Blood Levels of Polyamines in Patients with Brain Tumor: Special Reference to Relationship between Blood Levels of Polyamines and Histological Finding, Extension as well as Treatment of Tumor

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Summary

In 38 patients with brain tumor and 17 controls, the blood levels of polyamines were measured, and studies were made on the relationship between these levels and varieties of tumors. In the group of 21 patients with glioma, these levels were significantly higher than those in the control (p<0.01), and in 14 out of 21 patients (67%) the levels exceeded the upper limit of normal range. In one patient with reticulum cell sarcoma, these levels were remarkably high. In the group of 4 patients with metastatic brain tumor, these levels were significantly higher than those in the control (p<0.05), and in 2 of 4 patients the levels exceeded the upper limit of normal range. The levels were all within normal range in 4 patients with neurinoma, 3 patients with meningioma, 2 patients with pituitary adenoma, 1 patient with pinealoma, and 1 patient with cerebellar hemangioblastoma.

In 18 patients with glioma, studies were also made on the relationship between the blood levels of polyamines and the degree of histological malignancy and of extension of tumor. There was no significant difference in these levels between the control and the group of astrocytoma localized within a lobe, but the levels were significantly higher in the group of astrocytoma extending more widely and in the groups of anaplastic glioma or glioblastoma multiforme regardless of their size.

In patients with glioma, the blood levels of polyamines before and after treatment were compared. In 3 patients subjected to surgical excision, irradiation and chemotherapy these levels were all within normal range after treatment, but in a case treated by irradiation and chemotherapy the values increased immediately after treatment.

Key words:
polyamine, spermidine, spermine, cell proliferation, brain tumor, diagnosis

Introduction

At present most of the diagnostic methods for brain tumor are based on the characters of tumor as a space taking lesion, changes in the dynamics of circulation or the cerebral dysfunction. No practical method has been devised as yet to clarify the state of proliferation of the existing tumor. If it were possible to measure compounds which reflect the state of the proliferation of the tumor in the physiological fluid of the patient with brain tumor, estimation of these substances could be used to judge the character of tumor and the effectiveness of treatment.

Recently, attention has been paid to the relationship between cellular proliferation and polyamines such as spermidine and spermine. Polyamines are biosynthetized from methionine and ornithine as precursors, and postulated to act in such manner as to accelerate nucleic acid and protein synthesis and activate cellular proliferation. These compounds occur in considerable quantities in most animal and plant tissues examined, and increased concentrations
of polyamines have been reported in rapidly
growing tissue such as chicken embryo,\(^1\,^7,\,^10\)
and regenerating liver.\(^3,\,^8,\,10,\,11\) Dramatically

elevated activities of enzymes, which participate
in polyamine biosynthesis, are also found in such
rapidly growing neoplastic tissue as hepatoma
and sarcoma.\(^10\)

Many of the investigators who had compared
the levels of polyamines in the body fluid be-
tween patients with cancer and controls re-
ported that these levels had been higher in the
patients, suggesting that the measurements of
polyamines might be applicable to clinical exam-
ination.\(^2,\,4,\,5,\,6,\,10,\,14\)

The author measured the blood levels of poly-
amines in patients suffering from brain tumor
and studied the relationship between these levels
and the varieties of tumor, and also in patients
with glioma between these levels and the degree
of histological malignancy and of the extension
of tumor and the effect of the treatment upon the
blood levels. This paper deals with the results of
these studies.

**Materials and Methods**

Blood samples were collected from 38 patients
with brain tumor hospitalized in the Depart-
ment of Neurosurgery, the Brain Research In-
stitute, Niigata University. These included 21
patients with glioma (including 1 patient with
thalamic tumor whose histological diagnosis
was not verified), 1 patient with pinealoma, 1
patient with cerebellar hemangioblastoma, 1
patient with reticulum cell sarcoma, 4 patients
with neurinoma, 3 patients with meningioma, 2
patients with pituitary adenoma, and 4 patients
with metastatic brain tumor. As the control
group the blood levels of polyamines were meas-
ured in 17 healthy adults of 23 to 64 years (11
males and 6 females).

Changes in the polyamine levels in the blood
caused by surgical insult were investigated after
operation in 2 patients subjected to large cran-
iotomy; one of these patients suffered menin-
gioma (with 6800 ml of blood transfusion during
operation) and the other suffered cerebral
arteriovenous mulformation (with 1000ml of
blood transfusion).

An attempt was made to detect polyamines
from the cerebrospinal fluid in 9 patients with
brain tumor. These patients consisted of 6 pa-
tients with glioma (4 patients with anaplastic
glioma and 2 patients with glioblastoma multiforme), 1 patient with von Recklinghausen’s dis-
ease (combination of meningioma and neur-
inoma), 1 patient with teratoblastoma, and 1
patient with craniopharyngioma.

To exclude as much influence of infection as
possible, some patients were discarded from the
experiment when they were suspected of being
involved in infection from clinical symptoms, the
result of hematological examination, urinalysis
and chest X-ray films.

The isolation and estimation of polyamines
were carried out by the method of Otsuji, et al.\(^5\),
using ion exchange column chromatography
and cellulose-acetate membrane electrophoresis.
This method is described briefly as follows: 5 ml
of fresh heparinized blood or 15 to 20 ml of fresh
cerebrospinal fluid was deproteinized with TCA,
which was eliminated with ether. The samples
were then injected onto columns packed with
ion exchange resin: 1.0 × 6.0 cm, AG50W-X2
(200–400 mesh, H form); and polyamines were
washed out with 20 ml of 6N–HCl. The HCl
was evaporated by rotary evaporater, and the
residue was redissolved in 200 µl of 0.1N–HCl.
50 µl of this solution was subjected to cellulose-
acetate membrane electrophoresis at 13 volt/cm
for 70 min., and amines were quantified by stain-
ing with amidoblock 10B.

A histological diagnosis of brain tumor was
made with specimens obtained by operation or
autopsy.

In 18 patients with glioma, the patients were
classified into three groups according to the
degree of histological malignancy; a group of
astrocytoma, of anaplastic glioma and of glio-
blastoma multiforme. Also, these patients were
divided into two groups according to the degree
of tumor extension; a group of one lobe lesion in
which the tumor localized almost within one
lobe, and a group of multiple lobe lesion in
which the tumor extended beyond a single lobe.

The extension of tumor was judged from the
picture of angiography and the operative finding
in most patients or from autopsy findings in
patients who died within 1 to 2 months after the
measurement of polyamines.

**Results**

Figure 1 shows the result of the polyamine
determination in the blood from 17 controls and from 37 patients with brain tumor, and Table I tabulates these levels expressed with the mean (M ± standard deviation (SD)). The upper limit of the normal range (M + 2 SD of the controls) were 2.1 μg/ml for spermidine, 1.6 μg/ml for spermine and 3.3 μg/ml for spermidine plus spermine.

In the group of 21 patients with glioma, the number of patients, whose blood levels exceeded the upper limit of the normal range, were 9 patients (43%) each for spermidine and for spermine, 13 patients (62%) for spermidine plus spermine (Fig. 1). High values for polyamines were seen in 14 patients (67%), in whom at least one of the two compounds or the combination of these exceeded the upper limit of the normal range. The polyamine levels in this group expressed with M ± SD were 2.2 ± 1.0 μg/ml for spermidine, 1.5 ± 0.6 μg/ml for spermine and 3.6 ± 1.4 for spermidine plus spermine, and all of the values for these three were significantly higher in the group of glioma than in the control (p < 0.01) (Table 1).

No values for the blood polyamines were higher than the upper limit of the normal range (Fig. 1) in 4 patients with neurinoma, 3 patients with meningioma, and 2 patients with pituitary adenoma. Significant differences in these values were not found between the control group and any of these three groups (Table 1).

In the group of 4 patients with metastatic brain tumor, the blood values for polyamines were higher than the upper limit of the normal range (Fig. 1) in 2 patients (50%) and the levels of spermine and of spermidine plus spermine were significantly higher in this group than in the control (p < 0.05) (Table 1).

The blood values for polyamines were remarkably high in a patient with reticulum cell sarcoma, but were all within the normal range in a patient with pinealoma, and one with cerebellar hemangioblastoma (Fig. 1) (Table 1).

An attempt was made to detect polyamines from the cerebrospinal fluid in 9 patients with brain tumor, but this was in vain.

Figure 2 shows the values for blood polyamines in 18 patients with glioma classified on the basis of the degree of histological malignancy, and Table 2 tabulates these values expressed with M ± SD. The number of patients

<table>
<thead>
<tr>
<th>Blood polyanin, M ± SD (μg/ml)</th>
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<tbody>
<tr>
<td>No. of cases</td>
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<tr>
<td>-------------</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Glioma</td>
</tr>
<tr>
<td>Reticulum cell sarcoma</td>
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<tr>
<td>Pinealoma</td>
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<tr>
<td>Hemangioblastoma</td>
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<tr>
<td>Meningioma</td>
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<tr>
<td>Neurinoma</td>
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<tr>
<td>Pituitary adenoma</td>
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<td>Metastatic tumor</td>
</tr>
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</table>

*: p < 0.01  
*: p < 0.05
whose blood values exceeded the upper limit of the normal range were as follows: out of 5 patients with astrocytoma, one for spermidine plus spermine; out of 9 patients with anaplastic glioma, 5 each for spermidine and for spermine, and 8 for spermidine plus spermine; out of 4 patients with glioblastoma multiforme, 3 each for spermidine, for spermine and for spermidine plus spermine (Fig. 2). The polyamine levels expressed with M ± SD in these three groups were as follows: in the astrocytoma group, 1.7 ± 0.4 µg/ml for spermidine, 1.2 ± 0.3 µg/ml for spermine and 2.8 ± 0.5 µg/ml for spermidine plus spermine; in the anaplastic glioma group, 2.2 ± 0.8 µg/ml for spermidine, 1.7 ± 0.6 µg/ml for spermine and 3.9 ± 1.2 µg/ml for spermidine plus spermine; in the glioblastoma multiforme group, 2.9 ± 1.6 µg/ml for spermidine, 1.7 ± 0.6 µg/ml for spermine and 4.6 ± 1.6 µg/ml for spermidine plus spermine. The values for blood polyamines were significantly higher in all the patient groups than in the control group, except for the astrocytoma group in which no significant difference in the value for spermine was found between the control (Table 2). A tendency was seen that the higher the degree of histological malignancy, the higher became the blood levels, but there was no significant difference in these levels between any two groups classified on the basis of the degree of histological malignancy.

In these 18 patients with glioma, observation was made on the relationship between the degree of histological malignancy or the size of tumor and the blood levels of polyamines (Fig. 3) (Table 3). The number of the patients whose values exceeded the upper limit of the normal range were as follows: none out of 3 patients with astrocytoma localizing within a lobe; out of 2 patients with astrocytoma extending beyond a lobe, one for spermidine plus spermine; out of 6 patients with anaplastic glioma localizing within a lobe, 3 for spermidine, 2 for spermine and 5 for spermidine plus spermine; out of 3 patients with anaplastic glioma extending beyond a lobe, 2 for spermidine, 3 each for spermine and for spermidine plus spermine; out of 4 patients with glioblastoma multiforme all of which extended beyond a lobe, 3 each for spermidine, for spermine and for spermidine plus spermine (Fig. 3). The blood values expressed with M ± SD in these groups were as follows: in the group of astrocytoma localizing within a lobe, 1.6 ± 0.5

| Table 2 Blood levels of polyamines measured in patients with glioma classified by the degree of histological malignancy. Mean value ± Standard deviation |
|---|---|---|
| Blood polyamine, M ± SD (µg/ml) | No. of cases | Spermidine | Spermine | Spermidine + Spermine |
| Normal | 17 | 1.1 ± 0.5 | 0.8 ± 0.4 | 1.9 ± 0.7 |
| Astrocytoma | 5 | 1.7 ± 0.4 | 1.2 ± 0.3 | 2.8 ± 0.5 |
| Anaplastic glioma | 9 | 2.2 ± 0.8 | 1.7 ± 0.6 | 3.9 ± 1.2 |
| Glioblastoma multi. | 4 | 2.9 ± 1.6 | 1.7 ± 0.6 | 4.6 ± 1.6 |

*p < 0.01  b*p < 0.05
µg/ml for spermidine, 1.0 ± 0.3 µg/ml for spermine and 2.5 ± 0.2 µg/ml for spermidine plus spermine: in the group of astrocytoma extending beyond a lobe, 1.8 ± 0.3 µg/ml for spermidine, 1.5 ± 0.1 µg/ml for spermine and 3.3 ± 0.4 µg/ml for spermidine plus spermine: in the group of anaplastic glioma localizing within a lobe, 2.1 ± 0.8 µg/ml for spermidine, 1.5 ± 0.6 µg/ml for spermine and 3.6 ± 1.3 µg/ml for spermidine plus spermine: in the group of anaplastic glioma extending beyond a lobe, 2.4 ± 0.9 µg/ml for spermidine, 2.2 ± 0.4 µg/ml for spermine and 4.6 ± 0.7 µg/ml for spermidine plus spermine: in the group of glioblastoma multiforme, 2.9 ± 1.6 µg/ml for spermidine, 1.7 ± 0.6 µg/ml for spermine and 4.6 ± 1.6 µg/ml for spermidine plus spermine (Table 3). No significant difference in the values for spermidine, spermine and spermidine plus spermine were seen between the control group and the group of astrocytoma localizing within a lobe, or in the value for spermine between the control group and the group of astrocytoma extending beyond a lobe. However, the blood values were significantly higher in the group of anaplastic glioma and of glioblastoma multiforme, regardless of the tumor size, than in the control group. The larger the size of and the higher the degree of histological malignancy of tumor, the higher became the blood values for polyamines. But no significant differences in these values were found between any two of the groups mentioned above.

Figure 4 shows the sequence of the blood values for polyamines following surgical insult. Surgical insult resulted in the transient decrease of blood polyamines for 2 to 3 days, and increase over the preoperative level at 7 to 8 days after operation. Then the blood values declined slowly, and returned to the normal level by 20 to 30 days.

Four patients with glioma were studied before and after treatment in an effort to ascertain the

<table>
<thead>
<tr>
<th>Blood polyamine, M ± SD (µg/ml)</th>
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<tr>
<td>No. of cases</td>
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<tr>
<td>---------------</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Astrocytoma</td>
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<td>(&lt; one lobe)</td>
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<td>(≥ two lobe)</td>
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<tr>
<td>Anaplastic glioma</td>
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<td>(&lt; one lobe)</td>
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<td>(≥ two lobe)</td>
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<tr>
<td>Glioblastoma multi.</td>
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<td>(≥ two lobe)</td>
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* p < 0.01  \( ^{b} p < 0.05 \)

Fig. 4 Changes in the blood levels of polyamines with the lapse of time in the process of repair after operative insult.
effects on the blood levels of polyamines, and this study was carried out at the time avoiding the period influenced by operative insult (Table 4). Two out of 3 patients treated by surgical removal, irradiation and chemotherapy, had elevated levels of polyamines before treatment, and exhibited decrease to the normal range after the treatment. One of these three treated by the same ways, whose values had not been measured before, had normal levels after treatment. The remaining patient who was subjected to irradiation and chemotherapy but not to surgical removal, had normal levels of polyamines before treatment, but showed elevated levels immediately after the end of the treatment.

Table 4: Blood levels of polyamines measured in patients with glioma before and after treatment. Mean value ± Standard deviation

<table>
<thead>
<tr>
<th>Blood polyamine, M ± SD (μg/ml)</th>
<th>Spermidine</th>
<th>Spermine</th>
<th>Spermidine + Spermine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaplastic glioma\textsuperscript{a} before treatment</td>
<td>3.2</td>
<td>2.2</td>
<td>5.4</td>
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<tr>
<td>after treatment</td>
<td>1.4</td>
<td>1.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Anaplastic glioma\textsuperscript{a} before treatment</td>
<td>2.7</td>
<td>2.5</td>
<td>5.2</td>
</tr>
<tr>
<td>after treatment</td>
<td>1.9</td>
<td>1.1</td>
<td>3.0</td>
</tr>
<tr>
<td>Anaplastic glioma\textsuperscript{b} before treatment</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>after treatment</td>
<td>1.2</td>
<td>1.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Thalamic tumor\textsuperscript{b} before treatment</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>after treatment</td>
<td>2.0</td>
<td>2.1</td>
<td>4.1</td>
</tr>
</tbody>
</table>

\textsuperscript{a} treated by surgical removal, irradiation & chemotherapy.

\textsuperscript{b} treated by irradiation & chemotherapy.

\textsuperscript{*} histl. not verified.

Discussion

Some investigators compared the concentration of polyamines in the body fluid between patients with cancer and the controls. Among them, Russell, et al.\textsuperscript{12)}, Denton, et al.\textsuperscript{2)}, and Takeda, et al.\textsuperscript{4}) estimated the amount of polyamine excretion into the urine, and Morton, et al.\textsuperscript{4}), and Otsuji, et al.\textsuperscript{5,6)} the blood levels of polyamines. All the investigators mentioned above reported that the levels of polyamines had been higher in patients with cancer than in the normals. In their reports they listed leukemia, malignant lymphoma, various carcinoma and sarcoma as tumor which had given rise and an increase in polyamine levels in the body fluid. Brain tumor was mentioned in 4 cases of Russell, et al.\textsuperscript{12}) and 1 case of Otsuji, et al.\textsuperscript{6}) alone. Both authors\textsuperscript{6,12}) pointed out that the patients with brain tumor also had several fold higher levels of polyamines than the controls.

No papers seem to have been published on the estimation of the polyamine levels in the cerebrospinal fluid in patients with brain tumor. If it were possible to detect the compounds in the cerebrospinal fluid which were associated with the process of cell proliferation, the measurement of these compounds would be used more readily than those contained in the blood or urine for the estimation of growth of brain tumor without influence caused by any other organs. So an experiment was carried out on the basis of this assumption. In it, samples of cerebrospinal fluid amounting to as much as 15–20 ml each were collected from patients in whom anaplastic glioma, glioblastoma multiforme, or teratoblastoma of the third ventricle seemed to be proliferating actively. An attempt
was made to detect polyamines from them, but no positive results were obtained from any of the patients. The reason for this failure is unknown. It is desirable that a method of estimation will be established for such substances as reflecting the state of proliferation of tumor and contained in the cerebrospinal fluid.

The relationship between the types of tumor and the blood levels of polyamines was studied in patients with brain tumor before treatment. Concerning the proliferation of tumor, the characters which were postulated from these results obtained were almost the same characteristics as those which had been estimated from the clinical course and the histological findings exhibited so far by such patients with brain tumor. For example, pituitary adenoma, neurinoma and meningioma give rise to various symptoms which are associated with the site of occurrence, but these tumors take a slow clinical course and usually fail to exhibit so-called malignant changes in histological examination. Though only a limited number of patients were studied in the present investigation, the blood levels of polyamines were high in none of these patient. There was no significant difference in the blood levels between the groups of these patients and the control group. The results mentioned above suggest that such types of brain tumor may be those of benign tumor proliferating slowly.

In the two cell pattern pinealoma, tumor cells are sometimes disseminated into the ventricle and to the subarachnoid space of the spinal cord and histological examinations occasionally reveal distinct pictures of nuclear division in the epitheloid cells. These findings suggest that this kind of tumor may have been proliferating actively. But this tumor is produced in such regions that it oppresses the tegmentum of the mesencephalon to cause characteristic ocular symptoms or to cause increased intracranial pressure, so these symptoms enable the early diagnosis of the tumor before it grows into a large mass. Therefore, it is possible that no quantitative changes in metabolism related to the proliferation of tumor may be reflected to any substance contained in the blood. Such being the case, it seems reasonable to presume that quantitative changes in polyamines contained in the blood may be influenced not only by the type of tumor actually developing but also by the size of the tumor.

The blood levels of polyamines were high in more than a half of the patients with glioma, and were significantly higher (p<0.01) in these patients than in the control. Furthermore, a tendency was seen among the patients with glioma that the higher the degree of histological malignancy and the greater the size of tumor in lesions of similar histological changes, the higher became the blood levels of polyamines, although the differences in these levels were not so outstanding. It is a histological characteristic of glioma that this kind of tumor grows in an infiltrating manner showing no clear boundary from the normal tissue, and that every part of the lesion of tumor does not always present the same histological finding. Therefore, it is always pointed out that one is apt to make a mistake to discuss the character of the tumor by examining only one part of the lesion of tumor. Most of the patients with glioma studied in the present investigation were alive, and in many of them the histological diagnosis and the judgement of tumor size were made on the basis of the findings obtained by surgical operation and angiography. Accordingly, it should be borne in mind that the relationship between the histological findings or the size of tumor and the blood levels of polyamines were not so accurately evaluated.

Russell, et al. measured urine polyamine excretion before and after treatment, and they reported that urinary polyamines showed a remarkable fall in patients with ovarian, brain or testicular tumor after surgical removal, and that another patient with acute myelocytic leukemia had elevated levels of urinary polyamines before treatment, exhibited a several fold increase during chemotherapy, and showed normal levels during a remission period.

Denton, et al. measured urinary excretion of polyamines in patients with acute myelocytic leukemia, and mentioned that there was a marked increase in the excretion of spermine over pretreatment values in a patient during chemotherapy that was effective as judged by a fall in the peripheral count of blast cells, and that another patient who did not respond to chemotherapy showed no such changes in polyamine excretion.

The blood levels of polyamines were estimated in patients with glioma at regular intervals during the period of treatment. The values were within the normal range after treat-
ment in patients treated by surgical removal, irradiation and chemotherapy. In another patient with thalamic tumor in whom no surgical removal had been performed on account of the localization and who had been subjected to irradiation and chemotherapy, the blood levels of polyamines were found to be rather high immediately after the end of the treatment. A tendency was observed in the patients studied that the blood levels of polyamines became rather high during the period of similar treatment and returned to the normal range several days after the end of this period, although there was no mentioning about it in this paper. Therefore, it is possible for the last patient with thalamic tumor to show normal blood levels of polyamines some days after the end of the treatment.

Conclusions

Measurement of the blood levels of polyamines is presumed to be a useful method to estimate the state of proliferation of brain tumor. The results reported in this paper suggest that measurement of polyamines in the blood may lead to diagnosis, judgement of the effectiveness of the treatment and prognostic information in patients with brain tumor too, as in patients with malignant tumor of other organs. To ascertain these assumption, further studies need to be tried in many patients with brain tumor.

Acknowledgements

The author wishes to express his hearty thanks to Professor Komei Ueki, major adviser, for his valuable advice and review of this manuscript. I am also grateful to Dr. Kenichi Tanimura for his helpful suggestions.

References