Clinical Significance of Ki-67 Staining Index in Acoustic Neurinoma

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Abstract

The correlation between various clinical parameters and MIB-1 (Ki-67) staining indices was evaluated in 58 acoustic neurinomas. The index ranged from 0.37% to 6.61% (mean 1.70%), and did not correlate with age, sex, or initial tumor volume. Sixteen of the 18 tumors removed subtotally or partially showed regrowth, and two showed a volume reduction. The 16 patients with regrowth were divided into two groups according to Ki-67 staining index, $<2.00\%$ and $\geq 2.00\%$. These groups had a significant difference in tumor doubling time (TDT). In addition, there was a significant logarithmic correlation between Ki-67 staining index and TDT. Ki-67 staining index can be used as an index of regrowth rate in partially or subtotally removed acoustic neurinomas. Intraoperative assessment of Ki-67 staining index may be useful for better management planning as well as the estimation of prognosis.

Key words: acoustic neurinoma, growth kinetics, Ki-67 staining index, MIB-1

Introduction

Modern microsurgical techniques and intraoperative monitoring have greatly improved the operative results for acoustic neurinoma.^{7,22)} However, the mortality and morbidity are not zero, and the facial nerve is not always preserved.⁸⁾ Recently, the surgical procedure for acoustic neurinoma has shifted toward less invasive surgery preserving function, so, for example, tumor attached to functionally important structures such as the facial nerve should be intentionally left to avoid functional morbidity.²¹⁾ There is also increasing interest in stereotactic radiosurgery for acoustic neurinoma, which efficiently arrests tumor growth without major complications.^{5,18,26)} The growth rate of acoustic neurinomas is highly variable,^{1,4,13,14,25,27)} so a reliable way to estimate the growth rate of acoustic neurinomas would be of great clinical value for the planning of patient management.

Study of cell kinetics has shown that the proliferative potential of brain tumors can be analyzed immunohistochemically using antibodies against deoxyribonucleic acid polymerase α , proliferating cell nuclear antigen (PCNA), bromodeoxyuridine, or Ki-

67 antigen.^{2,11,12,15,19,23)} All these methods have inherent limitations, but MIB-1 (Ki-67) immunohistochemistry can be applied to routinely processed surgical specimens.^{3,6)}

The present study measured the tumor doubling time (TDT) and evaluated the correlation between various clinical parameters of acoustic neurinomas and Ki-67 staining index in formalin-fixed and paraffin-embedded sections.

Materials and Methods

Sixty-seven patients, 43 females and 24 males aged 17–78 years (mean 52.2 years), with acoustic neurinomas without stigmata of neurofibromatosis were operated on between April 1979 and March 1993. Clinical charts and all available radiological studies were reviewed.

Forty of the 67 patients underwent follow-up imaging studies at more than 1 year after the first operation, with the mean follow-up period of 4.5 years (range 1.0–11.9 years). The 40 patients were divided into four groups according to the extent of removal: total removal (T), tumor was totally removed; nearly total removal (NT), a small portion of the tumor remained at operation but no residual tumor was observed in the postoperative radiological studies; sub-

total removal (ST), more than 90% of tumor was removed; and partial removal (P), tumor was partially removed.²¹⁾

Preoperative tumor size was calculated by the method of Linsky *et al.*,¹⁶⁾ based on either computed tomography (CT) or magnetic resonance (MR) imaging. Five parameters were measured: width (A), length (B), and height (C) of the extrameatal solid tumor, and diameter (X) and depth (Y) of the internal acoustic meatus. The total tumor volume was calculated as intracanalicular tumor volume [$\pi(X)(X)(Y)/6$] plus extrameatal tumor volume [$4\pi(D/2)^3/3$], where $D = (A + B + C)/3$. Postoperative follow-up tumor volume was calculated by the following method, since the residual tumors usually showed irregular shape. Tumors on contiguous slices of known thickness were traced on graph papers and the areas were calculated, from which the total volume was estimated. Cystic portions were not included in the tumor volume. TDT was calculated by the formula,

$$\text{TDT} = t \log 2 / \log (V_1/V_0)$$

where V_0 is the immediate postoperative volume (cm^3), V_1 is the volume (cm^3) at the follow-up examination, and t is the follow-up period (years).

Ki-67 staining index was determined in 58 of the 67 patients. Nine patients were excluded; two patients died of postoperative complications, and seven others were inappropriate since the specimens were too small or poorly preserved. Specimens were deparaffinized and rehydrated. Intrinsic peroxidase was blocked with 3% H_2O_2 . Specimens were placed in boiled 10 mM citrate buffer (pH 6.0) and heated three times, each for 5 minutes, at maximum power (800 W) in a microwave oven.³⁾

Immunohistochemical staining for MIB-1

Fig. 1 Photomicrograph showing several MIB-1-positive nuclei. $\times 200$.

monoclonal antibody (Immunotech, Marseille, France) (1/50) was carried out using a LSAB kit (DAKO, Glostrup, Denmark). The Ki-67 staining indices were determined by photographing randomly selected light microscopic fields at a magnification of $\times 200$ or $\times 400$. The number of stained and unstained nuclei of tumor cells (a total of at least 2000 tumor cells for each specimen) were counted (Fig. 1). The staining index was defined as the percentage of Ki-67-positive nuclei.

Correlations between the TDT of acoustic neurinoma, age, sex, tumor size, and Ki-67 staining index were analyzed using the Mann-Whitney U test and Pearson's correlation coefficient.

Results

The Ki-67 staining index for the 58 patients ranged from 0.37% to 6.61% (mean 1.70%). The Ki-67 staining index did not correlate with age ($p < 0.46$) or preoperative tumor volume ($p < 0.72$), and did not differ significantly between females and males (Mann-Whitney U test, $p < 0.64$). The frequency of the Ki-67 staining index had a lognormal distribution (Fig. 2).

The tumor volume was measured in 38 of the 40 patients (Table 1). Two patients were excluded because of postoperative hemorrhage or poor image quality. Twenty patients were categorized in the T or NT group, 13 in the ST, and the remaining five in the P groups. Two of the 20 patients in the T and NT groups had regrowth (Table 1). Both had relatively high Ki-67 staining indices, 1.92% and 2.93%. The TDT could not be estimated in these two patients, as the immediate postoperative CT scans or MR images

Fig. 2 Frequency distribution of Ki-67 staining index in 58 acoustic neurinomas.

Table 1 Extent of removal and regrowth rate in 38 patients

Operative removal	No. of cases	No. of regrowth (%)
T	11	1 (9)
NT	9	1 (11)
ST	13	11 (85)
P	5	5 (100)

Table 2 Summary of 16 patients in ST and P groups with regrowth

Case No.	Age/ Sex	Volume (cm ³)		TDT (year)	t (year)	Ki-67 staining index (%)
		V ₀	V ₁			
1	47/F	0.15	0.24	2.22	1.55	2.24
2	59/M	2.10	14.30	0.98	2.70	3.82
3	30/F	15.30	39.20	2.72	3.69	1.53
4	51/M	5.20	15.80	2.10	3.36	1.00
5	74/F	1.20	9.90	3.31	10.07	1.68
6	62/F	9.73	22.50	1.22	1.48	2.42
7	18/F	0.20	4.58	0.70	3.18	4.78
8	50/F	0.73	1.00	17.70	8.02	1.21
9	36/M	0.78	2.67	2.33	4.14	1.14
10	65/F	3.44	19.40	1.66	4.14	2.50
11	46/F	0.51	0.86	1.98	1.49	1.16
12	48/F	1.54	2.71	1.13	1.00	3.40
13	34/F	1.01	1.60	8.34	5.54	1.04
14	63/F	0.40	0.61	4.50	2.77	1.58
15	51/M	0.23	0.59	2.72	3.70	2.52
16	63/F	0.26	0.37	6.14	3.01	0.86

t: follow-up period, TDT: $t \log 2 / \log (V_1/V_0)$, V₀: tumor volume measured on the films of immediate postoperative study, V₁: tumor volume at the follow-up study.

did not demonstrate the residual tumor.

Sixteen of the 18 patients in the ST and P groups showed regrowth and two patients had a reduction in tumor volume (Table 1). The 16 patients with regrowth were divided into two groups according to Ki-67 staining index: seven patients with a value of

Fig. 3 Ki-67 staining indices plotted against TDT in 16 patients in ST and P groups with regrowth, showing a significant correlation ($Y = -0.994X + 0.614$, $r = 0.787$, $p < 0.001$).

2.00% or more and nine patients with a value of less than 2.00% (Tables 2 and 3). The TDT was 1.52 years and 5.46 years, respectively. The difference in the TDT between these two groups was statistically significant (Mann-Whitney U test, $p < 0.01$). There was a significant logarithmic correlation between the Ki-67 staining index and TDT ($Y = -0.994X + 0.614$, $r = 0.787$, $p < 0.001$) in these 16 tumors (Fig. 3).

Discussion

Most previous studies have failed to find any definitive relationship between tumor growth and various clinical factors such as length of follow-up period, patient age, or radiological or histological features.^{14,24} However, histopathological factors such as hyaline degeneration, cell density, and PCNA labeling index may be a factor affecting the regrowth

Table 3 Subgroups of the ST and P patients with regrowth according to Ki-67 staining index

Ki-67 staining index (%)	No. of patients	Mean age (year)	Sex		Follow-up period (year)*	TDT (year)*
			F	M		
< 2.00	9	49.7	7	2	4.68 ± 2.75	5.46 ± 5.06
≥ 2.00	7	50.0	5	2	2.54 ± 1.21	1.52 ± 0.73

*Values are expressed as mean ± SD.

rate.¹⁰⁾

The mean Ki-67 staining index of acoustic neurinoma was 0.2–1.3% in several small series.^{2,11,12,19,23)} The present study showed a slightly higher value of 1.70%. Lesser *et al.*¹⁵⁾ found that acoustic tumors can be divided into two groups with large differences in growth rate; the Ki-67 staining index in one group was five times higher than in the other. Our study showed similar results (Table 3), and the frequency distribution of Ki-67 staining indices was lognormal (Fig. 2).

In this study, the patients in the ST and P groups with residual tumor naturally showed a high rate of regrowth, whereas only two of 20 patients in the T and NT groups had regrowth. Tani *et al.*²¹⁾ also reported that none of the T group developed regrowth and that only two of 26 patients in the NT group showed regrowth. A longer follow-up period is necessary before concluding whether tumors in T and NT groups rarely regrow or not, since neurinomas generally have a low proliferation potential. We also found decreased tumor size during the follow-up period in two of 18 cases in the ST and P groups. Such regression of the remaining tumor has been previously reported,^{1,9,10,14)} and is probably due to ischemia of the remaining tumor tissue secondary to operative manipulation of the feeding arteries.²¹⁾

The average growth rate of acoustic neurinomas may be low in the elderly,¹⁷⁾ but we could not find any significant correlation between age and TDT. Therefore, our results do not support Silverstein *et al.*²⁰⁾ who advocated a conservative approach for all acoustic neurinomas in the elderly.

Most previous studies on the growth rate of acoustic neurinomas described the increase in the maximum diameter,^{1,4,10,25)} and only a few studies employed the change in the tumor volume as an indicator of growth rate or TDT.^{13,14)} Our results showed a good logarithmic correlation between the Ki-67 staining index and TDT of acoustic neurinoma (Fig. 3). In addition, the difference in TDT between patient groups with <2.00% and $\geq 2.00\%$ Ki-67 staining index was statistically significant (Table 3). Although proliferation or cell loss rates of the tumor might be modified by operative procedures, our results show that the Ki-67 staining index is useful as an index of regrowth rate in partially or subtotally removed acoustic neurinomas. Intraoperative assessment of the Ki-67 staining index using frozen sections may allow better management planning as well as the estimation of prognosis.

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Commentary

The authors have presented interesting data on the regrowth rate and MIB-1 (Ki-67) staining indices for acoustic neurinomas. There was a correlation noted between the doubling time and the Ki-67 staining index, using 2% of cells staining as a dividing point. They suggest that intraoperative staining for Ki-67 index might be of benefit during the resection.

Surgical intervention has in fact become more aggressive in some respects in that we are now operating earlier in an attempt to preserve hearing while obtaining complete tumor removal.¹⁾ Microsurgical excision of acoustic schwannomas has progressed to where we can now resect almost all (99%) of these tumors completely without great risk to the facial nerve. If this can be done, I see no reason to leave residual tumor. The only situations where we intentionally leave tumor are: 1) in patients where the last scrap of tumor is too adherent to the facial nerve to remove without potential injury to the nerve, 2) in those patients who absolutely refuse to allow dissection along the facial nerve for fear of paresis, or 3) where an aggressive intracapsular removal is done without dissection of the facial nerve to expedite surgery in the elderly. Intraoperative staining for Ki-67 might not alter those decisions.

Nevertheless, this information is interesting in learning more about the natural history of these tumors. As there was no correlation between Ki-67 index and age, the notion that tumors in older patients grow more slowly may come into question. However, only one patient in this series was over the age of 65.

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Until recently, it was difficult to predict the regrowth rate and prognosis of acoustic neurinomas. Now, the antibody to Ki-67 antigen, MIB-1, is available routinely for application to formalin-fixed, paraffin-embedded specimen to determine the proliferation potential of various CNS tumors including acoustic neurinomas. Some studies using Ki-67 staining index (SI) demonstrated that acoustic neurinomas have several distinct growth rates.¹⁻³⁾ The authors state

that Ki-67 SI is significantly related to tumor doubling time (TDT) and a Ki-67 SI of $\geq 2\%$ is associated with shorter TDT. The cutoff value of 2% appears to be tentative, because this study was performed on a relatively small number of cases. A much larger and longer-term follow-up study may provide more valid information to evaluate the biological behavior of acoustic neurinomas.

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