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Clinicopathological Study of Diffuse Type Brainstem Gliomas: Analysis of 40 Autopsy Cases

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Summary. **Diffuse type brainstem gliomas, one of the most malignant brain tumors, have an extremely poor prognosis. Accordingly, it is presumed that the proliferative potential of these tumors is very high, but to date there have been few reports about the cell kinetics of the brainstem gliomas because surgical resection of these tumors has been applied only in limited cases.**

Forty autopsy cases with diffuse type brainstem gliomas were evaluated for histological grading, tumor spread, growth potential and prognosis. To quantify the growth potentials of individual tumors, we use monoclonal antibodies MIB-1 and proliferating cell nuclear antigen (PCNA), and proliferating cell indices (PCIs) were examined.

In 34 glioblastomas (GMs), mean MIB-1 and PCNA proliferating cell indices (PCIs) were 20.4% and 37.0%, respectively. Their median survival time in 22 treated cases was 40 weeks.

In 5 anaplastic astrocytomas (AAs), mean PCNA PCIs was 10.8% and their median survival time in 4 treated cases was 91 weeks. The MIB-1 and PCNA PCIs of one astrocytoma (A) were 2.9% and 20.3%, respectively, and the survival time was 56 weeks. There was a significant difference in PCNA PCIs between GMs and AAs ($p<0.05$), and there was also a significant difference in survival time between GMs and AAs, A in treated cases ($p<0.05$). Supratentorial extension was more frequent in GMs than in AAs ($p<0.05$).

Our results suggest that the majority of diffuse type brainstem gliomas are GM and their proliferative potentials are probably as high as that of adult supratentorial glioblastoma. Furthermore, supratentorial extension and dissemination are relatively frequent. There are a few AA or A, and they are less infiltrative and proliferative and have a slightly better prognosis than GM.

Key words—brainstem glioma, diffuse type, autopsy, MIB-1, PCNA.

INTRODUCTION

Brainstem gliomas acount for approximately 10-20% of children's brain tumors.[1,12,13] Among these, poorly demarcated and diffusely infiltrating gliomas, that is the "diffuse type" brainstem gliomas[4,8,14,25,37] or so-called "pontine glioma", have been thought to be biologically malignant irrespective of their histological gradings. Autopsy studies[6,7,18,21,23,24,33,34] revealed substantial numbers of diffuse type brainstem gliomas to be malignant, but few reports referred to the degree of tumor spread to the cerebrum, cerebellum, and spinal cord.[23] The poor prognosis of these tumors led to a presumption of high proliferative potentials for these tumors. However, there have been no reports on the cell kinetics of the brainstem gliomas because surgical resection of these tumors has been applied in only limited cases. On the other hand, there is increasing evidence that indices of tumor cell proliferation correlate fairly well with the prognosis of individual patients with gliomas. Immunohistochemical studies with the thymidine analog bromodeoxyuridine (BrdUrd) have shown that a high BrdUrd labeling index (LI), or S-phase fraction, indicates a higher proliferative potential, greater biologcal malignancy, and shorter time to recurrence than a low LI.[15,16,17,27] Some proliferation-associated nuclear proteins, such as Ki-67 protein, the

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Abbreviations—A, astrocytoma; AA, anaplastic astrocytoma; ABC, avidin biotin peroxidase complex, BrdUrd, bromodeoxyuridine, CT, computed tomography, DAB, diaminobenzidine, GM, glioblastoma; H & E, hematoxylin and eosin, K-B, Klüver-Barrera, LI, labeling index; MRI, magnetic resonance imaging; PBS, phosphate-buffered saline, PCIs, proliferating cell indices, PCNA, proliferating cell nuclear antigen; WHO, world health organization

proliferating cell nuclear antigen (PCNA), have also been reported to be useful for quantifying growth potential.[9,10,20,26,35] Recently, there have been numerous reports about a close correlation between MIB-1 or PCNA PCIs and the biological malignancy of various types of brain tumors, including supratentorial gliomas. These antibodies are suitable for use in formalin fixed paraffin embedded tissues. In this study, we have examined the histological features, degree of tumor spread, and proliferative potentials measured by using MIB-1 and PCNA monoclonal antibodies in 40 autopsy cases with diffuse type brainstem gliomas. The relationship between these factors and patient's prognosis is also studied.

MATERIALS AND METHODS

Patients

A total of 72 patients with brainstem glioma which were verified by autopsy, biopsy and/or CT, MRI were admitted to Niigata University Hospital between January 1962 and December 1996. The tumor arising primarily within the midbrain was excluded in this study. Sixty-four cases were a diffuse type[25,37], and the other 8 cases were a localized type.[25,37] The majority of these patients have already died and some of them have been subjected to autopsy. Forty autopsy specimens which were thought to be the diffuse type by clinical and histological findings were available for this study. These included 21 males and 19 females from 3 to 46 years of age, (mean: 11.9 years) at the time of admission. The majority of patients had radiotherapy and chemotherapy, and some cases had surgical intervention. The clinical data are summarized in Table 1.

Histological examination

Forty autopsy specimens were examined for this study. Sixteen cases received systemic autopsies including the whole spinal cord, and in 24 cases examination was limited to the intracranial tissues including the upper cervical cord. Each autopsy specimen was fixed in 10% formalin or 4% paraformaldehyde for 2 to 4 weeks. After fixation, the cerebrum was cut serially at a 10 mm thickness, and the brainstem and cerebellum were cut at a 5 mm thickness. The spinal cord was cut at an approximately 10 mm thickness at the upper cervical (C1-2), lower cervical (C6-7), thoracic (Th7-8), and lumbar portions (L3-4). Representative parts of the tumor, cerebrum, cerebellum, brainstem and spinal cord were embedded in paraffin. Each block was serially sectioned at a 4 μm thickness and used for hematoxylin and eosin (H & E), Klüver-Barrera (K-B) and immunohistochemical staining. The histology was classified according to WHO classification.[19] If the tumor had heterogeneity, a higher histological grade was elected. Parenchymal and leptomeningeal spread were examined microscopically in all sections of the tumor, cerebrum, brainstem, cerebellum and spinal cord.

Immunohistochemistry of Ki-67 (MIB1) and PCNA

Thirty-eight specimens were available for these immunohistochemical studies. A representative part of the tumor was serially sectioned and used for MIB-1 and PCNA studies. Each section was deparaffinized, placed in a 10 mM Citrate buffer (PH 6.0), and heated at 93℃ for 15 min. in a commercial microwave.[36] Then the sections were placed in 0.3% H_2O_2 in methanol for inhibition of endogeneous peroxidase for 30 min.. They were then reacted with nomal horse serum for 10 min., and further with the following primary antibodies at 4℃ overnight: MIB-1 (Immunotech S.A., Marseille, France) diluted 1 : 100 in phosphate-buffered saline (PBS) containing 0.05% tween 20 and PCNA (Dakopatts, Glostrup, Denmark) diluted 1 : 100 in PBS. The sections were washed in PBS three times and were reacted with biotinylated horse anti-mouse IgG for 60 min.. Then the sections were reacted with avidin biotin peroxidase complex (ABC) with a Vectastain ABC kit (Vector, Burlingame, CA, USA) for 60 min., developed with diaminobenzidine (DAB), and counterstained with hematoxylin. The MIB-1 and PCNA PCIs were calculated as a percentage of immunostained nuclei, excluding the nuclei of vascular components and hematogeneous cells. More than 1000 cells were counted in several areas of tissue in which positive nuclei were evenly distributed. The most actively proliferating areas were examined for each MIB-1 and PCNA PCIs.

Statistical analysis

Survival was measured from the date of admission to that of death. The Kaplan-Meier Method was used to estimate survival distribution, and a generalized Wilcoxon Method was used to compare it. The correlation between the MIB-1 and PCNA PCIs and survival time was determined by the linear regression analysis. The statistical significance of differences of MIB-1 and PCNA PCIs among histological grades and differences between clinical factors and survival time were determined by the t-test for unpaired data using Stat view IV statistical software (Abacus

Table 1. Clinical summary and MIB-1, PCNA PCIs of diffuse type brainstem glioma

Case	Age[a]	Sex	Site	Histology	Resect	XRT[b]	Chemo[c]	Tumor extension[d]	MIB-1 PCIs(%)	PCNA PCIs(%)	Survival[e] (weeks)
1	5y7m	F	P	GM	−	+	−	1	17.7	83.0	20
2	18y	M	P	GM	−	+	−	2	10.4	34.7	32
3	14y	M	P	GM	−	−	−	2	35.9	29.9	16
4	6y3m	F	P	A	−	+	−	1	2.9	20.3	56
5	35y	F	P	GM	partial	+	−	2	NE	37.7	44
6	4y9m	F	P	GM	−	+	−	1	14.8	31.5	36
7	6y6m	M	P	GM	−	−	−	2	21.1	17.3	12
8	28y	M	MO	GM	−	−	−	3	20.7	35.2	14
9	6y6m	F	P	GM	−	−	−	1	18.3	34.3	4
10	6y1m	M	P	GM	−	+	−	1	11.6	23.7	60
11	8y8m	M	P	GM	−	+	−	3	NE	16.0	40
12	4y1m	F	P	GM	−	−	−	1	NE	NE	2
13	42y	M	P	AA	−	−	−	1	NE	13.2	32
14	46y	M	P	GM	−	+	−	4	NE	NE	44
15	8y8m	F	P	GM	−	−	−	3	27.8	44.9	6
16	16y	F	P	GM	partial	−	−	2	9.6	NE	10
17	24y	M	P	GM	−	−	−	2	34.2	46.1	20
18	5y3m	F	P	GM	−	−	−	1	NE	24.3	8
19	8y10m	M	P	GM	−	−	−	1	45.4	49.6	6
20	8y3m	F	P	GM	−	−	−	1	ND	ND	6
21	4y6m	M	P	GM	−	50Gy	−	4	19.5	52.7	42
22	15y	F	P	GM	−	50Gy	−	4	10.4	34.6	24
23	8y0m	F	P	AA	−	44Gy	−	1	NE	3.9	25
24	5y4m	M	P	GM	−	−	−	1	27.1	47.8	8
25	6y2m	M	P	GM	−	56Gy	+	2	21.4	37.6	48
26	5y5m	F	P	GM	−	57Gy	+	1	NE	NE	34
27	4y0m	M	P	AA	−	52Gy	+	1	NE	15.3	91
28	6y9m	M	P	GM	−	59Gy	+	2	15.4	14.7	18
29	10y0m	F	P	GM	−	58Gy	+	1	21.5	32.2	32
30	4y10m	F	P	GM	−	95Gy	+	3	NE	14.9	24
31	7y3m	M	P	GM	−	80Gy	+	4	NE	70.1	48
32	31y	F	P	AA	−	60Gy	+	1	NE	10.7	336
33	7y2m	M	P	GM	−	80Gy	+	4	8.0	21.6	40
34	16y	F	P	GM	partial	86Gy	+	4	19.6	40.5	92
35	3y7m	M	P	GM	biopsy	60Gy	+	4	ND	ND	44
36	15y	M	P	AA	biopsy	93Gy	+	3	NE	NE	92
37	7y10m	F	P	GM	partial	50Gy	+	1	20.8	46.4	44
38	15y	M	MO	GM	subtotal	50Gy	+	4	25.5	68.9	48
39	25y	M	P	GM	biopsy	50Gy	+	2	13.7	18.5	56
40	4y7m	M	P	GM	−	46.8Gy	+	2	19.8	28.3	32

Resect, resection; XRT, radiotherapy; Chemo, chemotherapy; y, years; m, months; M, male; F, female; P, pons MO, medulla oblongata; GM, glioblastoma multiforme; AA, anaplastic astrocytoma; A, astrocytoma
NE, not evaluable; ND, not done.
[a] Age is at the time of admission; [b] + indicate that the dose of radiotherapy is unclear; [c] + indicate combination chemotherapy using nimustine (ACNU), ranimustine (MCNU), vincristine, etoposide and cisplatin; [d] The number is same as the number in Fig. 2. [e] Survival is measured from the date of admission.

Concepts, Berkeley, CA, USA). The statistical significance of differences between histological grades and tumor extension was determined by chi-square statistics.

RESULTS

The pathological data obtained are presented in Table 1 with clinical data.

Histology and tumor extension

The original tumor location was estimated from CT, MRI and/or autopsy findings (Fig. 1). The majority of the original tumor location was the pons (38 cases), and only 2 patients had a possible origin in the medulla oblongata. The pontine tumors consisted of 32 glioblastomas (GM), 5 anaplastic astrocytomas (AA), and 1 astrocytoma (A). The 2 tumors of the medulla oblongata were GM.

There was no extracranial metastasis. Proximal cervical cord involvement was seen in 3 cases (cases 17, 25, 32). Direct supratentorial invasion was observed in 18 cases (cases 2, 3, 5, 7, 14, 16, 17, 21, 22, 25, 28, 31, 33-35, 38-40) and extensive invasion of the cerebral white matter beyond the thalamus or basal ganglia was observed in 9 cases (cases 3, 5, 14, 22, 31, 34, 35, 39, 40). Cerebellar invasion was seen in 14 cases (cases 2, 7, 8, 10, 12, 21, 22, 25-27, 32, 35, 38, 40). Subarachnoid dissemination was observed in 13 cases (cases 8, 11, 14, 15, 21, 22, 30, 31, 33-36, 38), and in 6 cases among them dissemination was widely extended to the supratentorial and spinal subarachnoid space (cases 11, 14, 30, 31, 33, 38); ventricular dissemination was observed in 5 cases (cases 11, 21, 30, 34, 38). We divided the 40 autopsy cases into 4 groups according to the direct invasion and dissemination characteristics shown in Fig. 2. In 16 GMs, the tumors grew within the brain stem with (type III, N=4) or without (type I, N=12) leptomeningeal dissemination; in the remaining 18 GMs, the gliomas spread beyond the brain stem with (type IV, N=8) or without (type II, N=10) subarachnoid seedings. In 5 AAs, 4 were type I and 1 was type II. One astrocytoma showed type I growth (Fig. 2). Supratentorial extension was more frequent in GMs than in AAs ($p<0.05$).

MIB1 and PCNA PCIs

The MIB-1 immunohistochemistry and PCNA staining (Fig. 1) were evaluable in 66% (25/38) and 87% (33/38), respectively. In 13 cases. MIB-1 stainings could not be evaluated because of a lack of nuclear stainings (8 cases) or diffuse nonspecific cytoplasmic stainings (5 cases). PCNA stainings could not be evaluated in 5 cases because of a lack of nuclear staining (1 case) or diffuse nonspecific cytoplasmic stainings (4 cases). All these cases were not obviously related to period of storage, period of brain death, period of fixation, kinds of solution of the fixation, the interval from death to autopsy or season of the autopsy. Furthermore, inner positive control was not examined in this study. In GMs, MIB-1 PCIs ranged 8.0-45.4% (mean 20.4%) and PCNA PCIs ranged 14.7-83.0% (mean 37.0%) (Fig. 3). In AAs, mean PCNA PCIs was 10.8%, which was significantly lower than that of GMs ($p<0.05$) (Fig. 3). The MIB-1 and PCNA PCIs of A were 2.9% and 20.3%, respectively. On the PCNA staining, there was a wide variety of staining density, and sometimes the evaluation of positive or negative could not easily be compared with MIB-1 staining. We judged PCNA staining to be positive when strong nuclear staining was observed or the nuclear staining was dotted and absolutely limited to the nucleus, even though the staining density was slightly weak.

Survival time and prognostic factors

Median survival was 40 weeks for GMs and 74 weeks for AAs and A in treated cases. One astrocytoma patient survived 56 weeks. A significant difference in survival time was found between GMs and AAs, A ($p<0.05$) (Fig. 4). All patients except for a 7-year survivor were dead within 2 years, and median survival time was 32 weeks.

For GMs with radiotherapy, mean survival time was 41.0 weeks, which was significantly longer than that of GMs without radiotherapy (mean survival time 9.3 weeks) ($p<0.05$).

Also, in GMs, linear regression analysis showed a slight correlation which was not significant between MIB-1 PCIs and survival time ($R=-0.327$, $p=0.119$).

DISCUSSION

Histological characteristics of diffuse type brainstem glioma

In terms of the histology of the diffuse type brainstem glioma, about two-third of the tumors have been classified as high grade glioma in autopsy studies.[6,7,18,21,23,24,33,34] In our study, the incidence of high grade glioma was 97.5% (39/40); this was higher than that reported in previous reports. In biopsy studies, however, the reported incidence of high

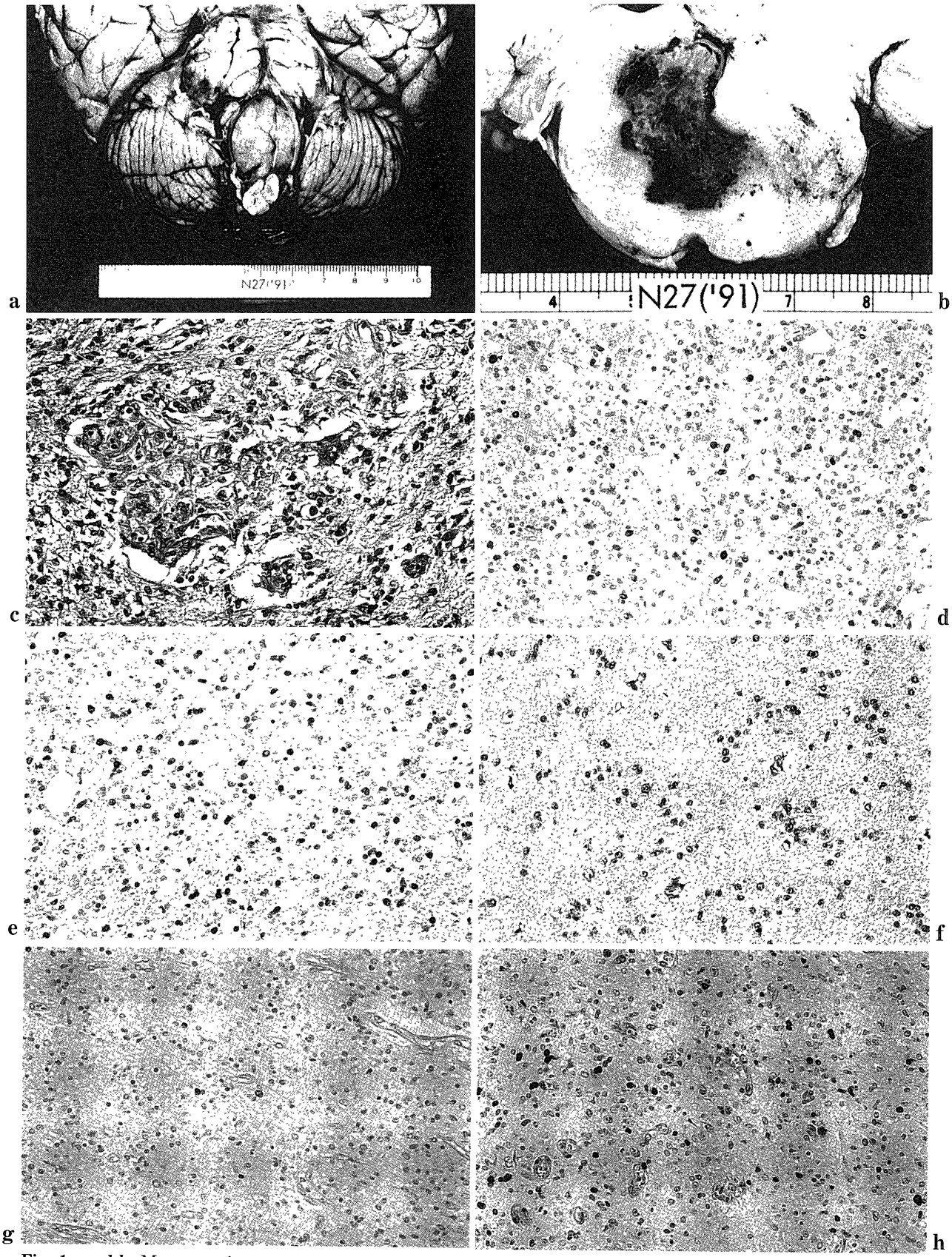

Fig. 1a and b. Macroscopic appearence of the diffuse type brainstem glioma from case 37. **c.** Photomicrogram of the tumor tissue from case 37 showed glioblastoma (hematoxylin and eosin ×180). **d.** The MIB-1 proliferating cell index (PCI) of case 37 was 20.8% (×180) and **e.** PCNA PCI was 46.4% (×180). **f.** Another tumor tissue from case 4 shows astrocytoma (hematoxylin and eosin ×180). **g.** MIB-1 PCI of case 4 was 2.9% (×180) and **h.** PCNA PCI was 20.3% (×180).

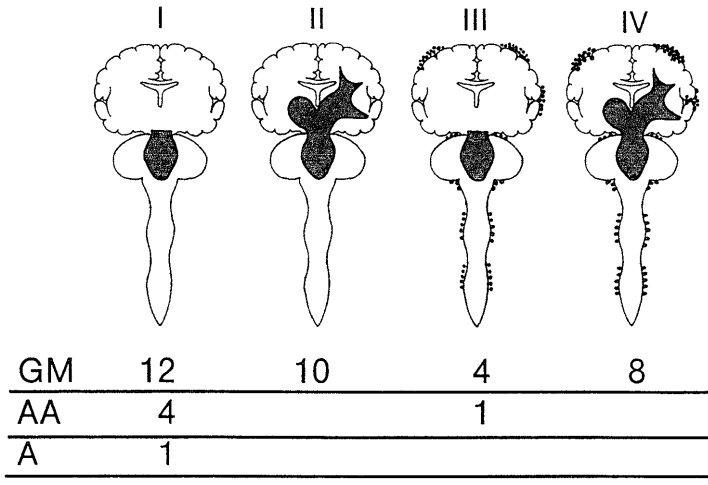

Fig. 2. Four types of tumor extension were observed. In 34 glioblastomas, 12 cases showed type I growth (the tumor grew within the brainstem without leptomeningeal dissemination), 10 cases showed type II growth (the tumor spread beyond the brainstem without subarachnoid seeding), 4 cases showed type III growth (the tumor grew within the brainstem with leptomeningeal dissemination), and 8 cases showed type IV growth (the tumor spread beyond the brainstem with subarachnoid seeding). In 5 anaplastic astrocytomas, 4 were type I and 1 type III growth. One astrocytoma showed type I growth.

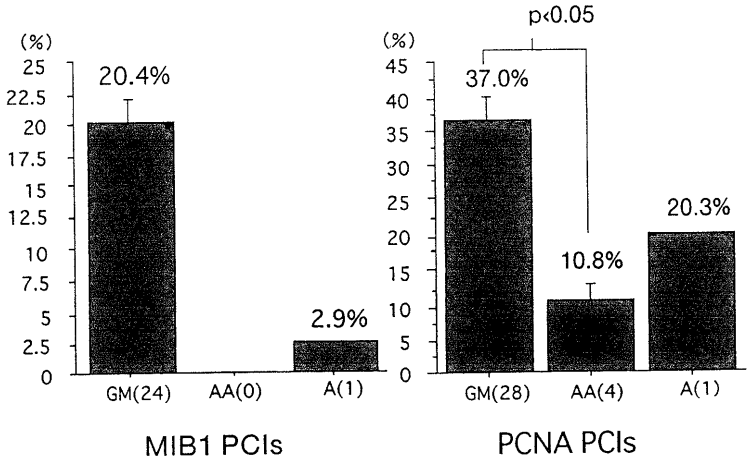

Fig. 3. Comparison of MIB-1 and PCNA indices among glioblastoma (GM), anaplastic astrocytoma (AA) and astrocytoma (A). Parentheses indicate number of cases.

grade glioma varied from 22% to 67%.[3,22,32,38] This histological discrepancy between biopsy and autopsy studies is attributed to sampling errors or the histological heterogeneity of these tumors.[2,5,6,11,38]

In our study, half of the untreated early stage tumors had histological heterogeneity, and the central parts of the tumors were more malignant and had more neovascularization than the periphery of the tumors. These tumors were classified as GM because of the existence of small parts of glioblastoma tissue, though most of the tumor tissues were classified as AAs or As according to WHO classifica-

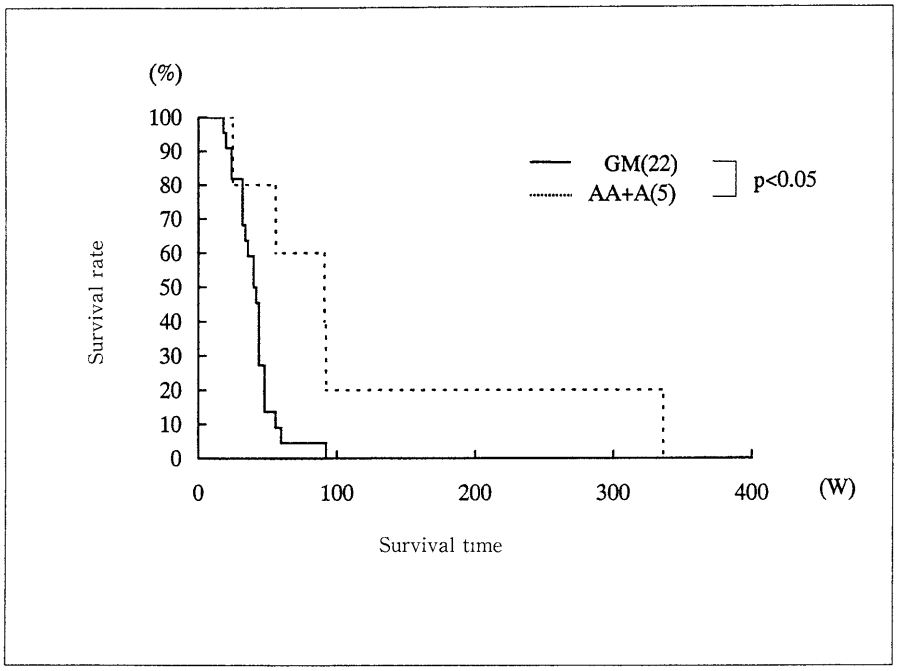

Fig. 4. Kaplan-Meier survival curves of glioblastoma (GM) and anaplastic astrocytoma, astrocytoma (AA+A) in treated cases.

tion because of the absence of necrosis and vascular proliferation. Thus, the incidence of high grade glioma was higher than that of previous reports, and the biopsy specimens were more likely to be diagnosed as more benign than their true tumor grade. We therefore speculated that one of the histological characteristics of diffuse type brainstem gliomas was the paucity of necrosis and vascular proliferation.

As for the tumor extension, there are very few autopsy studies concerning supratentorial and spinal extensions. Mantravadi et al.[23] reported on 23 autopsy studies, with 3 cases each of direct supratentorial extension and direct spinal extension, whereas cerebellar extension was observed in 10 cases. In that report, however, supratentorial extension was examined only about the temporal lobe. In our study, direct supratentorial extension was observed in 18 cases, cerebellar extension was observed in 14 cases, and spinal extention was observed in 3 cases; thus, the supratentorial extension was relatively frequent. Concerning leptomeningeal dissemination, Littman et al.,[22] Mantravadi et al.,[23] Packer et al.,[30] and Matsutani et al.[24] reported the incidences of the meningeal dissemination evaluated at autopsy as 17%, 17%, 33% and 17%, respectively. In our large series, 13 cases (33%) of leptomeningeal dissemination were observed, this incidence being as high as that of supratentorial GM,[29] with widespread subarachnoid dissemination observed in 6 of the 13 cases. We therefore also speculated that relatively frequent supratentorial extension and leptomeningeal dissemination were characteristics of the diffuse type brainstem glioma.

Proliferative potentials and prognostic factors

There were significant differences in survival time between GMs and AAs, A. There were also significant differences in PCNA PCIs between GMs and AAs, so it is certain that PCIs relates to biological malignancy of the diffuse type bainstem glioma like other brain tumors. However, we could not evaluate MIB-1 PCIs in AAs, so we could not find any significant differences between MIB-1 PCIs and histological grading. Also, there was no significant correlation between MIB-1, PCNA PCIs and patient survival time. Still, a slight correlation was observed between MIB-1 PCIs and survival time. These patients soon suffer from respiratory and swallowing disturbances and tetraplegia and are forced to be bedridden, finally developing pneumonia. In this way, the patient survival time is possibly shortened by these complications. This study also included both old and recent cases, so the recent progression of microsurgical technique and radiochemotherapy allowed a prolon-

gation of patient survival time prolong. These problems make it difficult to evaluate the relationship between MIB-1 and PCNA PCIs and patient survival time.

As for clinical factors, radiotherapy obviously improved the patients' prognosis, but no effect of chemotherapy was observed in this study.

Finally, in this study, the mean MIB-1 PCIs of GMs was 20.4% and this MIB-1 PCIs was an high as that of adult supratentorial GM reported in previous studies.[28,31] We therefore speculate that the proliferative potential of diffuse type brainstem gliomas is as high as that of adult supratentorial GM.

REFERENCES

1) Abramson N, Raben M, Cavanaugh PJ: Brain tumors in children: Analysis of 136 cases *Radiology* **112:** 669-672, 1974.
2) Albright AL, Guthkelch AN, Packer RJ, Price RA, Rourke LB: Prognostic factors in pediatric brainstem gliomas. *J Neurosurg* **65:** 751-755, 1986.
3) Albright AL, Price RA, Guthkelch AN: Brain stem gliomas of the children: A clinicopathological study. *Cancer* **52:** 2313-2319, 1983.
4) Berger MS, Edwards MSB, LaMasters D, Davic RL, Wilson CB: Pediatric brain stem tumors: Radiographic, pathological, and clinical correlations. *Neurosurgery* **12:** 298-302, 1983.
5) Bodian M, Lawson D: The intracranial neoplastic disease of childhood: a description of their natural history based on a clinic-pathological study of 129 cases *Br J Surg* **40:** 368-392, 1952.
6) Bray PF, Carter S, Taveras JM: Brainstem tumors in children. *Neurology* **8:** 1-7, 1958.
7) Buckley RC: Pontine gliomas: A pathologic study and classification of twenty-five cases. *Archs Pathol* **9:** 779-819, 1930.
8) Epstein F, McCleary EL: Intrinsic brainstem tumors of childhood: Surgical indications. *J Neurosurg* **64:** 11-15, 1998.
9) Gerdes J, Lemke H, Baisch H, Wacker H-H, Schwab U, Stein H: Cell cycle analysis of a cell-proliferation associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol* **133:** 1710-1715, 1984.
10) Gerdes J, Schwab U, Lemke H, Stein H: Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer* **31:** 13-20, 1983.
11) Golden GS, Ghtak NR, Hirano A, French JH: Malignant glioma of the brain-stem: a clinicopathological analysis of 13 cases. *J Neurol Neurosurg Psychit* **35:** 732-738, 1972.
12) Hara M, Takeuchi K: A temporal study of survival of patients with pontine gliomas. *J Neurol* **216:** 189-196, 1997.
13) Hoffman HJ, Becker L, Craven MA: A clinically and pathologically distinct group of benign brainstem gliomas. *Neurosurgery* **7:** 243-248, 1980.
14) Hoffman HJ: Modern management of brainstem glioma, Kageyama N (eds): Proceedings of the first international symposium on pediatric neurooncology. The University of Nagoya Press, Japan, 1987, p 134-135.
15) Hoshino T, Prados M, Wilson CB, Cho KG, Lee KS, Davis RL: Prognostic implications of the bromodeoxyuridine labeling index of human gliomas. *J Neurosurg* **71:** 335-341, 1989.
16) Hoshino T, Rodriguez LA, Cho KG, Lee KS, Wilson CB, Edwards MSB, Levin VA, Davis RL: Prognostic implications of proliferative potential of low-grade astrocytomas. *J Neurosurg* **69:** 839-842, 1988.
17) Ito S, Hoshino T, Shibuya M, Prados MD, Edwards MSB, Davis RL: Proliferative characteristics of juvenile pilocytic astrocytomas determined by bromodeoxyuridine labeling. *Neurosurgery* **31:** 413-419, 1992.
18) Jackson IJ, Thomson RK: Pediatric Neurosurgery, Charles C Thomas Publisher, Springfield, Ilinois, 1959.
19) Kleiheus P, Cavenee WK (eds): Pathology and Genetics of Tumors of the Nervous System. International Agency for Research on Cancer, Lyon, 2000, p22-44.
20) Kurki P, Vanderlaan M, Dolbeare F, Gray T, Tan EM: Expression of proliferating cell nuclear antigen (PCNA)/ cyclin during cell cycle. *Exp Cell Res* **166:** 209-219, 1986.
21) Lassman LP, Arjona VE: Pontine gliomas of childhood. *Lancet* **1:** 913-915, 1967.
22) Littman P, Jarret P, BilaniuK LT, Rork LB, Zimmerman RA, Bruce DA, Carabell SC, Schut L: Pediatric brain stem gliomas. *Cancer* **45:** 2787-2792, 1980.
23) Mantravadi RVP, Phatak R, Bellur S, Liebner EJ, Haas R: Brain stem gliomas: An autopsy study of 25 cases. *Cancer* **49:** 1294-1296, 1982.
24) Matsutani M, Sano K: Brainstem gliomas in children. *Shouni no Noshinkei* **2:** 411-416, 1978. (Japanese with English abstract)
25) Mori S, Tanaka R, Takeda N, Yoshida S: Brain stem gliomas in children: Correlation between CT findings and histology and prognosis. *Shouni no Noshinkei* **14:** 359-364, 1989. (Japanese with English abstract)
26) Miyachi K, Fritzler MJ, Tan EM: Autoantibody to a nuclear antigen in proliferating cells. *J Immunol* **121:** 2228-2234, 1978.
27) Nagashima T, DeArmond SJ, Murovic J, Hishino T: Immunocytochemical demonstration of S-phase cells by anti-bromodeoxyuridine monoclonal antibody in human brain tumor tissues. *Acta Neuropathol (Berl)* **67:** 155-159, 1985.
28) Onda K, Davis RL, Shibuya M, Wilson CB, Hoshino

T: Correlation between the bromodeoxyuridine labeling index and the MIB-1 and Ki-67 proliferating cell indices in cerebral gliomas. *Cancer* **74:** 1921-1926, 1994.

29) Onda K, Tanaka R, Takahashi H, Takeda N, Ikuta F: Cerebral glioblastoma with cerebrospinal fluid dissemination: a clinicopathological study of 14 cases examined by complete autopsy. *Neurosurgery* **25:** 533-540, 1989.

30) Paker RJ, Allen J, Nielsen S, Petito C, Deck M, Jereb B: Brainstem glioma: clinical manifestations of meningeal gliomatosis. *Ann Neurol* **14:** 177-182, 1983.

31) Pollack IF, Campbell JW, Hamilton RL, Julio Martinez A, Bozik ME: Proliferation index as a predictor of prognosis in malignant gliomas of childhood. *Cancer* **79:** 849-856, 1997.

32) Reigel DH, Scarff TB, Woodford JE: Biopsy of pediatric brain stem tumors. *Child's Brain* **5:** 329-340, 1979.

33) Renier WO, Gabreels FJM: Evaluation of diagnosis and non-surgical therapy in 24 children with a pontine tumour. *Neuropediatrics* **11:** 262-273, 1980.

34) Russell DS, Rubinstein LJ: Pathology of tumours of the nervous system, Sixth edition, Edward Arnold Publishers, London, 2000, p 335-339.

35) Sasaki K, Murakami T, Kawasaki M, Takahashi M: The cell cycle associated change of the Ki-67 reactive nuclear antigen expression. *J Cell Physiol* **133:** 579-584, 1987.

36) Shi S-R, Key ME, Kalra KL: Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem* **39:** 741-748, 1991.

37) Tanaka R: Brainstem glioma. Pediatric Neurosurgery. Igaku-Shoin, Tokyo, 1992, p 520-534.

38) Tomita T, McLone DG, Naidich TP: Brain stem gliomas in childhood: Rational approach and treatment. *J Neurooncol* **2:** 117-122, 1984.