Histopathological Subtypes of Medulloblastoma According to WHO Classification (2007): An 8-year Retrospective Study

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ABSTRACT

Objective: To provide histopathological subtypes of medulloblastomas (according to 2007 WHO Classification of tumors of the central nervous system) that were previously diagnosed during 1998 to 2005 in Siriraj Hospital, and to characterize the immunophenotypic patterns of medulloblastomas in correlation with these subtypes.

Methods: All diagnosed medulloblastomas collected during 1998 to 2005 in the Department of Pathology, Siriraj Hospital were reviewed and classified according to WHO classification (2007) by H&E staining. Furthermore, we performed immunohistochemical studies, including synaptophysin, glial fibrillary acidic protein (GFAP), neurofilament, and Ki-67 to characterize the immunophenotypical patterns of medulloblastoma correlated with morphological variants. Statistic analyses were Tukey HSD, Mann-Whitney test, and Fisher's exact test.

Results: Of 41 medulloblastomas, 32 cases were classic type (78.05%), 6 cases were large cell/anaplastic variant (14.63%), and 3 cases were desmoplastic variant (7.32%). This study showed significantly increased Ki-67 indices in the large cell/anaplastic variant compared to either classic medulloblastoma or desmoplastic/nodular variant (p <0.05). There is no statistical difference in immunophenotypes of synaptophysin, GFAP, or neurofilament between each subtype.

Conclusion: According to 2007 WHO classification of tumor of the central nervous system 2007, in our study, the majority was medulloblastoma classic type, followed by large cell/anaplastic and desmoplastic/nodular variants, respectively. Statistical significance of Ki-67 indices suggests its applicable adjuvant diagnostic tool to distinguish the large cell/anaplastic variant from others.

Keywords: Medulloblastoma; large cell/anaplastic; Ki-67; WHO classification; immunohistochemical study

Siriraj Med J 2010;62:202-205

E-journal: http://www.sirirajmedj.com

Medulloblastoma (MB) is a highly malignant embryonal tumor of the cerebellum and in the roof of the forth ventricle. In childhood, it is the most common malignant primary central nervous system tumor and is encountered in approximately one-quarter of all intracranial tumors. The peak incidence of medulloblastoma occurs near the end of the first decade of life.

In WHO classification 2007[1], medulloblastoma is categorized into classic type, desmoplastic/nodular, medulloblastoma with extensive nodularity, large cell, and anaplastic medulloblastoma (Table 1). The worst prognosis is associated with the degree of anaplasia, whereas prognosis between classic and nodular subtypes remains controversial.[2-7] The histologic classification of medulloblastoma is helpful in predicting the prognosis of patients. Other studies suggested that immunohistochemical studies are useful to characterize subtypes of pediatric medulloblastoma and predict clinical outcome.[8,9]

MATERIALS AND METHODS

This study is a retrospective study on cases diagnosed as medulloblastoma obtained from the anatomical pathology records of the Department of Pathology, Faculty of Medicine Siriraj Hospital, from January 1st, 1998 to December 31st, 2005. These cases were retrieved from a computer filing system. Second biopsies of

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TABLE 1. World Health Organization classification of embryonal tumors of the central nervous system.

2000

- Medulloepithelioma
- Ependymoblastoma
- Medulloblastoma
  - Desmoplastic medulloblastoma
  - Large-cell medulloblastoma
  - Medullomyoblastoma
  - Melanotic medulloblastoma
- Supratentorial PNET
  - Neuroblastoma
  - Ganglioneuroblastoma
- Atypical teratoid/rhabdoid tumor

2007

- Medulloepithelioma
- Ependymoblastoma
- Medulloblastoma
  - Desmoplastic/nodular medulloblastoma
  - Medulloblastoma with extensive nodularity
  - Large-cell medulloblastoma
  - Anaplastic medulloblastoma
- CNS primitive neuroectodermal tumour
  - CNS Neuroblastoma
  - CNS Ganglioneuroblastoma
  - Medulloepithelioma
  - Ependymoblastoma
- Atypical teratoid/rhabdoid tumor

the same patients during the selected period or consultation cases from outside the center were excluded. The hematoxylin and eosin (H&E) and reticulin-stained slides of the included cases were reviewed and classified according to WHO classification 2007[1] by three observers.

Immunohistochemical studies were performed on representative formalin-fixed, paraffin-embedded tissue sections in all cases, except for 2 cases with unavailable tissue blocks, using antibodies against the Glial Fibrillary Acidic Protein (GFAP), synaptophysin, neurofilament (NF), and Ki-67 (Table 2). Paraffin sections, 3 μm thick, were cut, mounted on coated slides and left to dry overnight in an oven at 56°C. Sections were dewaxed and endogenous peroxidase activity blocked in a 3% solution of hydrogen peroxide in methanol and then pressure cooked for 90 seconds in a pressure cooker using a 10 mmol citrate buffer at pH6 and cooled down for at least 20 minutes at room temperature and then running tap water for 5 minutes. Thereafter, the sections were washed in phosphate buffer saline (10 minutes) again and blocking non-specific reagent was applied for 20 minutes by 2% Bovine serum albumin. The sections were covered with primary antibodies overnight at room temperature then washed in phosphate buffer saline for 10 minutes. The detection system was Dako Cytomation Envision system-HRP labeled polymer (incubate sections for 30 minutes) with diaminobenzidine as the chromogen. The slides were counterstained with hematoxylin.

For immunostaining interpretations, we used criteria that were modified from that of Son EI, et al,[8] in which the immunoreactivity of tumor cells with the selected antibodies was determined by staining intensity. The immunostaining was interpreted as positive if the tumor cells demonstrated strong staining intensity (detectable under x40 magnification). The stained tumor cells that were only detectable under x100 or higher magnification were scored as weak staining and, therefore, defined as negative. In our observation, GFAP can express, with similar staining patterns, both in the glial differentiated foci of the tumors and in the non-neoplastic glial fibers that are derived from adjacent brain tissues. Because of this reason, we decided not to interpret GFAP staining that presented at the periphery of the tumor islands in the tissue sections. For proliferation index, Ki 67-positive cells were counted per 1,000 cells in each case, and then scored by percentage. Due to the biphasic pattern of desmoplastic medulloblastoma, the sum of Ki 67-positive cells per 500 cells within the reticulin-free nodules and the reticulin-rich internodular regions was performed in each case and the percentage of Ki 67-positive cells was calculated into an average value. For a small subset of classic medulloblastomas that contained significant nodular zones of neuronal differentiation (so called "neuropil island"),[10] Ki-67 was scored by the same method used for the desmoplastic variant. For classic medulloblastoma and large cell/anaplastic variant, randomly selected areas were counted. Comparison of proliferation index (Ki-67) between subtypes of medulloblastoma was performed by the method of Tukey HSD. Fisher's exact test was used comparing GFAP, synaptophysin, and neurofilament expression between each subtype.

TABLE 2. Antibodies used.

Antigen	Clone/dilution (overnight)	Source	Pretreatment
GFAP	6F2/ 1:5000	DAKO, DENMARK	Pressure cooker
SYN	SY38/ 1:2000	Produktionsvej 42	Pressure cooker
NF	2F11/ 1:1000	DK-2600 Glostrup	Pressure cooker
Ki-67	MIB-1/ 1:6000	Denmark	Pressure cooker

GFAP, glial fibrillary acidic protein; SYN, synaptophysin; NF, neurofilament

RESULTS

Three subtypes of medulloblastoma were identified in our study sample: classic, desmoplastic, and large cell/anaplastic medulloblastoma. Classic medulloblastoma was composed of round-to-oval or carrot-shaped, rather uniform cells with hyperchromatic nuclei and scant cytoplasms arranged in varying proportion of Homer-Wright rosettes (Fig 1A) and closely packed sheets (Fig 1B) or rows. Desmoplastic medulloblastoma was characterized by pale, reticulin-free nodules with neuronal maturation, and alternating reticulin-rich zones consisting of tumor cells morphologically similar to that of classic medulloblastoma (Fig 2). This variant of medulloblastoma was easier to recognize by using reticulin stain in highlighting the reticulin-rich zone surrounding the reticulin-free nodule. The reticulin-free nodules contained tumor cells with neurocytic appearance with small nuclei within a neuropil background. Large cell/ anaplastic medulloblastoma had different histologic features from that of classic cases, including increased nuclear atypia, abundant apoptotic bodies, high mitotic rate and vesicular nuclei with macronucleoli (Fig 3).

Of 41 medulloblastomas, the ages ranged from 1 to 41 years, 87.8% were less than 18 years of age,

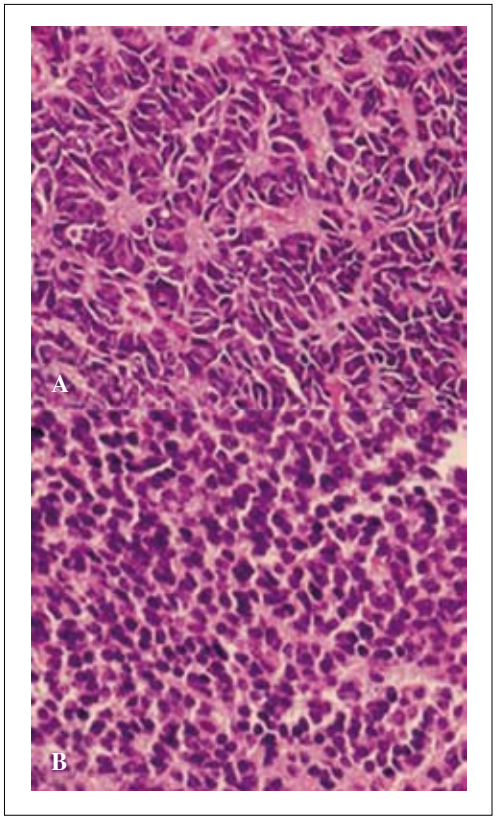

Fig 1. Photomicrograph of classic medulloblastoma showing Homer-Wright rosettes (A) and closely packed uniform primitive cells (B) (Paraffin section; H&E staining, 400x).

12.2% were more than 18 years of age. Thirty-two cases (78.05%) were classic type, 6 cases (14.63%) were large cell/anaplastic variant, and 3 cases (7.32%) were desmoplastic variant. Medulloblastoma with extensive nodularity variant was not observed in the sample.

Immunostaining was performed in 39 cases. GFAP was expressed in 28 from 32 cases (93%) of classic medulloblastoma, all 3 cases (100%) of desmoplastic variant, and 4 from 6 cases (60%) in large cell/anaplastic variant. Synaptophysin was expressed in 27 from 32 cases (90%) of classic medulloblastoma, all 3 cases (100%) of desmoplastic variant, and 4 from 6 cases (60%) of large cell/anaplastic variant. Neurofilament was expressed in 11 from 32 cases (25%) of classic medulloblastoma, none (0%) of desmoplastic variant, and 1 from 6 cases (25%) of large cell/anaplastic variant. Comparison of these patterns of immunoreactivity between subtypes yielded no significantly statistical difference.

Immunohistochemical study for Ki-67 was used

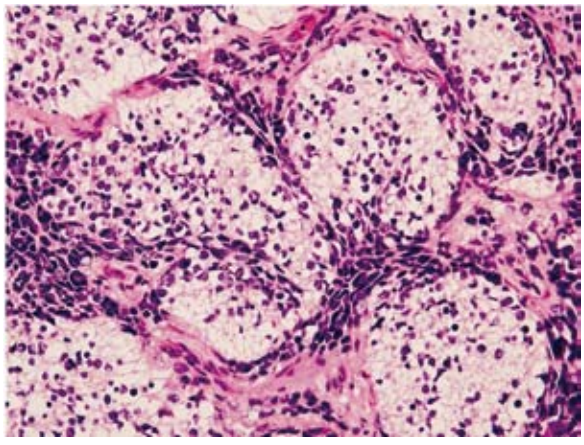

Fig 2. Photomicrograph of desmoplastic medulloblastoma showing pale neurocytic differentiated, reticulin-free nodules alternating with internodular zones (Paraffin section; H&E staining, 400x).

for labeling all proliferating cells in these tumors. The proliferation indices were 5.349 (SD = 4.096) in classic medulloblastoma, 11.033 (SD = 6.627) in desmoplastic variant, and 19.617 (SD = 6.546) in the large cell/anaplastic variant. Statistical analysis indicated the significantly higher proliferation index of the large cell/anaplastic variant compared to those of classic form and desmoplastic variants ($p < 0.05$) (Table 3, Fig 4).

DISCUSSION

Medulloblastoma is a malignant, invasive embryonal tumor of the cerebellum with preferential manifestations in children, predominantly neuronal differentiation, and an inherent tendency to metastasize via cerebrospinal fluid pathways.[1] According to WHO Classification of Tumours of the Central Nervous System 2007[1], medulloblastomas, in addition to cases with typical morphology, are further classified into 4 variants that are identified histologically and having some relevance for clinical outcome (desmoplastic/nodular medulloblastoma, medulloblastoma with extensive nodularity, anaplastic medulloblastoma and large cell medulloblastoma) and 2 patterns of differentiation that are recognized histologically, but do not have a distinct clinical or pathological significance [medullomyoblastoma (myogenic differentiation) and melanocytic medulloblastoma (melanocytic differentiation)]. As large cell medulloblastoma and anaplastic medulloblastoma have many histologic features in common (including increase in nuclear atypia, abundant apoptotic bodies, high mitotic rate and vesicular nuclei with macronucleoli), the new WHO Classification[1] suggests that precise distinction between

TABLE 3. Multiple comparison of proliferation index (Ki-67) between each subtype (Tukey HSD).

(A) Histological subtypes	(B) Histological subtypes	Mean difference (A-B)	Sig.
Large cell/Anaplastic	Desmoplastic	8.5833(*)	.036
	Classic	14.2677(*)	.000
Desmoplastic	Large cell/Anaplastic	-8.5833(*)	.036
	Classic	5.6843	.125
Classic	Large cell/Anaplastic	-14.2677(*)	.000
	Desmoplastic	-5.6843	.125

*The mean difference is significant at the .05 level.

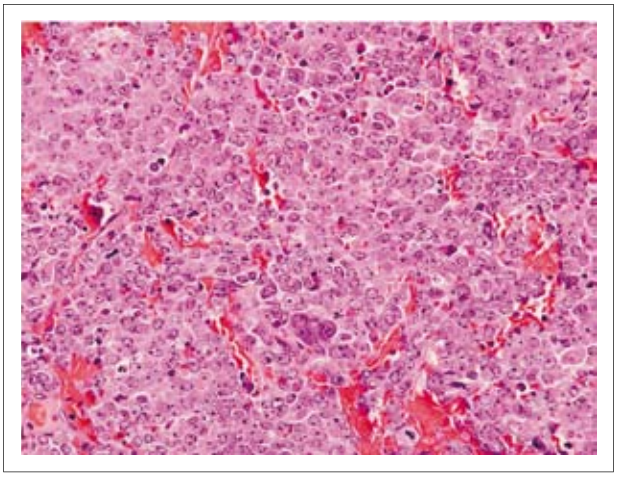

Fig 3. Photomicrograph of large cell/anaplastic medulloblastoma showing enlarged nuclei with nuclear atypia, vesicular nuclei with macronucleoli, and abundant apoptotic bodies (Paraffin section; H&E staining, 400x).

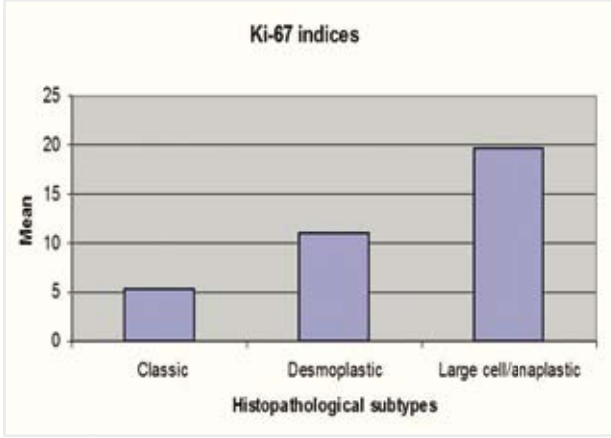

Fig 4. Proliferation indices labeled by Ki-67 immunostaining comparing between subtypes of medulloblastoma.

these two variants should be based on the presence of marked nuclear pleomorphism, polygonal cytologic feature with frequent nuclear molding and cell-cell wrapping; all of which are usually noted in anaplastic medulloblastoma.

Since different variants of medulloblastoma have different clinical outcomes, we, therefore, attempted to precisely subclassify the previously diagnosed cases as medulloblastoma according to the new WHO classification. Among the 41 cases of medulloblastoma in our study, the majority were classic medulloblastomas, followed by large cell/anaplastic and desmoplastic/nodular variants, respectively. However, none of our cases was medulloblastoma with extensive nodularity. Immunohistochemical studies showed significantly increased Ki-67 indices in large cell/anaplastic variants compared to classic medulloblastoma as well as desmoplastic/nodular variant ($p < 0.05$). Ki-67 immunostaining is applied to detect a nuclear antigen expressed in all non-G_0 phases of the cell cycle, i.e. all proliferating cells. In general, Ki-67 indices have been shown to be of prognostic relevance.[11] Thus, recognition of large cell/anaplastic medulloblastoma by using Ki-67 labeling indices was found to be a practical method in our study, and was compatible with the previous study by Son EI, et al.[8]

In our study, there were 2 and 3 cases of large cell/anaplastic and classic cases that were positive only with GFAP while all the neuronal markers were negative; these results might be considered as an atypical immunophenotype of the tumor since many medulloblastomas demonstrate neuronal antigens at least focally and infrequently express GFAP. In our opinion, the cases with an atypical immunophenotype but having a morphologic feature of medulloblastoma should not be misdiagnosed as high-grade gliomas. Comparing to the previous study by Son EI, et al[8], our results were, however, different in which we could not identify any statistical difference in expression of synaptophysin, GFAP, or neurofilament between large cell/anaplastic and other subtypes. Therefore, we cannot conclude that using a immunohistochemical panel of synaptophysin, GFAP, and neurofilament would aid in distinguishing between large cell/anaplastic and other subtypes of medulloblastoma.

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