal endothelial proliferation, no necrosis, and differential transitional zone to normal brain. 2. Diffuse astrocytoma (WHO grade II). Histopathogical examination revealed well-differentiated fibrillar or gemistotic neoplastic astrocyte, a loose structural background, and microcystic tumor matrix. Cellularity increases sufficiently with atipia nucleus. 3. Anaplastic astrocytoma (WHO grade III). Histopathological examination revealed increased cellularity, well-differentiated nucleus atipia, and predominant mitosis activity. The mitosis is abnormal. Glioblastoma multiforme (WHO grade IV). Histopathological examination revealed pleomorphism and poor-differentiation. Mitotic activity is fast. There are microvascular proliferation and/or necrosis. The study was carried out at Neurosurgery Outpatient Clinic, Neurosurgical wards, and the Department of Anatomic Pathology, Dr Soetomo Hospital, for 4 months, from August 2004 to November 2005. To analyze correlation between the expression of p53 mutant and histopathological grading, we used Spearman correlation test. To analyze the difference of p53 mutant expression in various histopathological grading, we used t-test and chi-square test.

RESULTS

From January 2002 to August 2005, there were 32 definitive operations to astrocytoma tumor patients. From those 33 patients, 20 individuals (60.6%) were male, and the rest 13 individuals (39.4%) were female. The youngest age of the patient was 4 years and the oldest was 62 years with averaga age 30.91 years. Most of the patients aged 37 years, comprising 3 individuals (4.1%) and if grouping according to age interval wa smade, most of the patients aged 31-40 years, comprising 12 individuals (36.4%).

Table 1. Patients characteristics

	Patients (n=33)	Percentage (%)
1. Sex		
Male	20	60.6
Female	13	39.4
2. Age		
Youngest	4 years	
Oldest	62 years	
Predominant	37 years	
01 - 10 years	4	12.1
11 - 20 years	6	18.2
21 - 30 years	4	12.1
31 - 40 years	12	36.4
41 - 50 years	2	6.1
51 - 60 years	4	12.1
61 - 70 years	1	3

Specimen was taken from patients who had undergone operation. Definitive diagnosis based on histopatho-logical examination was mostly diffuse astrocytoma

(WHO grade II), consisting of 16 cases (48.5%), while the others, Pilocytic Astrocytoma (WHO grade I) was cases (15.2%), anaplastik astrocytoma (WHO grade III) 10 cases (30.3%), glioblastoma multiforme 2 cases (6.1%).

Table 2. Histopathological characteristics

	Patients (n=33)	Percentage (%)
1. Specimen Operation	33	100%
2. PA Diagnosis		
Pilocytic Astrocytoma (WHO *Grade* I)	5	15.2
Diffuse Astrocytoma (WHO *Grade* II)	16	48.5
Anaplastic Astrocytoma (WHO *Grade* III)	10	30.3
Glioblastoma Multiform (WHO *grade* IV)	2	6.1

Based on k-means cluster analysis there were four category of p53 mutant protein, i.e.: 1. Grade I (mild): if the mean (median) of p53 mutant expression = 3; 2. Grade II (moderate), if the mean (median) of p53 mutant expression = 11; 3. Grade III (severe), if the mean (median) of p53 mutant expression = 21; and 4. Grade IV (very severe), if the mean (median) of p53 mutant expression = 32. The 33 obtained specimens were subjected to immunohistochemical staining. We obtained 21 specimens (63.6%) with mild p53 mutant expression, 8 with moderate expression (24.2%), 2 with severe expression (9.1%), and 1 with very severe expression (3%).

Table 3. Results of immunohistochemical staining

Mutant p53 protein expression	Patients (n= 33)	Percentage (%)
1. Grade I (Mild)	21	63.6
2. Grade II (Moderate)	8	24.2
3. Grade III (Severe)	2	9.1
4. Grade IV (Very Severe)	1	3.0

Based on differential degree groupings, 5 (15.2%) patients with Pilocytic Astrocytoma (WHO grade I) showed p53 mutant protein expression grade I (mild) expression, while protein expression in mild, severe, and very severe degree were absent. In patients with diffuse astrocytoma (WHO grade II), 14 patients (42.4%) had mild grade expression and 2 patients (6.1%) had moderate expression. From 10 patients with anaplastic astrocytoma, 2 patients (6.1%) showed mild expression, 6 patients (18.2%) had moderate expression and 2 patients (6.1%) had severe expression. In patients with glioblastoma multiforme (WHO grade IV), the expression of grade III (severe) was found in 1 patient (3.0%) and 1 patient (3.0%) was found to had grade IV (very severe) expression.

Table 4. p53 expression distribution according to the grade of differentiation

Mutant p53 protein expression	WHO I	WHO II	WHO III	WHO IV	TOTAL
1.Grade I (Mild)	5 (15.2%)	14 (42.4%)	2 (6.1%)		21 (63.6%)
2. Grade II (Moderate)		2 (6.1 %)	6 (18.2%)		8 (24.2%)
3. Grade III (Severe)			2 (6.1%)	1 (3.0%)	3 (9.1%)
4. Grade III (Very Severe)				1 (3.0%)	1 (3.0%)
TOTAL	5	16	10	2	33

From statistical analysis using Pearson's Chi-Square test ($p = 0.000$; $p < 0.05$), we found different p43 mutant protein in various histopathological gradings in astrocytoma. Based on the histopathological grading, WHO grade I only showed grade I (mild) p53 protein expression, while grade II (moderate), grade III (severe) and grade IV (very severe) were not obtained. In WHO grade II, the grade I p53 expression (moderate) increased to 14 (42.4%), and 2 patients (6.1%) were found with grade III (severe) expression, which was not found in WHO grade I. In WHO grade III, the percentage of patients showing grade IV (severe) expression increased to 6 patients (18.2%), more than that in WHO grade II, which was 2 patients (6.2%). In WHO grade IV, there was 1 patient (3.0%) with grade IV (severe) and 1 patient (3.0%) with grade IV (very severe), in very severe grade was not found in grade I, II and III. When we observed each grading, according to grade increase, the p53 expression was also found to increase. Results of statistical analysis using Spearman rho correlation test revealed correlation between p53 mutant expression and the increase of differentiation degree ($p = 0.0001$) with correlation coefficient 0.736.

Based on the patients' age, it was found that there was correlation between age increase and mutant p53 protein expression. The older the patient's age, the higher the mutant p53 expression. Table 5 also shows correlation

between age and the increase of WHO grading. The older he age, the higher the WHO grading.

Table 5. Correlation between p53 mutant expression and WHO histopathological grading and age.

	Mutant p53 expression	WHO grading
WHO grading	r = 0.736 p = 0.0001	
Age	r = 0.434 p = 0.012	r = 0.641 p = 0.0001

The mean of mutant p53 protein expression in men was higher than that in women. Results of statistical test revealed $p = 0.047$ ($p < 0.05$), indicating difference in the expression of mutant p53 protein between men and women. The histopathological grading in men was also higher than that in women, in which the statistical test showed $p = 0.011$ ($p < 0.05$). After being tested with multiple linear regression, it was found that only histopathological grading that had effect on the expression with correlation strength of 0.545, while sex and age were not significant.

Table 6. The difference of mutant p53 protein expression and WHO grading between men and women

	Sex		Statistical test	P value	Notes
	M	F			
p53 expression	9.09 ± 8.57	4.63 ± 3.48	t = 0.047	0.047	Significant
WHO Grading (%)					
I	2 (10.0 %)	3 (23.1 %)	z = -2.550	0.011	Significant
II	7 (35.0 %)	9 (69.2 %)			
III	9 (45.0 %)	1 (7.7 %)			
IV	2 (10.0 %)	0 (0.0 %)			

Table 7. The effect of histopathological grading, age, and sex on the expression of mutant p53 protein

Variables	B	t value	p value	Notes
WHO Grading	6,418	3,855	0.001	S
Age	4,257E – 02	0.568	0.575	NS
Sex	-0.225	-0.112	0,912	NS
Constant	-8,433	-3,234	0,003	

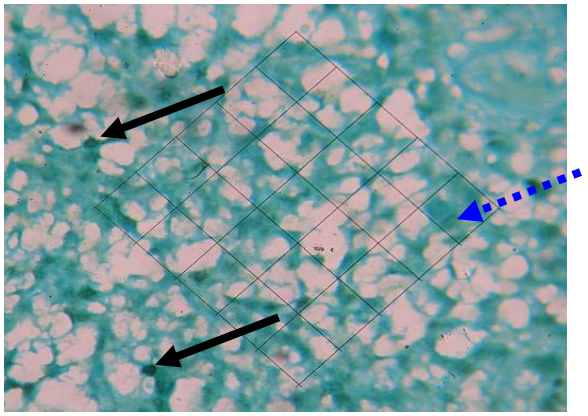

Figure 1. Observable tumor cells showing positive expression with mutant p53 protein (black arrow), while interrupted blue arrow shows tumor cells without the expression of mutant p53 protein in astrocytoma tumor of WHO grade III (anaplastic astrocytoma) (T 628/04, magnification 400x with Olympus microscope).

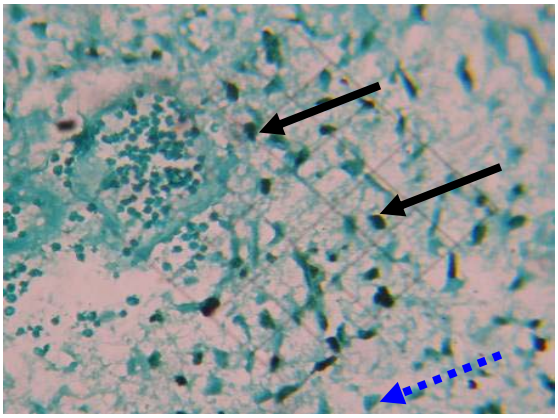

Figure 2. Positive tumor cells with mutant p53 in nucleus (black arrow) and blue interrupted arrow shows no mutant p53 protein expresion from grade IV astrocytoma tumor (glioblastoma multiforme) (PA 5779/03, magnification 400x with Olympus microscope).

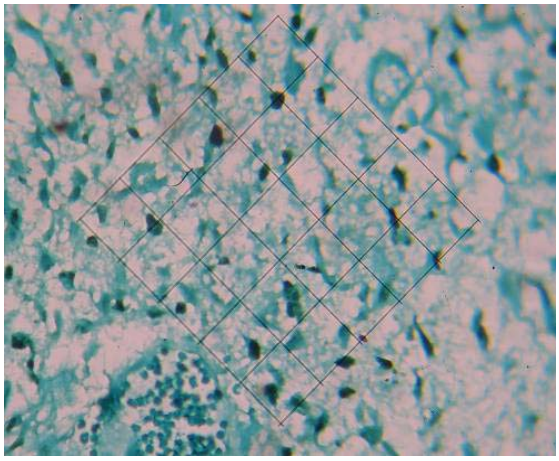

Figure 3. Positive tumor cells with mutant p53 in nucleus (black arrow), and blue interrupted arrow shows no expression of mutant p53 from grade IV astrocytoma tumor (glioblastoma multiforme) (PA 5779/03, magnification 400x with Olympus microscope).

DISCUSSION

From 33 patients we obtained from January 2002 to August 2005, male patients were in higher number than female patients, where 60.6% of the patients were male. This was different from the retrospective descriptive study carried out in four hospitals in Surabaya (Dr Soetomo, RKZ, Adi Husada, and Darmo Hospitals). In year 2002-2004 most of astrocytoma tumor patients were female (56% of the cases) (Anab & Wahyuhadi 2005). Data from WHO classification of tumors, the incidence of astrocytoma tumor in men is higher than in women (1.8 : 1). The occurrence of the tumor in men is averagely in age 52.2 and women in age 37.2. The older the age of the patient, the higher the histopathological grading (Kleihues 2000). In this study, the tumor was mostly found in patients aged 31-40 years old, with mean of 30.91. Most of the patients were admitted at WHO grade II (diffuse astocytoma), comprising 48.5% of the patients. Data between 2002-2004 revealed 34.11% of the patients were admitted with WHO grade III. This proved that the awareness of the patients about their disease is very low, as indicated by remarkable number of patiens with grade II and III. From 4 year data, we found only 5 patients came with WHO grade I.

Specimens we obtained in this study mostly had diffuse astrocytoma (WHO grade II), comprising 48.5%, while 30.3% were anaplastic astrocytoma (WHO grade III), pilocytic astrocytoma (WHO grade I) comprising 15.2%, and the rest, 6.1% were glioblastoma multiforme (WHO grade IV). Nayak et al. (2004) collected

histopathological data from 152 patients. They found 9% pilocytic astrocytoma (WHO grade I), 25% diffuse astrocytoma (WHO grade II), 19% anaplastic astrocytoma (WHO grade III), 46% glioblastoma multiforme (WHO grade IV). The above data shows difference in the incidence, particularly the GBM, in which this study found only 6.1% GBM patients, while Nayak et al. found 46%. The presence of difference in such epidemological data in Indonesia and western countries indicates, first, that there remains many aspects that should be investigated regarding the behavior of atrocytoma tumor in Indonesia, and, second, there is an advance in the treatment and live expectancy, providing more responsibilities to the clinicians in treatment follow-up since the patients in Indonesia had relatively younger age.

Correlation between mutant p53 expression and histopathological grading according to WHO

The higher the histopathological grading, the more p53 mutation occurs, so that the expression of p53 protein becomes higher. From the results of statistical analysis using Spearman rho correlation test, it was found that there was correlation between mutant p53 expression and histopathological grading in astrocytoma tumor ($p < 0.0001$) with a high correlation strength and had positive value. This study confirmed that of Litofsky et al. who found correlation between the increase of mutant p53 protein expression and the increase of histopathological grading. This suggestion was also coined by other authors, such was Weixin et al. and Put-Ti-Noi et al. (Litofsky et al. 1997; Put-Ti-Noi et al. 2004; Weixin et al. 2000).

This study also confirmed the suggestion that mutant p53 protein does not inhibit proliferation or cell cycle in G1 phase, so that DNA damage becomes irreversible and mutation becomes fixed in the dividing cells, with the result that the cells are lead to malignant transformation. Abnormal p53 protein produced by mutant p53 gene is also capable in binding normal p53 gene products and inhibit its function as cell proliferation inhibitor (Kumar et al. 2005). Tumor cells containing mutant p53 gene will be resistant against apoptosis, so that tumor lacking these normal p53 genes will have more progressive growth. The capability of p53 to control apoptosis as a response against DNA damage also has practical implications in cancer therapy. Radiation and chemotherapy are two primary cancer therapies that have effect to induce DNA damage and apoptosis. Tumor cells that remains having normal p53 gene are more responsive against therapy than those containing mutant p53 (Kumar et al. 2005; Stratton 1996).

Correlation between mutant p53 expression and the patients' age and sex

This study shows that the older the age of the patients, the higher the expression of mutant p53 mutant. From the result of statistical analysis with Spearman rho correlation test, we found a strong correlation $r = 0.0434$ with $p = 0.012$. The age also affected the histopathological grading, in which the higher the patients' age, the higher the histopathological grading ($r = 0.641$, $p=0.0001$). Similar results were also reported by Flowers et al who found correlation between age increase and histopathological grading in which at least 50% of the patients with GBM had the disease at the age of more than 55 years (Flowers 2000). Regarding with sex, this study revealed significant correlation between sex and p53 expression. From statistical test, we obtained the value $p = 0.047$ ($p < 0.05$), indicating difference in mutant p53 protein in men and women. Similarly, the histopathological grading in men was higher than in women, with $p = 0.011$ ($p < 0.05$). Thus, although it can be concluded that the higher the histopathological grading, the stronger the mutant p53 protein expression, the expression of p53 protein cannot stand by itself. We cannot use it as a single determinant in determining disease prognosis, as it is also affected by other factors, such as age, sex, and other factors (location), which was not accounted for in this study.

CONCLUSIONS

It can be concluded that there is difference in mutant p53 expression in each histopathological grading of astrocytoma tumor, and there is significant correlation between mutant p53 expression and the increase of astrocytoma histopathological grading. The higher the grading, the higher the expression of mutant p53. Therefore, this study proved that mutant p53 expression can be used as genetic-based molecular marker that has prognostic value for astrocytoma tumor. The higher the expression of mutant p53, the higher the histopathological grading, the worse the prognosis. Further studies are required to involve larger sample, and should be carried out retrospectively in order to elaborate the role of mutant p53 expression in astrocytoma tumor. Further continuous studies are also needed on the correlation between mutant p53 expression and survival rate as well as on radiotherapy and chemotherapy, either neoadjuvant, chemosensitivity, or the type that would be given. Since various studies on the expression of mutant p53 in astrocytoma had given different results, a deeper study on factors (receptor, hormonal, age, sex, etc.) affecting mutant p53 expression on the emergence of astrocytoma maligancy is also needed.

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