^{201}Tl 뇌 SPECT을 이용한 신경교종의 평가

가톨릭대학교 의과대학 핵의학과, 신경외과,[1] 병리학교실,[2] 내과학교실[3]
손형선 · 김의녕 · 김성훈 · 유이령 · 정용안 · 정수교 · 홍용길[1] · 이연수[2] · 최보영[3]

Evaluation of Glioma with Thallium-201 Brain SPECT: The Correlation with ^{1}H MR Spectroscopy and Pathology

Hyung sun Sohn, M.D., **Euy-Neyng Kim,** M.D., **Sung-Hoon Kim,** M.D., **Ie-Ryung Yoo,** M.D., **Yong-An Chung,** M.D., **Soo-Kyo Chung,** M.D., **Yong-Gil Hong,** M.D.,[1] **Youn-Soo Lee,** M.D.[2] and **Bo-Young Choe,** Ph.D.[3]

Departments of Nuclear Medicine, Neurosurgery,[1] Pathology and Biomedical Engineering,[2] College of Medicine,[3] St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea

Abstract

Purpose: Thallim-201 (^{201}Tl) brain SPECT and proton (^{1}H) magnetic resonance spectroscopy (MRS) have been used to evaluate tumor grade and viability of glioma. We assessed the correlations between ^{201}Tl brain index or spectrum of metabolites of ^{1}H MRS and grade of glioma or histopathologic findings. **Materials and Methods:** We studied 17 patients (4 astrocytoma, 7 anaplastic astrocytoma and 6 glioblastoma). On ^{201}Tl Brain SPECT, ^{201}Tl index was measured as the ratio of average counts for region of interest to those for the contralateral normal brain. On ^{1}H MRS, we calculated choline (Cho) /creatine (Cr) ratio and N-acetylaspartate (NAA)/Cr ratio in ROI defined as tumor center. Histopathologic findings were graded by Ki-67 index, cellularity, mitosis, pleomorphism, necrosis and endothelial proliferation. An unpaired t test and statistical correlations were performed to evaluate these data. **Results:** Tl-index showed the best correlation with Ki-67 index ($p<0.01$), less correlations with cellularity, mitosis, and endothelial proliferation, but no correlation with results of MRS, pleomorphism, or necrosis. The findings of MRS did not correlate with all of the above. The cases of glioblastoma demonstrated a higher Tl-index, Cho/Cr ratio, Ki-67 index and lower NAA/Cr ratio, albeit without statistical significance. **Conclusion:** Even though ^{201}Tl brain SPECT did not correlate directly with grade of malignancy, it may still be useful in determining biological aggressiveness of tumor and prognosis of patients because it correlated well with Ki-67 index, a growth fraction of glioma, cellularity, mitosis and endothelial proliferation. **(Korean J Nucl Med 2000;34:465-77)**

Key Words: Brain, Glioma, ^{201}Tl, SPECT, ^{1}H MRS, Ki-67 index

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Corresponding Author: Euy-Neyng Kim, M.D., Department of Nuclear Medicine, St. Marys Hospital, 62 Youido-Dong, Youngdeungpo-Gu, Seoul 150-713, Korea
Tel: 82-2-3779-1289, Fax: 82-2-769-1722
E-mail: kpc@cmc.cuk.ac.kr
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Introduction

The pathology and biological activity of the brain tumor are very important to decide thera-

peutic plan and to predict the prognosis of the patient. To obtain the proper brain tumor pathology, image-guided stereotactic or craniotomic brain biopsy has been increasingly utilized.[1-3] Since not only the biopsy procedure is invasive and possibly harmful to patient, but also choosing the exact site for biopsy within the visualized abnormal lesion is not always easy, several imaging modalities to determine the grade of brain tumor have been used. There have been numerous reports on the usefulness of 201-Thallium (^{201}Tl) brain SPECT[4-6] and proton (^{1}H) magnetic resonance spectroscopy (MRS)[7-9] to differentiate or grade brain tumor. Since these modalities utilize metabolic changes or functional characteristics of tumors, they can be more reliable in grading brain tumor than conventional CT or MR, which represents merely anatomic changes or breakage of blood brain barrier by tumor. To our knowledge, there are few reports on the evaluation of glioma with ^{201}Tl brain SPECT or ^{1}H MRS along with pathology.[10] The aim of this study is to evaluate the correlations between the preoperative grade of gliomas by ^{201}Tl brain SPECT, or ^{1}H MRS and histopathology.

Materials and Methods

1. Patients

The study population consisted of 17 patients with intracranial astrocytic gliomas (M:F=8:9; Ages, 11-71 years; mean, 43 years). Histological diagnosis for each patients is summarized in Table 1. Tumor sizes ranged from 2 to 9 cm. All patients were examined with contrast-enhanced CT, MRI and histologic confirmation of the lesion by biopsy or surgery. Informed consent was obtained in all cases from patients or guardians.

2. Tumor classification

The histological findings are summarized in Table 1. Astrocytic tumors were divided into three groups, i.e, astrocytoma, anaplastic astrocytoma and glioblastoma. Histopathologic findings are examined under microscopy. Degree of cellularity, mitosis, pleomorphism, necrosis, and endothelial proliferation of tumors were evaluated by a neuropathologist.

3. SPECT

Brain SPECT images were acquired 20 minutes after intravenous injection of 111 MBq (3 mCi) of ^{201}Tl (Nihon Medi-Physics Co., Ltd., Nishinomiya, Japan) using a multi-detector scanner (ECAM plus; Siemens, Erlangen, Germany) equipped with a low-energy, fan-beam collimator. Data were acquired for 0.7 to 1×10^{6} counts per slice on 64 $\times$64 matrix and with a 20% symmetric window at 74 keV. Continuous transaxial tomograms of the brain were reconstructed after filtered backprojection with a Butterworth (cutoff frequency 0.25 cycles/pixel, order 8) to reduce statistical noise. ^{201}Tl images were corrected for tissue attenuation using a standard commercial correction routine, which assumed uniform attenuation with the circular shape of the head.

4. Quantitative analysis

Regions of interest (ROI) were drawn manually over the selected area around the site of the greatest activity of ^{201}Tl in the tumor. If the lesion showed no definite ^{201}Tl uptake, CT or MRI was used to determine the appropriate ROI. The counterpart of ROI for control region was drawn over the contralateral analogous normal brain region. The ^{201}Tl index was defined as the ratio of average counts per pixel in the ROI to those in the control region.

5. MR Spectroscopy

All ^{1}H MRS studies were performed on a 1.5-T

MR imager (Vision plus; Siemens, Erlangen, Germany) with a standard birdcage head coil. We obtained orthogonal base images to delimit the ROI using T1-weighted sagittal spin echo (SE) images, T1-weighted coronal SE images, and T2-weighted axial SE images. Automatic MAP shim was performed during the measurement of base images. With reference to previously performed diagnostic images including ^{201}Tl brain SPECT, position of the volume of interest (VOI) was settled on the orthogonal base images. The visualized lesion of abnormal signal intensity on the base images was included at the central portion of VOI as much as possible. We improved magnetic field homogeneity using selective local shim across the VOI. Then we readjusted frequency and improved suppression of water signal. MRS raw data were achieved using multivoxel PRESS (point-resolved spectroscopy) sequence (1500/135 [repetition time msec/echo time msec], 180° flip angle, 4-mm section thickness, voxel size=15×15×15 mm^3, 16×16 phase-encoding steps, field of view (FOV)=240 mm, number of acquisition=4). VOI and number of voxels in the VOI were adjusted to the size of lesion on the base images. At the end of ^{1}H MRS data acquisition, the raw data file and base images were exported to UNIX workstation and evaluated. On ^{1}H MRS obtained within 3 days after ^{201}Tl brain SPECT, we measured contents of choline (Cho), creatine (Cr) and N-acetylaspartate (NAA) in ROI defined as tumor center and calculated Cho/Cr and NAA/Cr ratio.

6. Ki-67 Immunohistochemical assay

Astrocytic tumor sections mounted on paraffin-coated slides were air-dried overnight. The Ki-67 antibody at a 1:20 dilution in 0.05 M Tris-HCL prepared in 0.01 M phosphate-buffered saline, pH 7.6 was added to the section. The specimens were stained according to the alkaline phosphatase anti-alkaline phosphatase method. The preparations were then examined under a light microscope. Five to ten randomly selected fields were photographed (magnification, ×200) and 500 to 1000 cells were evaluated. The labeling indices of Ki-67 were calculated by the following equation and were used for detecting tumor proliferation:

Labeling index (%)=(number of positive-stained cells)/(total number of cells)×100.

7. Statistical analysis

All quantitative data were presented as mean±1×standard deviation (SD). Comparisons of quantitative data were performed by unpaired t-test. A p value below 0.05 was considered statistically significant.

Results

Histological diagnoses in patients with brain tumors, results of ^{201}Tl SPECT (top), MRS, and Ki-67 index are summarized in Tables 1 and 2. Fig. 1 shows ^{201}Tl SPECT, MRI (middle) and ^{1}H MRS (bottom) images in patients with astrocytoma (left), anaplastic astrocytoma (middle) and glioblastoma (right). There were tendencies of increasing ^{201}Tl indices, Ki-67 indices, Cho/Cr ratio and decreasing NAA/Cr ratio with increasing malignant potential of glioma (Table 3, Fig. 2), albeit without statistical significance. ^{201}Tl index showed the best correlation with Ki-67 index ($r=0.90$, $p<0.01$, Fig. 3A) and less correlations with mitosis ($r=0.48$, $p<0.05$, Fig. 3B), cellularity ($r=0.7$, $p<0.05$, Fig. 3C) and endothelial proliferation ($r=0.53$, $p<0.05$, Fig. 3D). ^{201}Tl index demonstrated no correlations with the results of ^{1}H MRS or numbers of pleomorphic cells (Fig. 3E-G). The Cho/Cr ratio of MRS demonstrated no significant correlation with ^{201}Tl index, Ki-67 index, or histopathologic findings (Fig. 4A-E).

Table 1. Data of the Patients

Pt. No.	Age	Sex	Final diagnosis	Cellularity	Mitosis*	Pleomo rphism	Necrosis	Endo. prolif.
1	43	M	GBM	3	3	3	−	+
2	56	M	GBM	2	1	2	+	+
3	42	F	GBM	2	25	3	+++	+
4	61	M	GBM	NA	NA	NA	NA	NA
5	23	F	GBM	2	1	2	+	+
6	65	M	GBM	3	20	2	+	+
7	33	M	Ana.astrocytoma	1	0	3	−	−
8	21	F	Ana.astrocytoma	2	1	2	−	−
9	45	F	Ana.astrocytoma	1	1	1	−	−
10	37	F	Ana.astrocytoma	1	1	1	−	−
11	41	F	Ana.astrocytoma	2	4	2	−	−
12	37	F	Ana.astrocytoma	2	2	2	−	+
13	39	F	Ana.astrocytoma	3	14	1	−	+
14	71	M	Astrocytoma	1	1	1	−	−
15	11	M	Astrocytoma	1	2	1	−	−
16	56	F	Astrocytoma	NA	NA	NA	NA	NA
17	48	M	Astrocytoma	1	1	1	−	−

M, male; F, female; GBM, glioblastoma, Ana.; Astrocytoma, anaplastic astrocytoma, Endo.; Prolif., endothelial proliferation; NA, not available. * per 10 high power fields.

Table 2. Results of ^{201}Tl SPECT, Ki-67 Index and ^{1}H MRS

Pt. No.	Age	Sex	Final diagnosis	^{201}T1 index	Ki67 index	NAA/Cr	Cho/Cr
1	43	M	GBM	1.91	25	1.41	2.02
2	56	M	GBM	1.78	12	0.33	1.28
3	42	F	GBM	2.25	40	0.11	1.16
4	61	M	GBM	2.03	NA	0.90	1.42
5	23	F	GBM	2.35	5	1.13	3.22
6	65	M	GBM	4.99	95	1.14	2.06
7	33	M	Ana.astrocytoma	1.09	1	0.68	1.03
8	21	F	Ana.astrocytoma	2.30	10	0.22	2.32
9	45	F	Ana.astrocytoma	1.11	8	1.57	1.73
10	37	F	Ana.astrocytoma	1.40	0	1.09	1.61
11	41	F	Ana.astrocytoma	2.0	10	1.71	0.66
12	37	F	Ana.astrocytoma	2.0	3	1.26	1.01
13	39	F	Ana.astrocytoma	2.2	10	NA	NA
14	71	M	Astrocytoma	1.26	3	1.01	1.21
15	11	M	Astrocytoma	1.58	7	0.47	1.52
16	56	F	Astrocytoma	1.89	0	1.35	0.96
17	48	M	Astrocytoma	1.54	1	0.98	1.31-

^{201}T1 index, (average counts per pixel in the tumor)/(average counts per pixel in the contralateral region, Ki-67 index (%)=(number of positive-stained cells)/(total number of cells)×100.

Discussion

Regional accumulation of ^{201}Tl, which biologically behaves like potassium, is related to the changes in blood-brain barrier (BBB) permeability, regional blood flow and increased influx of this potassium analog directly into malignant tumor cells by the (Na^+-K^+)-ATPase pump.[11,12] Brismar et al[13] reported that the cellular uptake mechanism for ^{201}Tl was passive in glioma cell lines according to Nernsts equation and not Na^+/K^+ pump is functioning and the cells are viable. Also, ^{201}Tl uptake is related to cell growth rate.[11] Ishibashi[14] reported that as thallium uptake increased, increased tumor cell proliferation was observed by proliferating cell nuclear antigen.

Given the effectiveness of ^{201}Tl imaging in patients with brain tumors, it might be expected to play a substantial role in the predicting the prognosis of these patients. Clinical assessment of the therapeutic response is a major problem in the follow-up of patients with high-grade astrocytoma. Deterioration of clinical symptoms in a patient

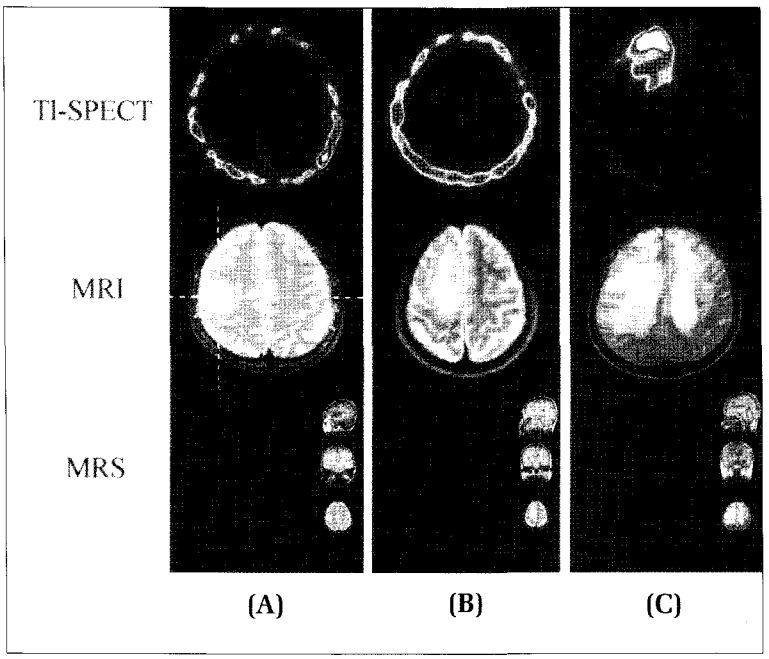


Fig. 1. Typical ^{201}Tl SPECT images. From left to right: images of astrocytoma (patient No. 13), anaplastic astrocytoma (patient No. 10) and glioblastoma (patient No. 3) are demonstrated. (top) ^{201}Tl SPECT, (middle) T2-weighted MR images, and (bottom) ^{1}H MRS.

Table 3. Results of Tl-index, Ki-67 index, and Results of ^{1}H MRS

	Tl-index	Ki-67 index	NAA/Cr	Cho/Cr
GBM	2.5±1.21	35.4±35.9	0.84±0.51	1.86±0.77
Ana. Astrocytoma	1.7±0.52	6±4.50	1.09±0.56	1.39±0.61
Astrocytoma	1.6±0.26	2.7±3.09	0.95±0.36	1.23±0.28

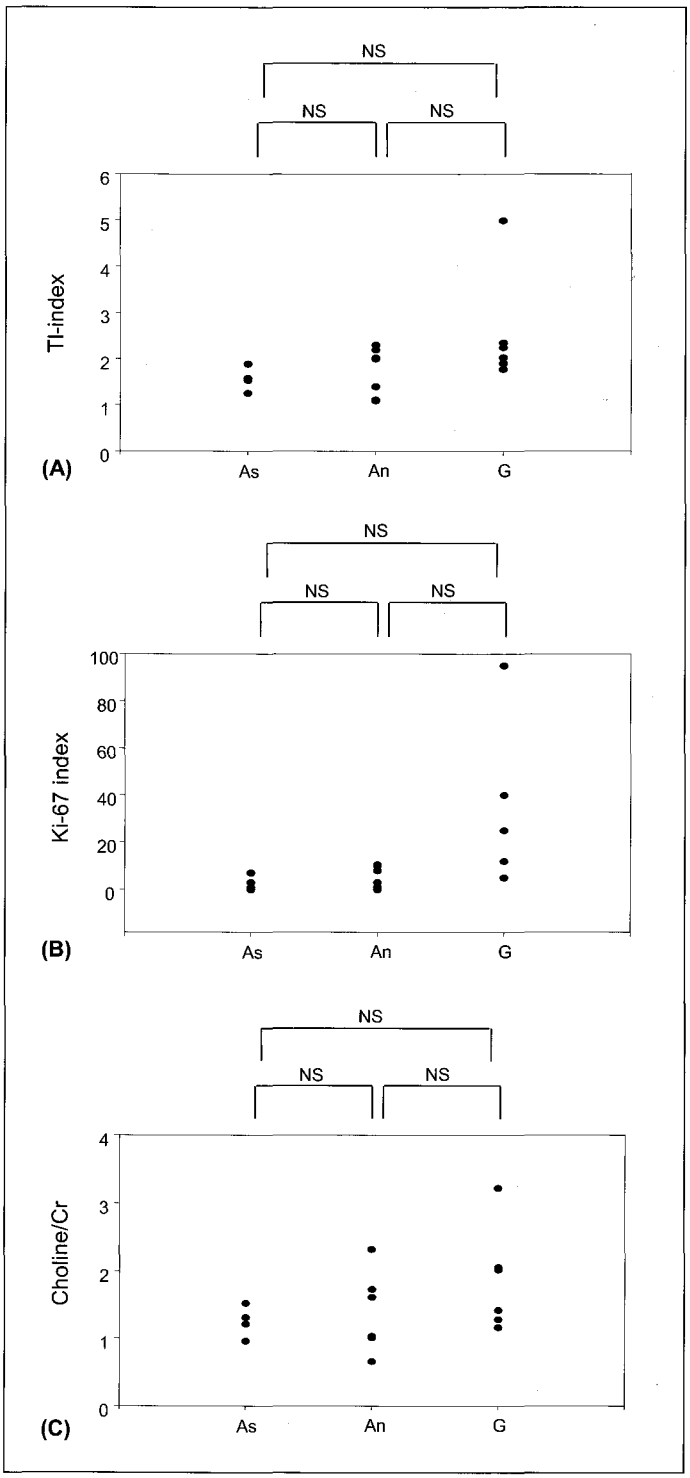
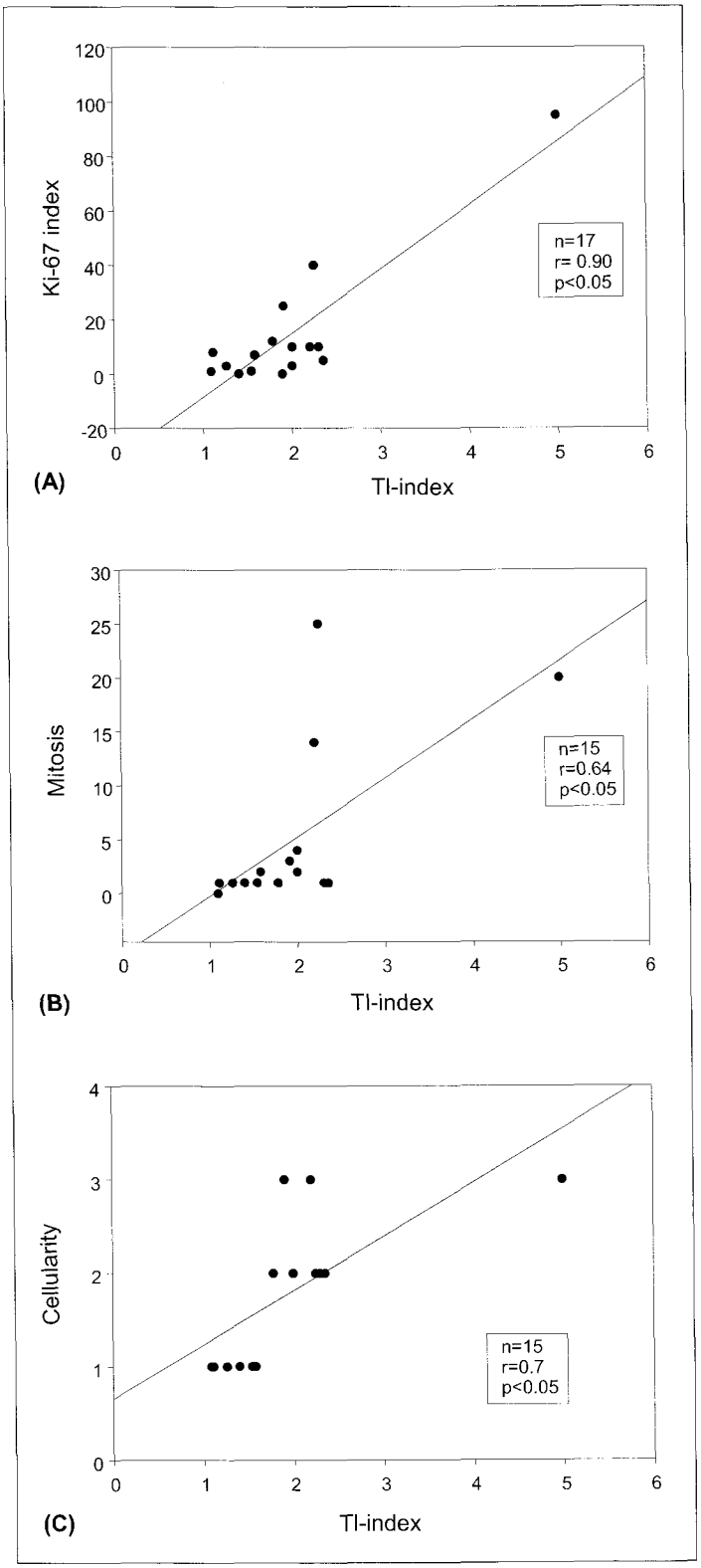
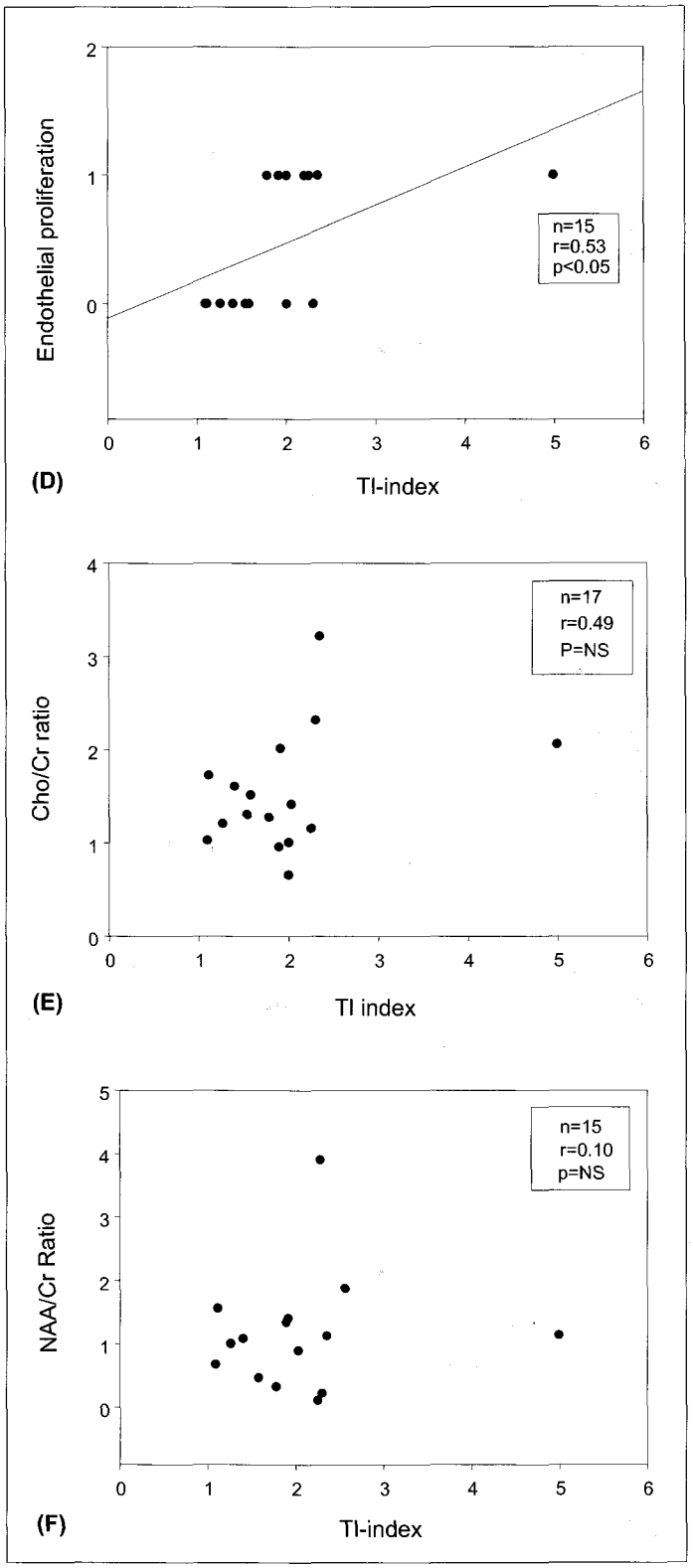


Fig. 2. Relationship between (a) Tl-index, (b) Ki-index, (c) MRS in astrocytoma (As), anaplastic astrocytoma (An) and glioblastoma (G).


Ki-67 index
TI-index
n=17
r= 0.90
p<0.05
(A)
Mitosis
TI-index
n=15
r=0.64
p<0.05
(B)
Cellularity
TI-index
n=15
r=0.7
p<0.05
(C)


Endothelial proliferation
n=15
r=0.53
p<0.05
TI-index
(D)
Cho/Cr ratio
n=17
r=0.49
P=NS
TI index
(E)
NAA/Cr Ratio
n=15
r=0.10
p=NS
TI-index
(F)

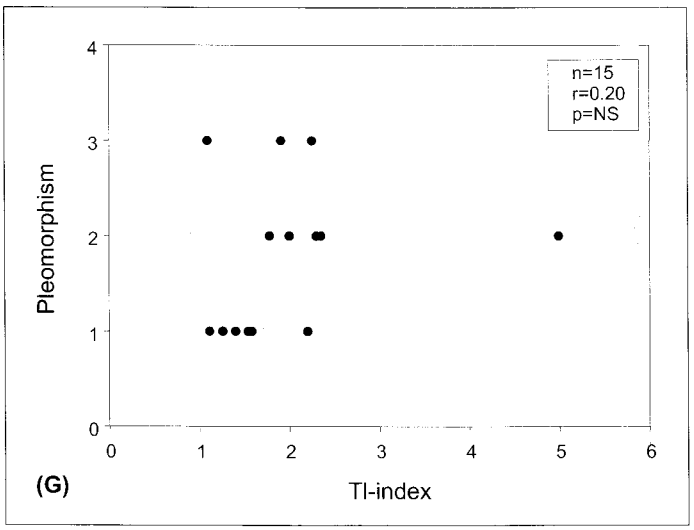


Fig. 3. Correlation between Tl-index and (A) Ki-67 index, (B) mitosis, (C) cellularity, (D) endothelial proliferation, (E) Cho/Cr ratio, (F) NAA/Cr ratio, and (G) number of pleomorphic cells. Tl-index has statistically significant correlations with Ki-67, mitosis, cellularity, and endothelial proliferation.

with a high-grade astrocytoma may be caused either by radiation therapy or tumor recurrence. CT and MRI, however, often fail to make this distinction. ^{201}Tl imaging may play a role in evaluating tumor grade and assessing therapeutic effectiveness.

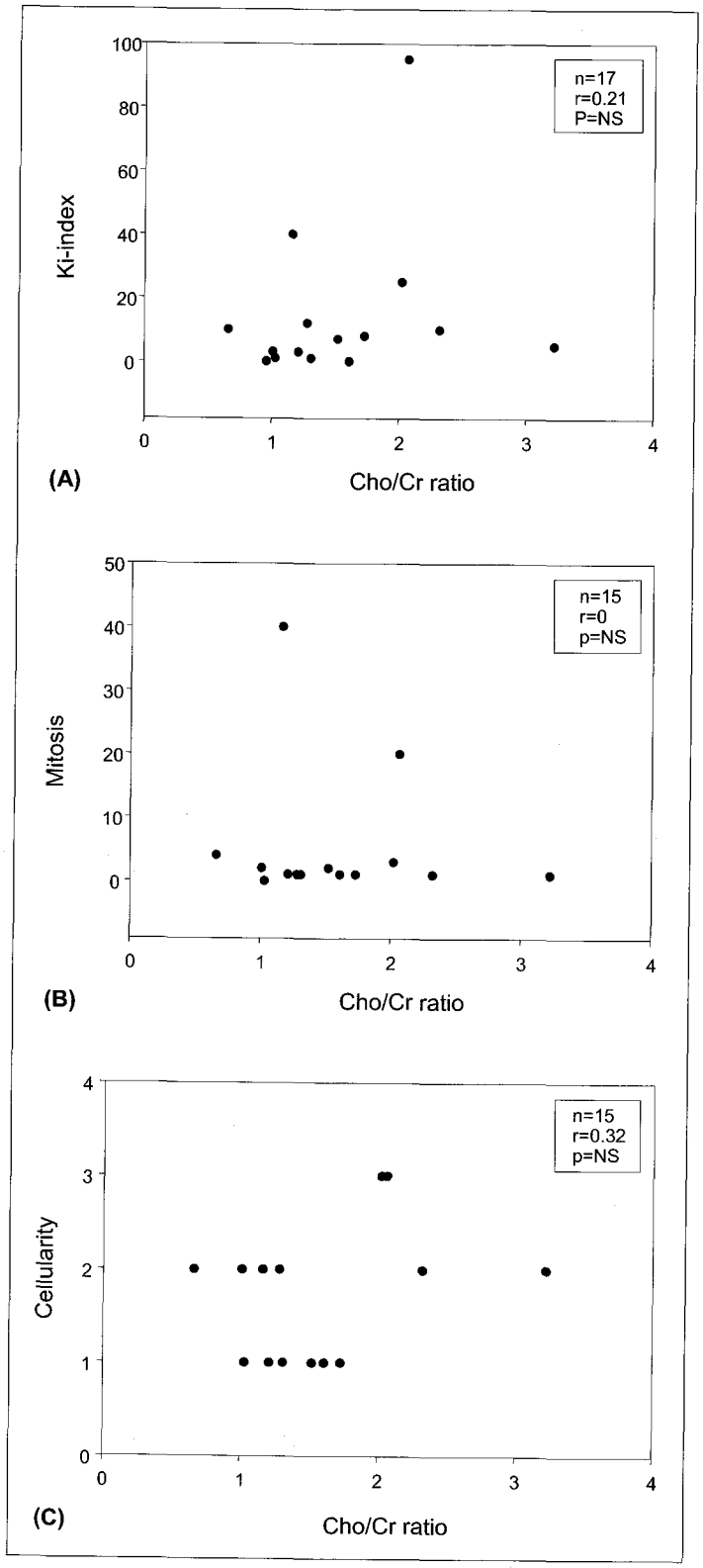
1. Thallium index in brain tumors

Our data showed that ^{201}Tl accumulated in both benign and malignant astrocytic tumors. Several researchers have focused on the relationship between ^{201}Tl uptake and degree of astrocytic tumors.[15,16] Kim et al.[15] described strong statistical differences between the ^{201}Tl index in low-grade and high-grade brain tumors. In 14 patients with biopsy- or autopsy- documented low-grade astrocystoma, the mean ^{201}Tl index was significantly lower than that of high-grade astrocytomas. Using a threshold index of 1.5 to distinguish low versus high-grade lesions, Kim et al[15] could predict malignancy grade with an accuracy of 89%.

In astrocytic tumors in our study, the lower the grade was, the lower the ^{201}Tl uptake was. But there was no significant difference between the ^{201}Tl index in astrocytoma, anaplastic astrocytoma, and glioblastoma. We speculated on the two possible reasons for the lack of significant difference. The first is that the number of patients in study group is too small for statistically meaningful result. The second possible reason would be that the heterogeneous nature of a brain tumor itself might have produced insignificant results. A large glioblastoma containing a large area of necrosis and hemorrhage within the mass might show a lower ^{201}Tl index than low grade gliomas which do not have necrosis or hemorrhage as in cases No. 2 and No. 3 in our study.

2. Ki-67 index in brain tumors

The present study compared Ki-67 and ^{201}Tl indices in patients with astrocytic tumors. Recent advances in immunobiology and molecular pathology have led to the development of techniques that can potentially obtain inaccessible information


n=17
r=0.21
P=NS
Ki-index
Cho/Cr ratio
(A)
n=15
r=0
p=NS
Mitosis
Cho/Cr ratio
(B)
n=15
r=0.32
p=NS
Cellularity
Cho/Cr ratio
(C)

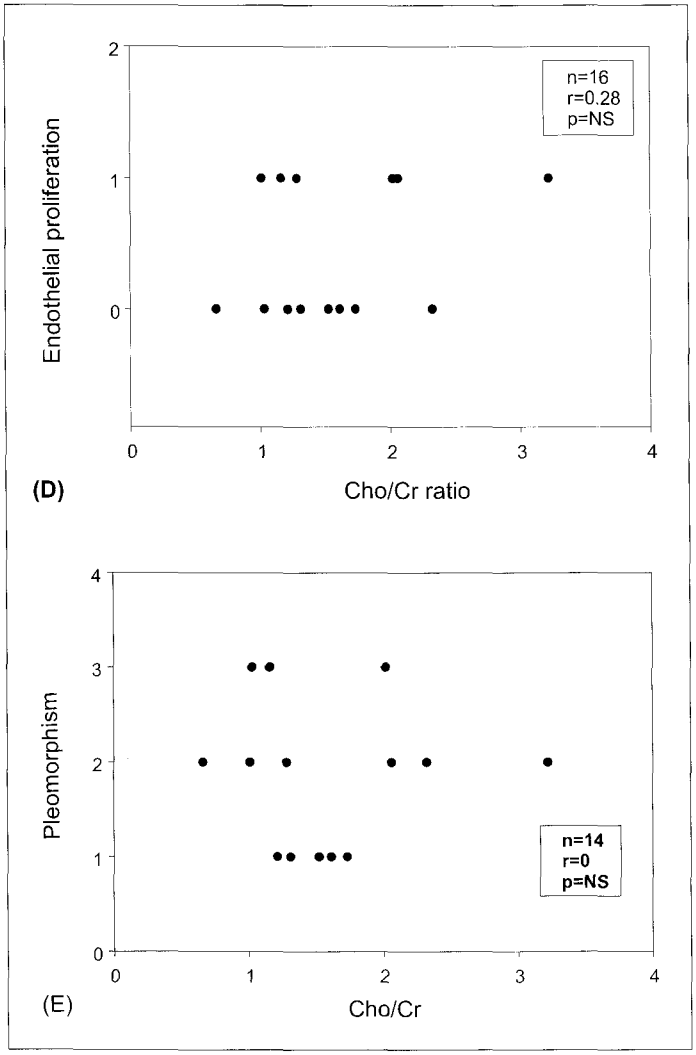


Fig. 4. Correlation between Cho/Cr ratio and (a) Ki-67 index, (b) mitosis, (c) cellularity, (d) endothelial proliferation, and (e) number of pleomorphic cells. There is no statistically significant correlation among them.

from tumor tissues otherwise inaccessible. Immunocytochemical investigation, using flow cytometry, also revealed that the antigen recognized by the Ki-67 antibody was closely associated with DNA.[17] Okada et al.[18] described a close correlation between Ki-67 positivity and histological grading in malignant lymphoma using FDG-PET. The drawback for FDG-PET is high cost and huge preparation. ^{201}Tl, on the other hand, is readily available in any hospital. Tihan et al.[19] Discussed that higher Ki-67 index indicated a worse outcome than suggested by the histologic grading and that a realistic prognostic upgrading of diffuse astrocytoma should be made only when labeling index for this marker was high.

In our study, there was no significant difference of Ki-67 indices between astrocytoma, anaplastic astrocytoma and glioblastoma. The possible two

reasons were discussed previously. But our data emphasize that the increase in ^{201}Tl index correlated well to the increase of the Ki-67 index, a growth fraction of glioma.

3. ^{1}H MRS

There are numerous reports about relationship between findings of ^{1}H MRS and grade of glioma. Ott et al.[7] reported that the differential diagnosis of the brain tumor spectra was limited because of intra-individual differences between spectra. However, the variations in metabolite concentrations, and especially the presence or absence of aliphatic signals, were proved to be indicators of the histologic grade of tumor. In general, on MRS, the Cho/Cr ratio increases when there is high cellular replication in active tumoral growth, while the NAA/Cr ratio decreases when there is loss of active alive neuron within the ROI. For example, in glioblastoma, increased malignant tumor cell replication with loss of active neuron makes increase of Cho/Cr ratio and decrease of NAA/Cr ratio. So it is possible to estimate the grade of glioma by the spectrum of the ^{1}H MRS. However MRS has fundamental limitations in that it is very sensitive or vulnerable to local magnetic filed inhomogeneity. A small disturbance of local magnetic field homogeneity due to ferromagnetic substance within the tumoral hemorrhagic components and vascular flow can deteriorate the results. In our study, there was no statistical correlation between the tumor grade nor between the ^{201}Tl index and results of MRS. This may be due to limitations of ^{1}H MRS.

Overall, the present data show a positive correlation between ^{201}Tl index and Ki-67 index and cellularity, but does not demonstrate statistically significant correlation between the ^{201}Tl index, results of MRS and microscopic grade of gliomas. The probable reasons for this insignificance are the small number of patients and heterogeneous internal nature of the tumors.

As shown by our results, ^{201}Tl index is more valuable than MRS in predicting invasiveness of the tumor and prognosis in patient with glioma because it reflects a growth fraction of glioma (Ki-67 index) and cellularity. In conclusion, we suggest that ^{201}Tl brain SPECT should remain an important noninvasive technique for quantitative description of brain tumors.

요 약

목적: Thallim-201 (^{201}Tl) 뇌 SPECT와 proton (^{1}H) magnetic resonance spectroscopy (MRS)는 뇌 신경교종의 악성도와 치료 후 종양의 생존여부를 평가하기 위하여 사용되어 왔다. 우리는 뇌 신경교종에서 ^{201}Tl brain 지수와 ^{1}H MRS 소견을 비교하여 보고 병리조직소견과 잘 일치하는지를 알아보고자 이 연구를 시행 하였다. **대상 및 방법**: 성상교세포종 4예, 미분화 성상교세포종 7예, 다형성 교모세포종 6예 등 모두 17예를 대상으로 하였다. ^{201}Tl 뇌 SPECT에서 ^{201}Tl 지수는 병변에 관심영역을 설정하고 계수한 평균값을 반대측 뇌에 관심영역을 설정하고 계수한 평균값으로 나누어 구하였다. ^{1}H MRS에서는 뇌종양 중앙에서 choline (Cho)/creatine (Cr) 비와 N-acetylaspartate (NAA)/Cr 비를 구하였다. 병리조직학으로는 Ki-67 지수, 세포충실도, 유사분열정도, 다형태성정도, 괴사 및 내피생성정도 등을 검사하였다. 통계방법으로는 unpaired t test와 상관관계분석을 사용하였다. **결과**: ^{201}Tl-지수는 Ki-67 지수와 좋은 상관관계가 있는 것으로 판명되었고 ($p<0.01$), 세포 충실도, 유사분열정도 및 내피생성정도 등과도 어느 정도 상관성이 있었다. 하지만 ^{1}H MRS 결과나 다형태성 및 괴사정도와는 무관하였다. ^{1}H MRS와 일치하는 병리소견이 없었다. 다형성교모세포종인 경우에는 성상교세포종보다 높은 ^{201}Tl-지수, Cho/Cr 비, Ki-67 지수 등을 나타내었고 낮은 NAA/Cr 비를 나타내었지만 통계적인 의미는 없었다. **결론**: 비록 ^{201}Tl 뇌 SPECT와 ^{1}H

MRS가 악성 뇌교종과 양성 뇌교종을 직접적으로 감별해 낼 수는 없었다. 하지만 ^{201}Tl 지수는 여러 가지 병리소견을 대변하므로 종양의 생물학적 생활성이나 환자의 예후 평가 등에 도움이 될 수 있을 것이다.

References

1) Earnest F, Kelly PJ, Scheithauer BW, Kall BA, Cascino TL, Ehman RL, et al. Cerebral astrocytomas: histopathologic correlation of MR and CT contrast enhancement with stereotactic biopsy. *Radiology* 1988;166:823-7.
2) Zhang J, Levesque MF, Wilson CL, Harper RM, Engel J Jr, Lufkin R, Behnke EJ. Multimodality imaging of brain structures for stereotactic surgery. *Radiology* 1990;175:435-41.
3) Black PM, Alexander E, Martin C, Moriarty T, Nabavi A, Wong TZ, et. al. Craniotomy for tumor treatment in an intraoperative magnetic resonance imaging unit. *Neurosurgery* 1999; 45:423-31.
4) Taki S, Kakuda K, Kakuma K, Kobayashi K, Ohashi M, Ito S, et, al. 201-Tl SPECT in the differential diagnosis of brain tumours. *Nucl Med Commun* 1999;20:637-45.
5) Roesdi MF, Postma TJ, Hoekstra OS, van Groeningen CJ, Wolbers JG, Heimans JJ. Thallium-201 SPECT as response parameter for PCV chemotherapy in recurrent glioma. *J Neurooncol* 1998;40:251-5.
6) Sugo N, Ohishi H, Kuroki T, Ohtsuka T, Kano T, Mito T, et al. ^{201}TlCl accumulation dynamics in glioma with different grades of malignancy: an investigation using dynamic SPECT. *No Shinkei Geka* 1998;26:881-7.
7) Ott D, Hennig J, Ernst T. Human brain tumors: assessment with in vivo proton MR spectroscopy. *Radiology* 1993;186:745-52
8) Warren KE, Frank JA, Black JL, Hill RS, Duyn JH, Aikin AA, et. al. Proton magnetic resonance spectroscopic imaging in children with recurrent primary brain tumors. *J Clin Oncol* 2000;18: 1020-6.
9) Esteve F, Grand S, Rubin C, Hoffmann D, Hoffmann D, Pasquier B, Graveron-Demilly D, et al. MR spectroscopy of bilateral thalamic gliomas. *AJNR* 1999;20:876-81.
10) Kumabe T, Shimizu H, Sonoda Y, Shirane R. Thallium-201 single-photon emission computed tomographic and proton magnetic resonance spectroscopic characteristics of intracranial ganglioglioma: three technical case reports. *Neurosurgery* 1999;45:183-7;
11) Kimelberg HK, Bowman C, Biddlecome S, Bourke RS. Cation transport and membrane potential properties of primary astroglial cultures from neonatal rat brains. *Brain Res* 1979;177: 533-50.
12) Elligsen JD, Thompson JE, Frey HE, Kruuv J. Correlation of (Na^+-K^+)-ATPase activity with growth of normal and transformed cells. *Exp Cell Res* 1974;87:233-40.
13) Brismar T, Collins VP, Kesselberg M. Thallium-201 uptake relates to membrane potential and potassium permeability in human glioma cells. *Brain Res* 1989;500:30-6.
14) Ishibashi M, Taguchi A, Sugita Y, Morita S, Kawamura S, Umezaki N, et. al. Thallium-201 in brain tumors: relationship between tumor cell activity in astrocytic tumor and proliferating cell nuclear antigen. *J Nucl Med* 1995;36:2201-6.
15) Kim KT, Black KL, Marciano D, Mazziotta JC, Guze BH, Grafton S, et al. Thallium-201 SPECT imaging of brain tumors: methods and results. *J Nucl Med* 1990;31:965-9.
16) Oriuchi N, Tamura M, Shibazaki T, Ohye C, Watanabe N, Tateno M, et al. Clinical evaluation of thallium-201 SPECT in supratentorial gliomas: relationship to histologic grade, prognosis and proliferative activities. *J Nucl Med* 1993;34: 2085-9.
17) Sasaki K, Matsumura K, Murakami T, Shinozaki F, Takahashi M. Intranuclear localization of the Ki-67 reactive antigen in HeLa cells. Flow cytometric analysis. *Biol Cell* 1990;68:129-32.
18) Okada J, Yoshikawa K, Itami M, Imaseki K, Uno K, Itami J, et al. Positron emission tomography using fluorine-18-fluorodeoxyglucose in malignant lymphoma: a comparison with proliferative activity. *J Nucl Med* 1992;33:325-9.
19) Tihan T, Davis R, Elowitz E, DiCostanzo D, Moll U Practical value of Ki-67 and p53 labeling indexes in stereotactic biopsies of diffuse and pilocytic astrocytomas. *Arch Pathol Lab Med* 2000;124:108-13.