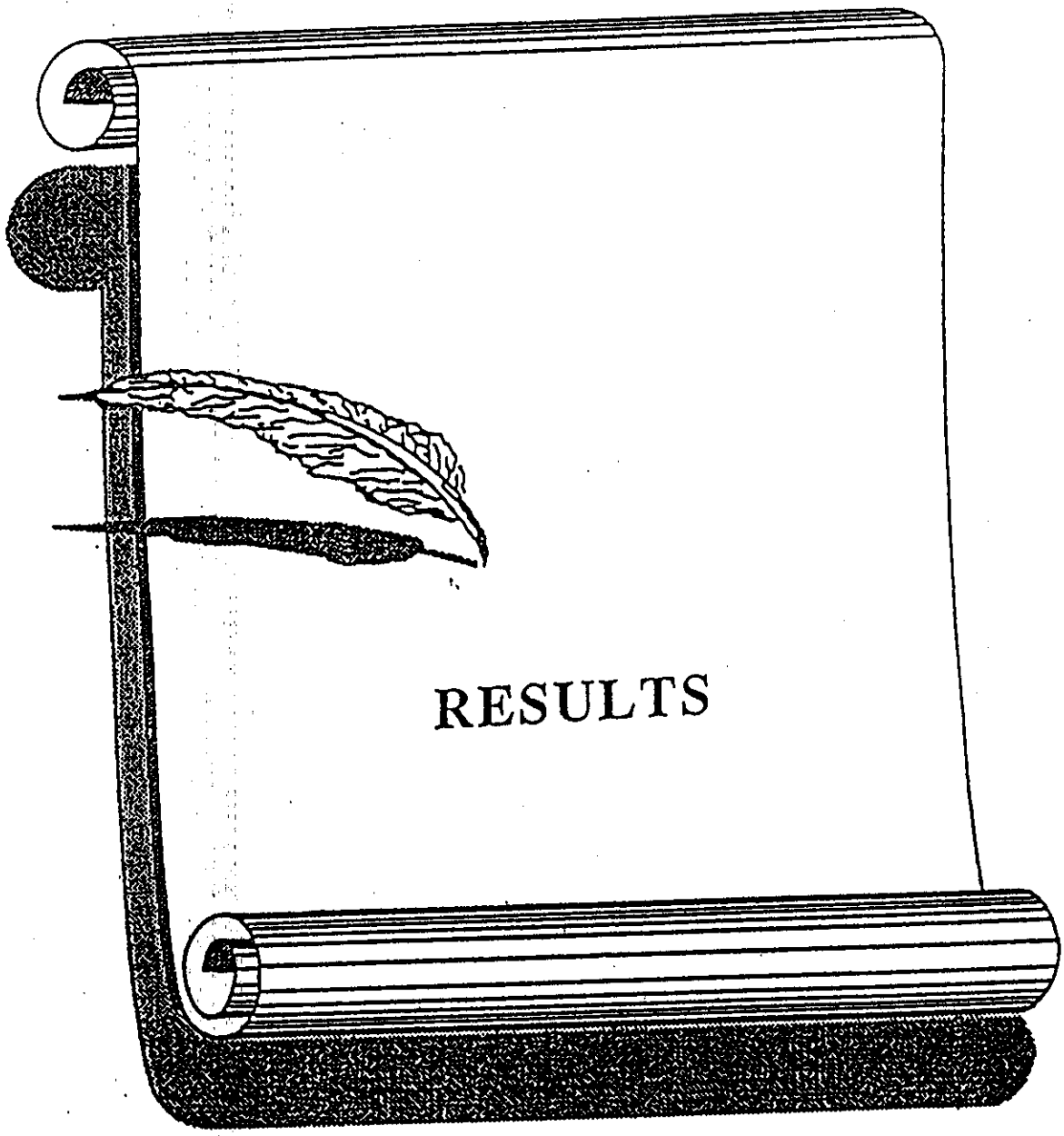

RESULTS

## * Histopathological Diagnosis :-

*- Tumor Type and Grade :*

In this work the forty patients with intracranial gliomas were studied and classified according to the world Health Organization and recorded ( **Table 3**) which illustrates the types and grades of these tumors .

*- Histopathological Features :-*

The histopathological features which were studied in this work are summarized in Tables **4 , 5 , 6** and **7** respectively .

*1- Cellular density and types :-*

( **Table 4** ) demonstrates the cellular density in the different types and grades of the forty cases with intracranial gliomas expressed from mild + to dense ++++ , the table also shows the type of cells forming these tumors , 42.5% of cases showed presence of uniform rounded to spindle shaped cells , the cases showing these cells mostly are astrocytoma grade 1 and II , an equal number 42.5% of cases mostly of grade III astrocytoma showed predominance of small anaplastic cells , while the last 15.0% of cases showed the presence of more than one type of cells in addition to large bizarre shaped and gemistocytic cells .

*2- Mitosis :-*

The number of mitosis was scored on a semiquantitative manner, it revealed the presence of a low mitotic count ( from zero to five ) per ten high power fields in cases of astrocytoma grade I and II , also in oligodendroglioma as well as choroid plexus papilloma , while cases of astrocytoma grade III , glioblastoma multiforme , ependymoma grade III , medulloblastoma , and gliosarcoma showed a mitotic count ranging from seven to twenty per ten high power fields .

*3- Cellular abnormalities and stromal features :-*

*a- Multinucleation and gemistocytes :-*

The study revealed the presence of multinucleated giant cells in 35% of cases while gemistocytes were obsrved in 30% of cases

*b- Stromal features :-*

Mild ( + ) and moderate ( ++ ) degree fibrosis was encountered in eight and one case respectively ( **Table 5** )

*4- Degenerative changes :-*

The degenerative changes were mainly necrosis 67.5% , pseudopalisading 7.5% and cystic degeneration 25% .(**Table 6**)Two types of necrosis were encountered "coagulative necrosis and liquifactive necrosis" However microcystes were present in 10 cases, cysts with proteinious material were detected in 2 cases only

5- *Vascular changes :-*

The vascular changes encountered in this work are summarized (Table 7) , which illulstrates ,

a- Increased vascularity compared with normal tissue due to an increase of either medium sized blood vessels or capillaries , it was expressed from mild increased in vascularity to marked .

b- The other vascular changes evaluated were the endothelial proliferation ( 17.5% ) , vessel wall hyalinization ( 65% ) , thrombosis ( 37.5% ) and tufts of pseudoglomeruli ( 12.5% )

Figs ( 13, 14, 15, 16, 17, 18 ,19 ,2o21demonstrates the normal control , the different grades of gliomas , and the different histopathological features studied in gliomas .

Table 3. Histopathological types and grades of 40 patients with intracranial gliomas.

| Diagnosis | Number of patients | Percent |
|---|---|---|
| Astro cytoma grade I | 3 | 7.5 |
| Astr cytoma grade II | 14 | 35.0 |
| Astro cytoma grade I I | 15 | 37.5 |
| Glioblastoma multiforme | 3 | 7.5 |
| Ependymoma grade 3 | 1 | 2.5 |
| Medulloblastoma | 1 | 2.5 |
| Oligodendroglioma | 1 | 2.5 |
| Choroid plexus papilloma | 1 | 2.5 |
| Gliosarcoma | 1 | 2.5 |
| Total | 40 | 100.0 |

Table 4. Cellular type and density in intracranial gliomas.

| Cellularity | Number of patients | Percent |
|---|---|---|
| Type of cells | | |
| Uniform rounded or spindle shaped | 17 | 42.5 |
| Small anaplastic | 17 | 42.5 |
| Others | 6 | 15.0 |
| Density | | |
| + | 3 | 7.5 |
| ++ | 16 | 40.0 |
| +++ | 19 | 47.5 |
| ++++ | 2 | 5.0 |

Key:
+ = Mild cellularity
++ = Moderate cellularity
+++ & ++++ = Dense cellularity
Others = Large bizarre shaped cells and gemistocytes.

Table 5. Cellular and stromal features in intracranial gliomas.

| Abnormalities | Number of patients | Percent |
|---|---|---|
| Multinucleation | | |
| - | 26 | 65.0 |
| + | 14 | 35.0 |
| Gemistocytes | | |
| - | 28 | 70.0 |
| + | 12 | 30.0 |
| Fibrosis | | |
| - | 31 | 77.5 |
| + | 8 | 20.0 |
| ++ | 1 | 2.5 |

Table 6. Degenerative changes in intracranial gliomas.

| Changes | Number of patients | Percent |
|---|---|---|
| Type of necrosis | | |
| Normal | 13 | 32.5 |
| Coagul | 15 | 37.5 |
| Coagul & Liqui | 12 | 30.0 |
| Pseudo pallisading | | |
| Negative | 37 | 92.5 |
| Positive | 3 | 7.5 |
| Microcysts | | |
| Negative | 30 | 75.0 |
| Positive | 10 | 25.0 |
| Microcysts with proteinious material | | |
| Negative | 38 | 95.0 |
| Positive | 2 | 5.0 |

Key:
Normal = Absence of necrosis
Coagul = Coagulative necrosis
Liqui = Liquifactive necrosis

Table 7. Vascular changes in intracranial gliomas.

| Changes | Number of patients | Percent |
|---|---|---|
| Increased vascularity | | |
| + | 19 | 47.5 |
| ++ | 18 | 45.0 |
| +++ | 3 | 7.5 |
| Endothelial proliferation | | |
| - | 33 | 82.5 |
| + | 7 | 17.5 |
| Hyalinization | | |
| - | 14 | 35.0 |
| + | 19 | 47.5 |
| ++ | 7 | 17.5 |
| Thrombosis | | |
| - | 25 | 62.5 |
| + | 12 | 30.0 |
| ++ | 3 | 7.5 |
| Pseudo glomeruli | | |
| - | 35 | 87.5 |
| + | 4 | 10.0 |
| ++ | 1 | 2.5 |

Fig 13 Photomicrograph of the normal brain tisssue from the cerebral cortex ( control ) H&E × 240

Fig 14 Photomicrograph of astrocytoma grade I with mild uniform rounded to oval astrocytes and very small thin walled capillary . H&E × 200

Fig 15 Photomicrograph of astrocytoma grade II with moderate cellularity , thickened wall blood vessels . H&E × 400

Fig 16 Photomicrograph of astrocytoma grade III with increased cellularity, pleomorphism , mitosis , dilated medium sized blood vessel .and giant cells H&E × 400

Fig 17 Photomicrograph showing astrocytoma grade III with gemistocytes , large bizarre shaped cells , increased vascularity H&E × 400

Fig 18 Photomicrograph showing astrocytoma grade III with endothelial pro liferation and hyalinization of blood vessel wall H&E × 200

Fig 19 Photomicrograph showing glioblastoma multiforme with increased cellularity , palisading , mitosis H&E × 400

Fig 20 Photomicrograph showing glioblastoma multiforme with increased vascularity , pseudo glomeruli formation and endothelial proliferation and lymphocytic infiltration H&E × 400

## State of cell proliferation estimated by Immunohistochemistry

***The staining pattern of ki- 67 :-***

The nucleoli of tumor cells were strongly stained with diffuse nuclear staining . Neither cytoplasm nor cell membrane were labelled . Fig ( 22 )

The distribution of Ki-67 labelled cells varied from field to field even within the same tumor . Most of the labelled cells were of the small anaplastic astrocytes , while the large bizzare shaped cells were mostly unlabelled .

**** Control group :-***

The normal brain tissue in this study failed to show any positive staining for histochemical study concerning proliferating cell marker Ki-67 . Fig ( 23 )

**** The glioma group :-***

The growth fraction estimated by immunohistochemical staining of Ki-67 showed that .

1-The frequency of Ki-67 positve cells was not related with either age or sex .

2- The labelling index varied greatly from case to case almost zero to fifty one% .

Fig 21 Photomicrograph showing glioblastoma multiforme with fibrosis , lymphocytic infiltration - giant cells and mitosis H&E × 400

The labelling index of Ki-67 in gliosarcoma was the highest index 51% , in medulloblastoma it was 47% . The astrocytoma grade III and glioblastoma multiformes showed labelling indices varying from 5.6% to 24% with an average of 12.22% and from 7.1% to 14.6% respectively . The only case of ependymoma grade III showed a labelling index of 12.1% , while the labelling index of Ki-67 was ranged from 1% to 3% and from 0% to 1% in astrocytoma grade II and 1 respectively . The oligodendroglioma case showed a Ki-67 labelling index of 1% while the choroid plexus papilloma failed to give any positive staining .

Figs { 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33 } .

Fig 22 Photomicrograph showing astrocytoma grade III with ki 67 positive cells with neuclear and nucleolar staining. PAP × 1000

Fig 23 Photomicrograph of the normal brain tisssue ( control ) with no response to Ki 67 PAP × 400.

Fig 24 Photomicrograph of choroid plexus papilloma with no response to Ki 67 PAP × 400.

Fig 25 Photomicrograph of astrocytoma grade III showing response to Ki 67. Ki 67 positive cells were observed in approximately 3.1%PAP × 400.

Fig 26 Photomicrograph of astrocytoma grade III showing response to Ki 67. Ki 67 positive cells were observed in approximately 10%PAP × 400.

Fig 27 Photomicrograph of gliosarcoma showing high labeling index for Ki 67 PAP × 400

Fig 28 Photomicrograph of astrocytoma grade III showing pseudorosette for mation H&E × 200 .

Fig 29 Photomicrograph of astrocytoma grade III showing pseudorosette pat tern for Ki 67 PAP × 1000 .

Fig 30 Photomicrograph of astrocytoma grade III showing response to Ki 67. Ki 67 positive cells were observed in approximately 24%PAP × 400.

Fig 31 Photomicrograph of astrocytoma grade III showing response to Ki 67. Ki 67 positive cells were observed in approximately 24%PAP × 1000.

Fig 32 Photomicrograph of medulloblastoma with uniform rounded cells H&E × 200 .

Fig 33 Photomicrograph of medulloblastoma with high Ki 67 response PAP × 400

## Results of correlation between Ki-67 labelling index and the histopathological diagnosis

A good correlation and a statistically significant difference between hitopathological grade of intracranial gliomas divided into low and high grades on one side and the Ki-67 labelling index on the other side has been detected . ( **Table 8** )

The correlation between Ki-67 index and the different histological criteria studied in this work were as follows ( **Tabels 9 ,10 , 11 , 12** ). **(Table 9)** shows the relation between Ki-67 labelling index on one side and cellular density as well as type of neoplastic cells on the other side . A highly significal diference was encountered , where the Ki-67 labelling index in tumors with dense cellularity +++ to ++++ showed a high mean value (15.56% ± 12.20%) , while in tumors with low density the mean labelling index of Ki-67 was 1.44% ± 1.02% . With regard to the Ki-67 index and type of neoplastic cells , it was found that the mean labelling index in tumors with small anaplastic astrocytes was significantly higher than the labelling index of Ki-67 in tumors formed of uniform rounded or spindle shaped cells and also tumors with other types of cells ( gemistocytes and giant bizarre cells ) . (12.56% ± 10.78% , 6.67% ± 12.73% , 4.55% ± 4.62% ) .

Table 8. KI 67 labelling index according to grading of intracranial gliomas.

| Diagnosis[a] | Number of cases | Mean±SD | P-value[b] |
|---|---|---|---|
| Low | 19 | 1.55± 1.00 | |
| High | 21 | 15.56±12.20 | <0.0001 |

a Diagnosis according to WHO classification:
Low = Grade I and II
High = Grade III and IV

b P-value for the difference between the groups, p-value <0.05 is considered significant, N.S.= Not significant.

Table 9. KI 67 labelling index according to cellularity of intracranial gliomas.

| Cellularity | Number of cases | Mean±SD | P-value[a] | ANOVA results[b] |
|---|---|---|---|---|
| Density | | | | |
| +/++ | 19 | 1.44± 1.02 | | |
| +++/++++ | 21 | 15.56±12.20 | <0.0001 | |
| Type of cells | | | | |
| Uniform rounded or spindle shaped | 17 | 6.67±12.73 | | B |
| Small anaplastic | 17 | 12.56±10.78 | | A |
| Others | 6 | 4.55± 4.62 | 0.0207 | B |

a P-value for the difference between the groups, p-value <0.05 is considered significant, N.S.= Not significant.

b ANOVA results = Analsysis of variance multiple comparisons results, means sharing the same letter are significantly different, p-value > 0.05.

***Results of ststistical correlation between mitosis and Ki-67 labelling index :-***

A significantly higher Ki-67 labelling index was encountered in tumors with high mitosis . Fig (34)

***- Cellular and stromal features :-***

A significant correlation was found between Ki-67 labelling index and presence of multinucleation and fibrosis .

No statistically significant difference in Ki-67 labelling index was encountered between tumors with or without gemistocytes . ( $P > 0.05$ ) ( **Table 10**)

***Degenerative changes :-***

The statistical analysis regarding the relation between Ki-67 index and degenerative changes ( **Table 11** ) indicated that tumors with necrosis had significantly higher index than those without necrosis $P < 0.0001$ . The index of Ki-67 was also higher in tumors with both types of necrosis (Coagulative and liquifactive ( $14.84\% \pm 14.49\%$ ) , than tumors without necrosis ( $1.22\% \pm 0.96\%$ ) There was no significant fifference between tumors with both types of necrosis and those with coagulative necrosis only .

There was also statistically significant difference in Ki-67 labelling index between tumors with and without microcysts (16.63% ±11.82% and 6.26% ± 10.04% ) P < 0.0001.

No significant difference in Ki-67 index was found between tumors with and without pseudopalisading or microcysts with proteinious material

***Vascular changes :-***

The results showed that (1) Tumors with increased vascularity had a higher Ki-67 labelling index than those with mild vascularity (14.87% ±12.58% versus 2.21% ± 3.49% ) P < 0.0001.

(2) Tumors with endothelial proliferation , hyalinization , thrombosis , and pseudoglomeruli had significantly higher Ki-67 labelling indices than those without these vascular changes .

( 15.97% ±15.00% versus 7.35% ± 10.00% ) P < 0323 . ( 15.37% ±12.29% versus 1.86% ± 2.48% ) with P. 0.0002 . ( 14.47% ±15.26% versus 5.48% ± 6.36% ) with P. 0.0229 . ( 10.14% ±3.26% versus 8.67% ± 12.04% ) with P. 0.0088 . **(Table 12 )**

Figure 34 Relationship between KI index and mitosis in brain tumor patients

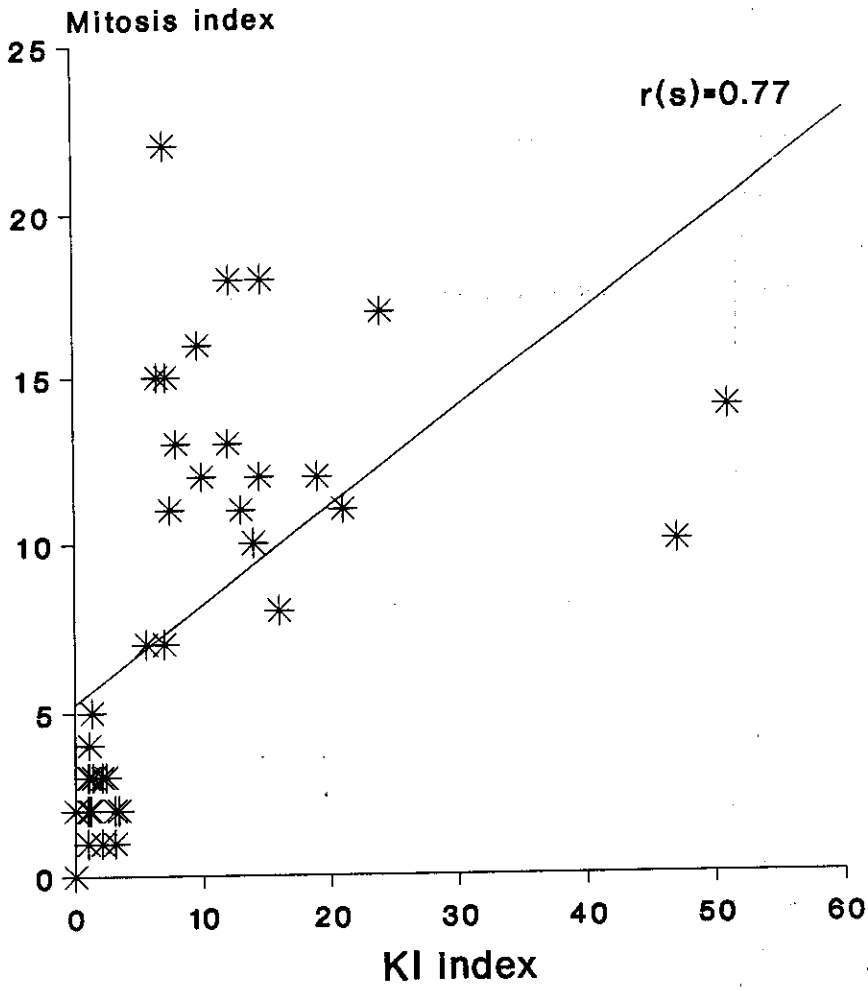


Table 10. KI 67 labelling index according to cellular and stromal features in intracranial gliomas.

| | Number of cases | Mean±SD | P-value[a] |
|---|---|---|---|
| Multinucleation | | | |
| - | 26 | 8.05±13.66 | |
| + | 14 | 10.34± 4.56 | 0.0038 |
| Gemistocytes | | | |
| - | 28 | 8.39±13.07 | |
| + | 12 | 9.94± 5.64 | N.S. |
| Fibrosis | | | |
| - | 31 | 7.42±10.06 | |
| +/++ | 9 | 13.79±14.43 | 0.0457 |

[a] P-value for the difference between the groups, p-value <0.05 is considered significant, N.S.= Not significant.

Table 11. KI 67 labelling index according to degenerative changes in intracranial gliomas.

| | Number of cases | Mean±SD | P-value[a] | ANOVA results[b] |
|---|---|---|---|---|
| Type of necrosis | | | | |
| Normal | 13 | 1.22± 0.96 | | B |
| Coagul | 12 | 9.64± 8.37 | | A |
| Coagul & Liqui | 15 | 14.84±14.39 | <0.0001 | A |
| Microcysts | | | | |
| Negative | 30 | 6.26±10.04 | | |
| Positive | 10 | 16.63±11.82 | <0.0001 | |

a P-value for the difference between the groups, p-value <0.05 is considered significant, N.S.= Not significant.

b ANOVA results = Analsysis of variance multiple comparisons results, means sharing the same letter are significantly different, p-value > 0.05.

Key:
Normal = Absence of necrosis
Coagul = Coagulative necrosis
Liqui = Liquifactive necrosis

Table 12. KI 67 labelling index according to vascular changes in intracranial gliomas.

| | Number of cases | Mean±SD | P-value[a] | ANOVA results[b] |
|---|---|---|---|---|
| Increased vascularity | | | | |
| + | 19 | 2.21± 3.49 | | |
| ++/+++ | 21 | 14.87±12.58 | <0.0001 | |
| Endothelial proliferation | | | | |
| - | 33 | 7.35±10.00 | | |
| + | 7 | 15.97±15.00 | 0.0323 | |
| Hyalinization | | | | |
| - | 14 | 1.86± 2.48 | | B |
| + | 19 | 11.61±11.91 | | A |
| ++ | 7 | 15.37±12.29 | 0.0002 | A |
| Thrombosis | | | | |
| - | 25 | 5.48± 6.36 | | |
| +/++ | 15 | 14.47±15.26 | 0.0229 | |
| Pseudo glommeruli | | | | |
| - | 35 | 8.67±12.04 | | |
| +/++ | 5 | 10.14± 3.26 | 0.0088 | |

a P-value for the difference between the groups, p-value <0.05 is considered significant, N.S.= Not significant.

b ANOVA results = Analsysis of variance multiple comparisons results, means sharing the same letter are significantly different, p-value > 0.05.

## The Immunohistochemical results of lymphocytic and Natural Killer cell infiltrates in gliomas

### * Control group

The normal brain tissue sections failed to show positive staining for T lymphocytes and their subsets as well as for natural killer cells .

### * Glioma group :-

The immunohistochemical staining was observed mainly in the cell membrane , no nuclear staining was detected . As shown in **table 13** , mild infiltration with CD3 + ve cells was encountered in 4 cases of low grade gliomas ( 21.05% ) . There was also mild infiltration with CD4 + ve and CD8 + ve cells in 2 and 1 cases respectively ( 10.52% , 5.3% ) . The degree of CD4 + ve and CD8 + ve cell infiltration in low grade gliomas was comparable . In high grade gliomas ( 21 cases ) , there was mild infiltration with CD3 + ve cells in 3 cases ( 14.3% ) moderate infiltration in 5 cases ( 23.8% ) and intense infiltration in 10 cases ( 47.6% ) . There was mild infiltration with CD4 + ve cells in 7 cases ( 33.3% ) , moderate infiltration in 7 cases ( 33.3% ) , while infiltration with CD8 + ve cells in high grade gliomas was mild in 9 cases (42.85%) , and moderate in another 9 cases ( 42.85% ) .

The table also shows that there is CD4+ ve and CD8 + ve cell infiltration in 2 and 1 cases , predominance of CD4 + ve cells in 2 cases and predominance of CD8 + ve cells in 4 cases . The results

also showed predominance of CD8 + ve ( suppressor/cytotoxic ) T lymphocytes in high grade gliomas .

***As regard natural killer cells , it was found that :-***

- Leu 7 + ve cell infiltration in high grade gliomas was restricted to 4 cases only , two of which were astrocytoma grade III and the other two were glioblastoma multiforme .

- Leu 11 + ve cell infiltration was encountered in 4 cases , 3 of which were astrocytoma grade III and 1 was glioblastoma multiforme .

- The lymphocytic and natural killer cellular infiltrates were closely associated with small and more often pathologic vessels , diffuse and scattered distribution of the stained cells was detected .

Fig ( 35 a 35 b 35 c, 36)

**Correlation between Ki-67 labelling index and T lymphocyte with its two subsets infiltrating gliomas**

( Table 14) - Tumors with marked T lymphocytic infiltration had significantly higher Ki-67 labelling index than those without - or with mild to moderate infiltration + : ++ ( 11.45% ± 5.47% , 7.34% ±15.41% , 8.97 ± 7.19 ) with P. value 0.0266 .

- Tumors with CD8 + ve ( suppressor / cytotoxic ) T lymphocyte infiltration had significantly higher Ki-67 labelling index than those without CD8 + ve cell infiltration . ( 10.89% ± 5.64% , 6.50% ± 4.36% ) P. 0.0017 .

No statistically significant difference in Ki-67 labelling index between tumors with or without CD4 + ve cell infiltration .

Detailed clinicopathologic and immunohistochemical staining findings are summarized in **table 15** .

Table 13 . T Lymphocytic infiltration in gliomas

| T Lymphocytic Infiltration | Low grade gliomas n (%) | High grade gliomas n (%) |
|---|---|---|
| CD 3 | | |
| - | 15 ( 78.9) | 3 (14.3) |
| + | 4 (21.05) | 3 (14.3) |
| ++ | | 5 (23.8) |
| +++ | | 10 (47.6) |
| CD4 | | |
| - | 17 (89.5) | 7 (33.3) |
| + | 2 (10.5) | 7 (33.3) |
| ++ | | 7 (33.3) |
| CD8 | | |
| - | 18 (94.7) | 3 (14.3) |
| + | 1 (5.3) | 9 (42.9) |
| ++ | | 9 (42.9) |
| Total | 19 | 21 |

Table 14. KI 67 labelling index according to T lymphocyte subsets infiltrating intracranial gliomas.

| | Number of cases | Mean±SD | P-value[a] | ANOVA results[b] |
|---|---|---|---|---|
| CD3 | | | | |
| - | 18 | 7.34±15.41 | | B |
| +/++ | 12 | 8.97± 7.19 | | B |
| +++ | 10 | 11.45± 5.47 | 0.0266 | A |
| CD4 | | | | |
| - | 24 | 8.28±13.95 | | |
| +/++ | 16 | 9.71± 5.71 | N.S. | |
| CD8 | | | | |
| - | 21 | 6.50±14.36 | | B |
| + | 10 | 11.97± 6.25 | | A |
| ++ | 9 | 10.89± 5.64 | 0.0017 | A |

a P-value for the difference between the groups, p-value <0.05 is considered significant, N.S.= Not significant.

b ANOVA results = Analsysis of variance multiple comparisons results, means sharing the same letter are significantly different, p-value > 0.05.

Table 15. Clinoco-pathologic and immunologic parameters in intracranial gliomas.

| | | | | Cellularity | | Degenerative changes: Necrosis | | | | | Vascular changes | | | | | Cellular abnormalities | | | | KI | Lymphatic infiltration | | | | | Follow- |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No | DIAG | Age | Sex | DEN | CEL | Type | Extent | PSE | MIC | MW | MS | PRO | HYL | THR | GLO | MIT | MUL | GEM | FIB | index | CD3 | CD4 | CD8 | LEU7 | LEU11 | up |
| 1 | I | 42 | M | + | 1 | - | - | - | - | - | - | - | - | - | - | 0 | - | - | - | 0.0 | - | - | - | - | - | Alive |
| 2 | I | 9 | M | + | 1 | - | - | - | - | - | - | - | - | - | - | 0 | - | - | - | 0.0 | - | - | - | - | - | Alive |
| 3 | I | 27 | F | + | 1 | - | - | - | - | - | + | - | - | - | - | 1 | - | - | - | 1.0 | - | - | - | - | - | Alive |
| 4 | II | 36 | F | ++ | 1+2 | - | - | - | - | - | + | - | - | - | - | 3 | - | - | - | 1.1 | - | - | - | - | - | Alive |
| 5 | II | 45 | M | ++ | 2 | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | 3.1 | - | - | - | - | - | Recur |
| 6 | II | 34 | M | ++ | 2 | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | 2.1 | - | - | - | - | - | Alive |
| 7 | II | 14 | M | ++ | 1+2 | Coagul. | 5 | - | - | - | + | - | - | - | - | 2 | - | - | - | 1.2 | + | - | - | - | - | Alive |
| 8 | II | 24 | M | ++ | 2 | Coagul. | 5 | - | - | - | + | - | - | - | - | 5 | - | - | - | 1.3 | - | - | - | - | - | Alive |
| 9 | II | 27 | F | ++ | 1 | Coagul. | 5 | - | - | - | + | - | - | - | - | 2 | - | - | - | 3.4 | - | - | - | - | - | Alive |
| 10 | II | 16 | M | ++ | 1+2 | - | - | - | - | - | - | - | - | - | - | 2 | - | - | - | 1.0 | + | - | + | - | - | Dead |
| 11 | II | 43 | F | ++ | 3+1 | - | - | - | - | - | + | - | + | - | - | 3 | - | - | - | 2.4 | - | - | - | - | - | Alive |
| 12 | II | 31 | F | ++ | 3+4 | Coagul. | 20 | - | - | - | + | - | + | - | - | 2 | + | + | + | 3.1 | - | - | - | - | - | Alive |
| 13 | II | 46 | F | ++ | 1 | - | - | - | - | - | + | - | + | + | - | 3 | - | - | - | 2.1 | + | + | - | - | - | Alive |
| 14 | II | 51 | M | ++ | 1 | - | - | - | - | - | - | - | - | - | - | 2 | - | - | - | 1.1 | - | - | - | - | - | Alive |
| 15 | II | 32 | M | ++ | 2+4 | Coagul. | 10 | - | - | - | + | - | + | + | - | 3 | - | - | - | 1.4 | - | - | - | - | - | Recur |
| 16 | II | 37 | M | ++ | 1+4 | C+L | 15 | - | - | - | + | - | + | + | - | 4 | - | - | - | 1.1 | + | + | - | - | - | Alive |
| 17 | II | 54 | F | ++ | 2 | - | - | - | - | - | - | - | + | - | - | 3 | - | - | - | 1.0 | - | - | - | - | - | Dead |
| 18 | III | 41 | M | +++ | 2+PR | Coagul. | 50 | - | - | - | ++ | - | ++ | + | + | 12 | + | - | - | 10.0 | ++ | - | ++ | - | + | Recur |
| 19 | III | 58 | M | +++ | 3+1 | Coagul. | 5-10 | - | - | - | + | - | + | - | - | 8 | + | - | - | 16.0 | + | + | + | - | - | Alive |
| 20 | III | 39 | M | +++ | 2+PR | Coagul. | >30 | - | - | - | + | - | + | - | - | 11 | - | - | - | 21.0 | ++ | - | + | - | - | Recur |
| 21 | III | 61 | M | +++ | 3+4 | C+L | >25 | - | + | - | ++ | - | ++ | + | - | 13 | - | + | + | 8.0 | +++ | ++ | ++ | - | - | Alive |
| 22 | III | 45 | M | +++ | 3+1 | C+L | >25 | - | + | - | + | - | ++ | + | - | 12 | - | + | + | 14.5 | + | - | + | - | - | Alive |
| 23 | III | 57 | M | +++ | 2+3 | Coagul. | 5 | - | + | - | ++ | + | + | + | - | 17 | - | + | - | 24.0 | +++ | ++ | ++ | - | + | Recur |
| 24 | III | 63 | M | +++ | 3+4 | C+L | >50 | - | + | + | + | + | ++ | + | + | 7 | + | + | - | 7.0 | ++ | + | ++ | - | - | Alive |
| 25 | III | 41 | F | +++ | 3 | Coagul. | >15 | - | + | - | + | - | + | - | - | 12 | + | - | - | 19.0 | + | - | + | - | - | Alive |
| 26 | III | 49 | F | +++ | 3+4 | C+L | >25 | - | - | - | ++ | - | ++ | + | - | 10 | + | - | - | 14.0 | +++ | + | + | - | - | Alive |
| 27 | III | 52 | M | +++ | 4+1 | Coagul. | >25 | - | - | - | ++ | - | + | ++ | - | 7 | + | + | + | 5.6 | +++ | ++ | ++ | - | - | Recur |
| 28 | III | 25 | F | +++ | 3+2 | C+L | 10 | - | - | - | + | + | + | - | - | 11 | - | - | - | 7.5 | ++ | ++ | + | + | - | Alive |

(continued next page)

Table 15. Continued

| | | | | Cellularity | | Degenerative changes: Necrosis | | | | | Vascular changes | | | | | Cellular abnormalities | | | | KI | Lymphatic infiltration | | | | | Follow- |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No | DIAG | Age | Sex | DEN | CEL | Type | Extent | PSE | MIC | MW | MS | PRO | HYL | THR | GLO | MIT | MUL | GEM | FIB | index | CD3 | CD4 | CD8 | LEU7 | LEU11 | up |
| 29 | III | 40 | M | ++++ | 3+5 | C+L | >30 | + | - | - | + | + | + | - | - | 15 | + | + | - | 7.2 | ++ | + | + | - | - | Alive |
| 30 | III | 52 | M | +++ | 2+3 | C+L | >50 | - | + | - | ++ | + | + | - | + | 13 | + | + | - | 12.0 | +++ | + | ++ | - | - | Recur |
| 31 | III | 43 | M | +++ | 3+5 | C+L | >50 | - | - | - | + | - | + | - | - | 15 | + | + | - | 6.5 | +++ | ++ | + | + | - | Alive |
| 32 | III | 41 | F | +++ | 2 | C+L | <25 | - | + | - | ++ | - | + | - | - | 11 | + | - | + | 13.0 | +++ | + | + | - | + | Recur |
| 33 | GLI | 46 | F | +++ | 1+2+3 | C+L | >50 | + | - | - | ++ | - | + | + | ++ | 18 | + | + | - | 14.6 | +++ | ++ | ++ | + | - | Recur |
| 34 | GLI | 37 | F | +++ | 3+5 | Coagul. | >25 | - | + | - | + | - | - | + | - | 16 | + | + | + | 9.7 | +++ | + | ++ | - | - | Alive |
| 35 | GLI | 51 | F | +++ | 4+5 | C+L | >50 | - | - | - | ++ | + | ++ | ++ | + | 22 | + | + | + | 7.1 | +++ | ++ | ++ | + | + | Alive |
| 36 | EPE | 18 | M | +++ | 6+RF | C+L | >25 | - | + | - | ++ | - | + | - | - | 18 | - | - | + | + 12.1 | - | - | - | - | - | Recur |
| 37 | MED | 27 | F | ++++ | 7+RF | C+L | >25 | - | + | + | + | + | ++ | + | - | 10 | - | - | - | 47.0 | - | - | - | - | - | Dead |
| 38 | SAR | 41 | F | +++ | 2+1 | C+L | >50 | - | - | - | ++ | - | + | ++ | - | 14 | - | - | + | 51.0 | - | - | - | - | - | Recur |
| 39 | OLI | 23 | M | ++ | 7 | - | - | - | - | - | - | - | - | - | - | 3 | - | - | - | 1.0 | - | - | - | - | - | Alive |
| 40 | CPP | 31 | M | ++ | 8+PF | - | - | - | - | - | + | - | - | - | - | 2 | - | - | - | 0.0 | - | - | - | - | - | Alive |

Abbreviations:
DIAG=Diagnosis, Age=Age in years, DEN=Density, CEL=Type of cell, PSE=Pseudopallisading, MIC=Microcysts, MW=Microcysts with proteinious material, VAS=Increased vascularity, PRO=Endothelial proliferation, HYL=Hyalinization, THR=Thrombosis, GLO=Pseudoglomeruli, MIT=Mitotic count /10 high power fields, MUL=Multinucleation, GEM=Gemistocytes, FIB=Fibrosis, CD3=Pan T lymphocytes, CD4=Helper T lymphocytes, CD8=Suppression/cytotoxic T lymphocytes, LEU7=Immature natural killer cells , LEU11=Mature natural killer cells.

1= rounded cells . 2= oval & spindle cells. 3= small anaplastic cells. 4= large bizarre cells. 5= fibrillated cells. 6= giant cells. 7= small uniform cells. 8= cuboidal cells.
PR= pseudorosette .

Fig 35 a Photomicrograph of astrocytoma grade III showing mild lymphocytic infiltration (cell membrane) + PAP × 1000 .

Fig 35 b Photomicrograph of astrocytoma grade III showing moderate lymphocytic infiltration (cell membrane) ++ PAP × 1000 .

Fig 35 c Photomicrograph of astrocytoma grade III showing dense lymphocytic infiltration (cell membrane) +++ PAP × 1000 .

Fig 36 Photomicrograph of astrocytoma grade III showing perivascular natural killer cell infiltration (cell membrane and cytoplasmic staining ) PAP × 1000 .

# Results of Cytophotometric studies

## *DNA distribution histograms*

### 1- *Control histogram*

The histogram of the normal control is presented in Fig (37) where the majority of cells were found in the diploid range

### 2- *The glioma's histograms :*

a- Astrocytoma grade I (3 cases ) showed a histogram characterized by having a single distinct model DNA value in the diploid region (2c) of normal cells , only a small number of cells deviated more than a few percent of the normal value Fig (38) .

b- Astrocytoma grade II ( 14 cases ) showed a histogram similar to the histogram of astrocytoma grade I but with a slight shift of the model DNA value towards the triploid level (3c) Fig (39)

c- Histogram of astrocytoma grade III(15 cases) revealed more than one peak and more shift to the right towards the tetraploid level and hyperploidy Fig (40)

d-Histogram of glioblstoma multiforme (3 cases) is presented in Fig (41)

it was characterized by marked aneuploidy with erratic distribution of DNA values .The individual DNA values were ranged

from levels near 2c up to values more than 12c with most DNA level measurements above 4c .

e-Histogram of ependymoma grade III (1 case ) is presented in Fig (42) , it was comparable to histogram of asrtocytoma grade III with slight shift to right and most of the measurements between 2c and 4c

f- Histogram of medulloblastoma (1 case ) is presented in Fig (43) ,where there was more than one peak and marked aneuploidy and most measurements above 4c .

g-Histogram of gliosarcoma (1 case ) is presented in Fig (44) .it was characterized by irregular aneuploidy and shift to right with some measurements reaching 16 c .

h-Histogram of choroid plexus papilloma (1 case ) Fig (45) , was comparable to histogram of astrocytoma grade I where about 70% of measurements were in the diploid range (2 c ) .

i-Histogram of oligodendroglioma ( 1 case ) Fig (46) ,was characterized by one peak in the diploid range and another peak on the right (6 c) with slight irregular aneuploid line ) . All cases of intracranial gliomas studied showed aneuploid pattern in histograms .

Figure 37. DNA distribution in normal controls.

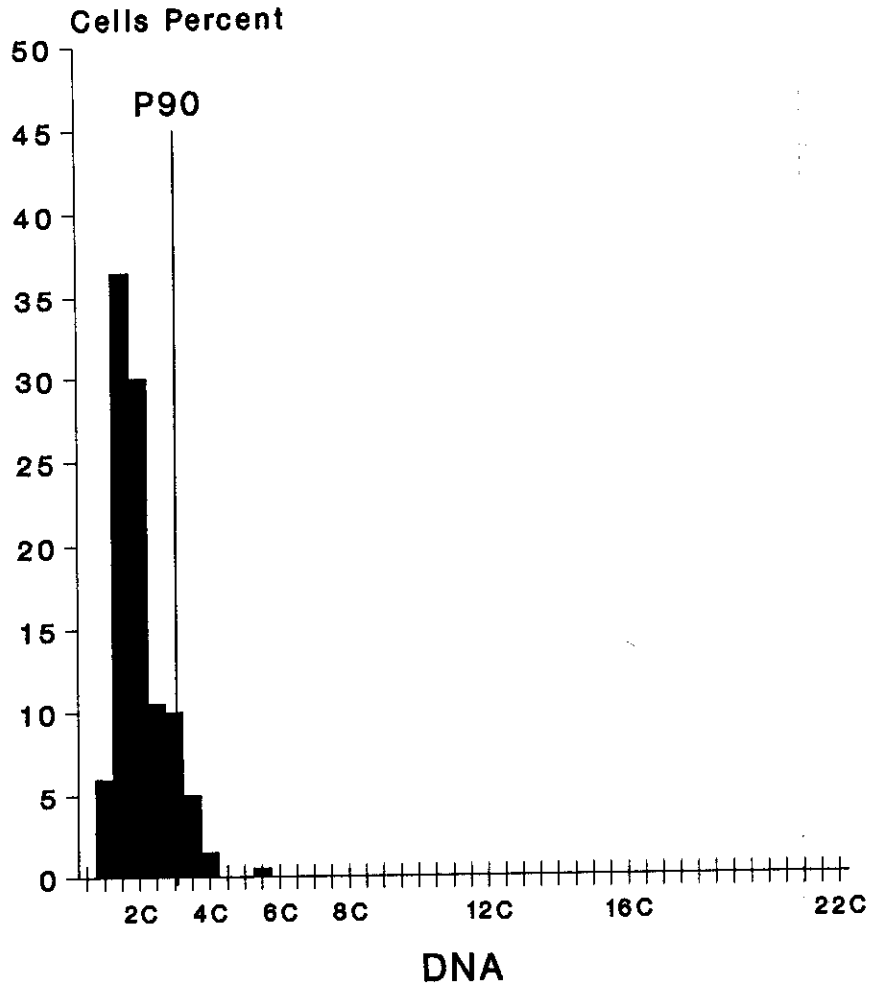


Figure 38. DNA distribution in astrocytoma grade I patients.

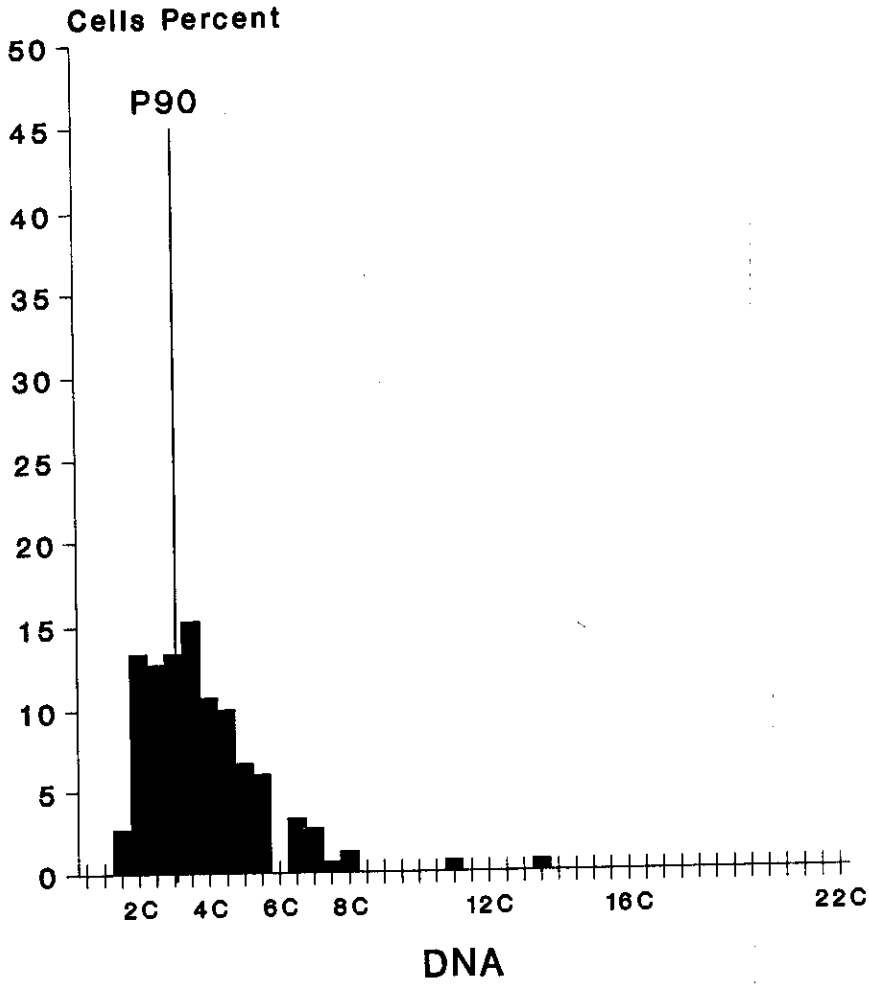


Figure 39. DNA distribution in astrocytoma grade II patients.

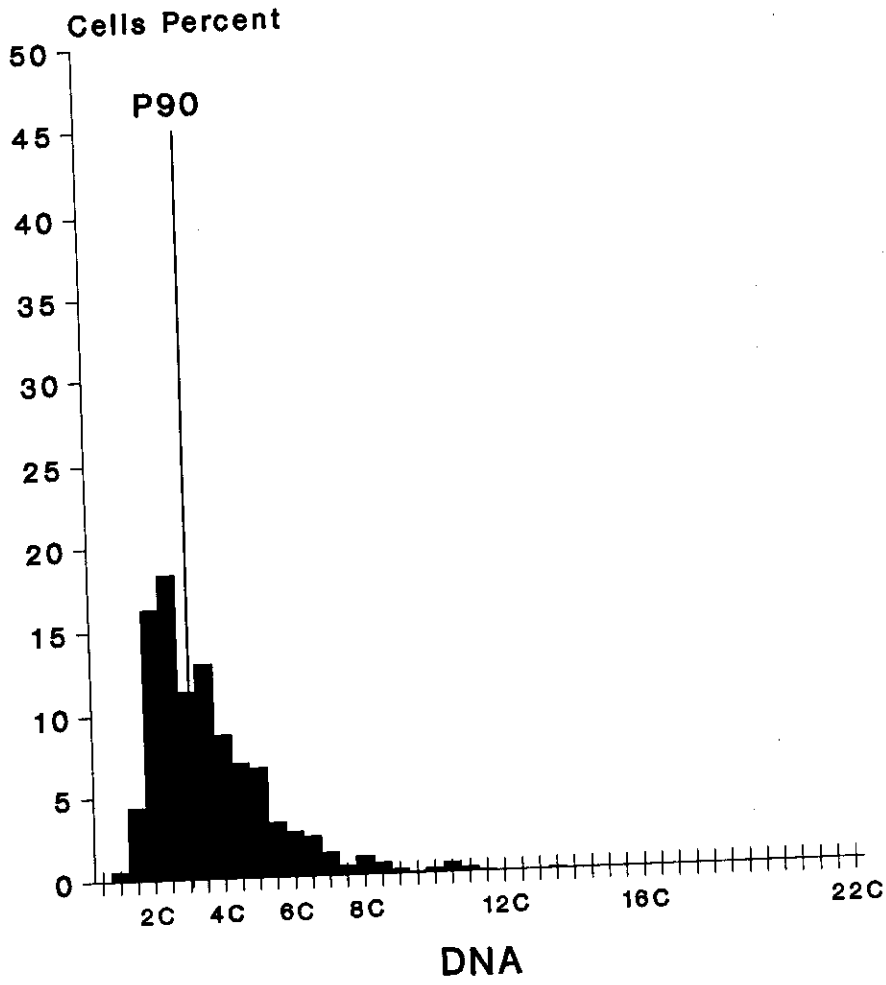


Figure 40. DNA distribution in astrocytoma grade III patients.

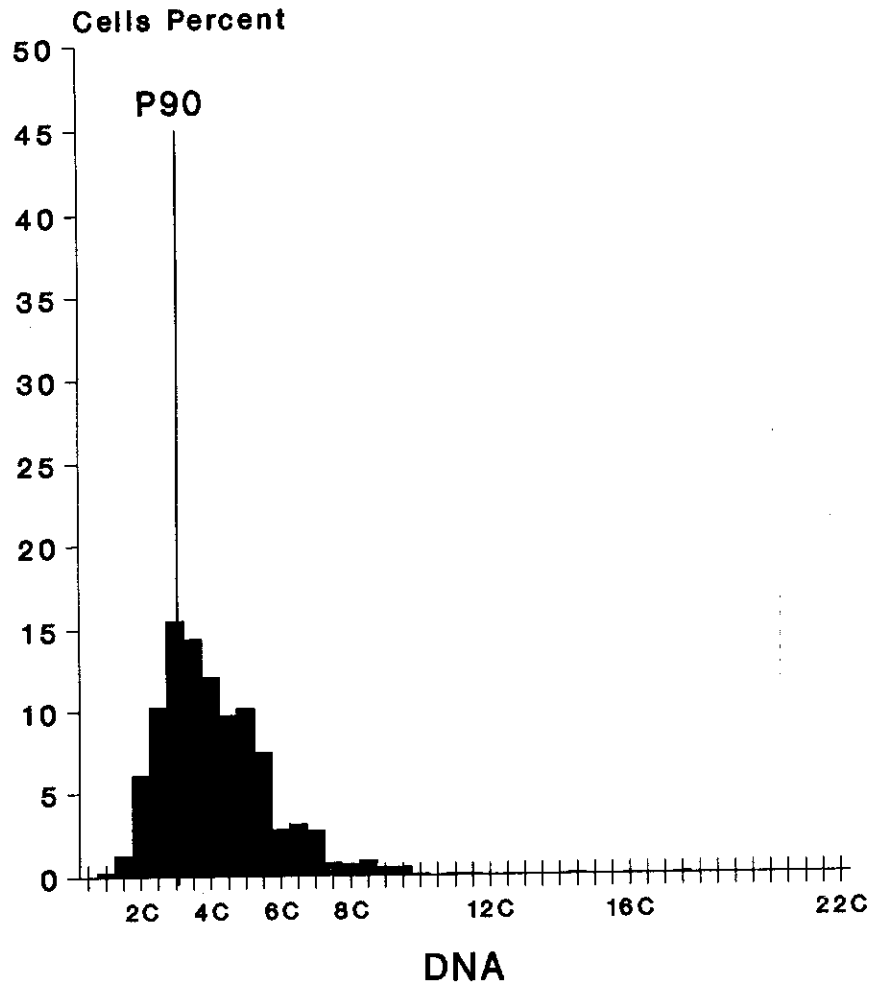


Figure 41. DNA distribution in glioblastoma multiforme patients.

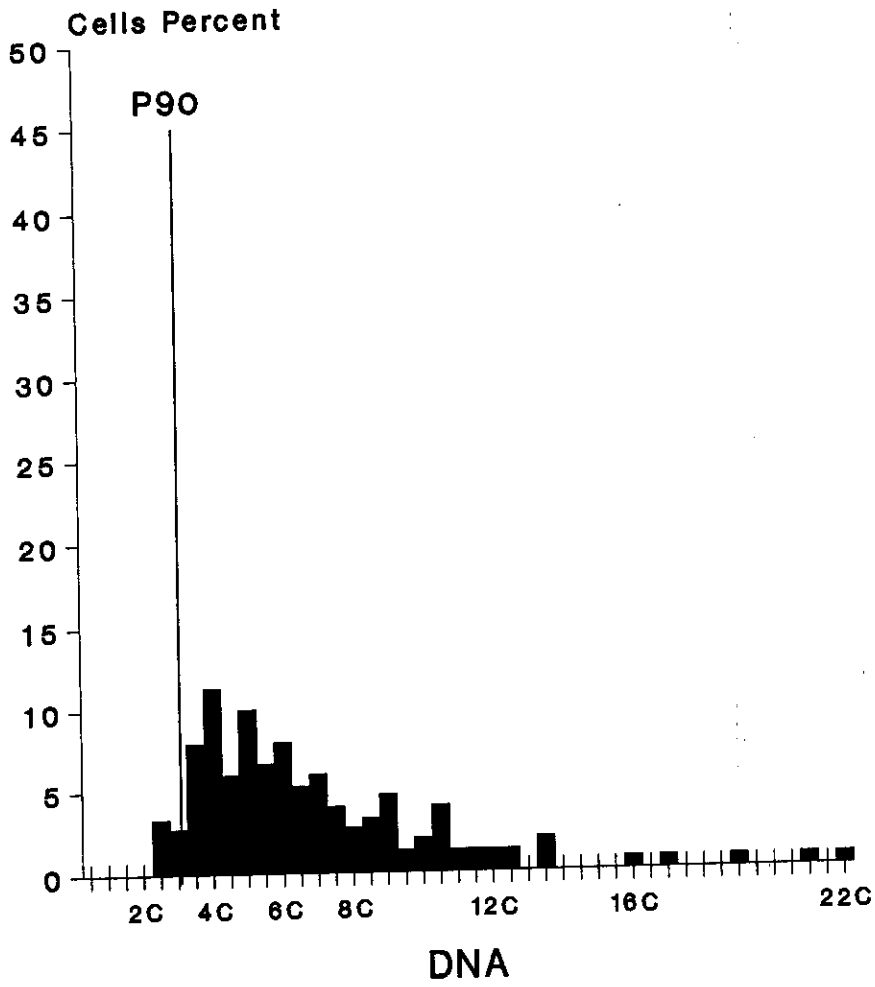


Figure 42. DNA distribution in ependymoma GIII patients.

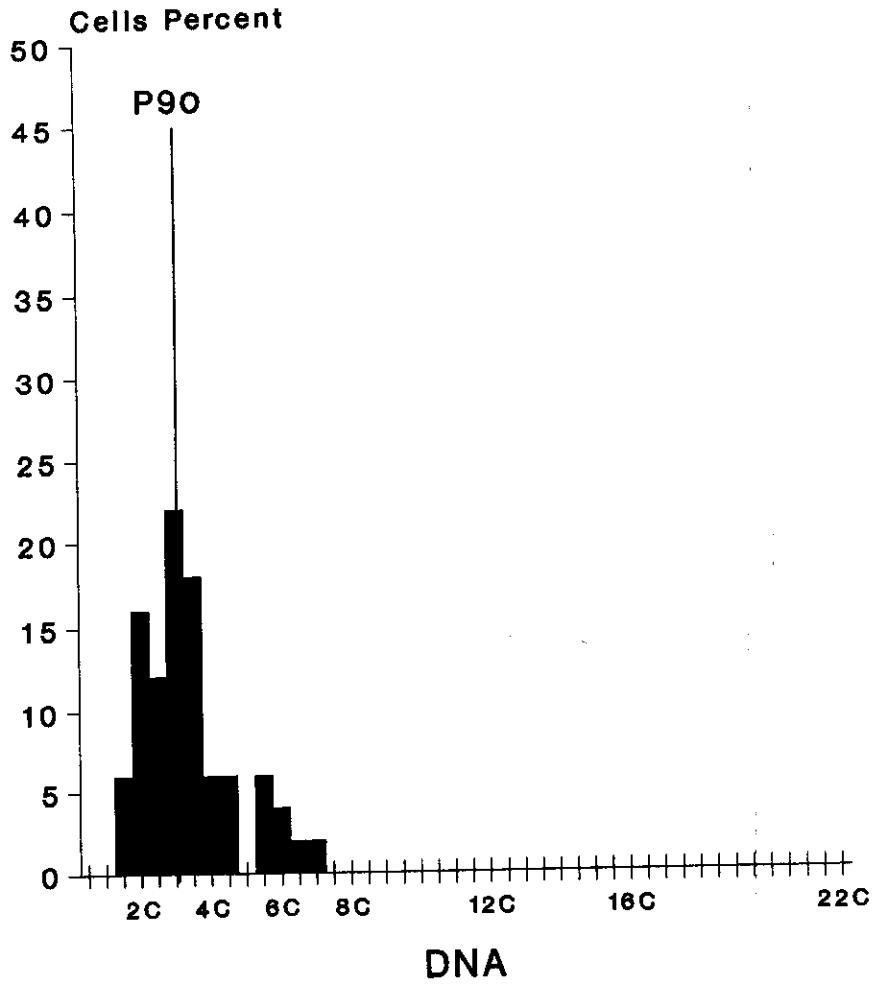


Figure 43. DNA distribution in medullo blastoma patients.

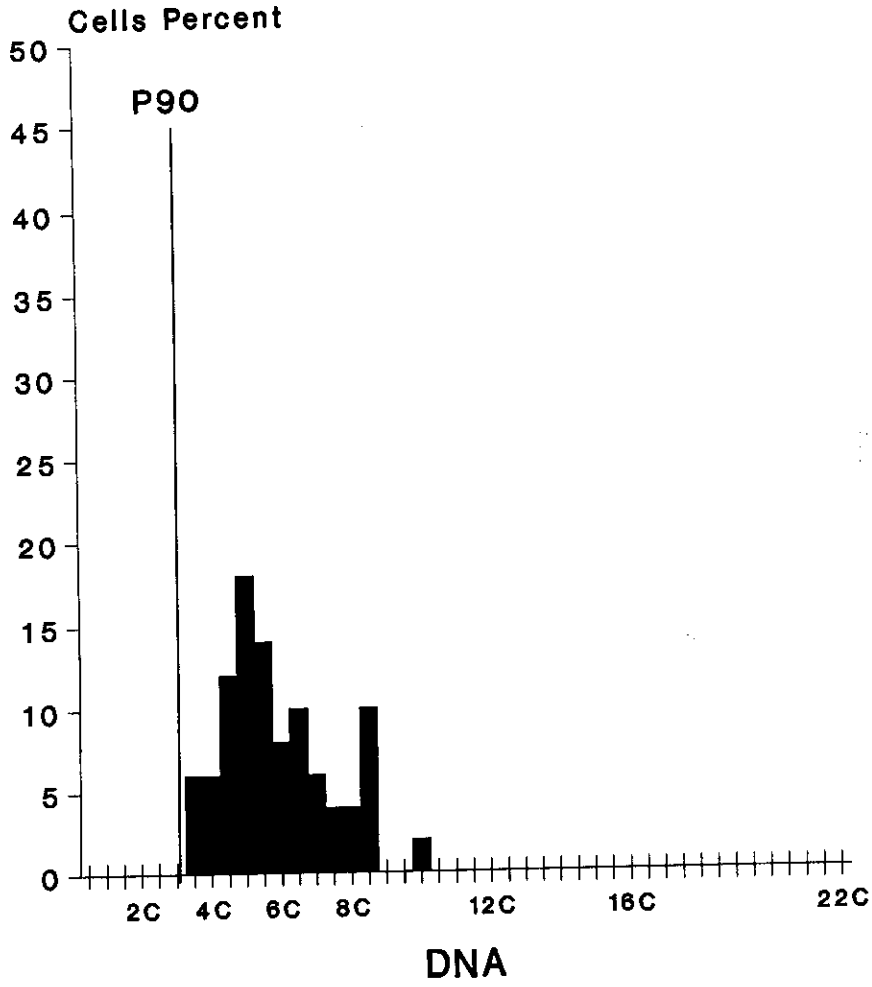


Figure 44. DNA distribution in glio-sarcoma patients.

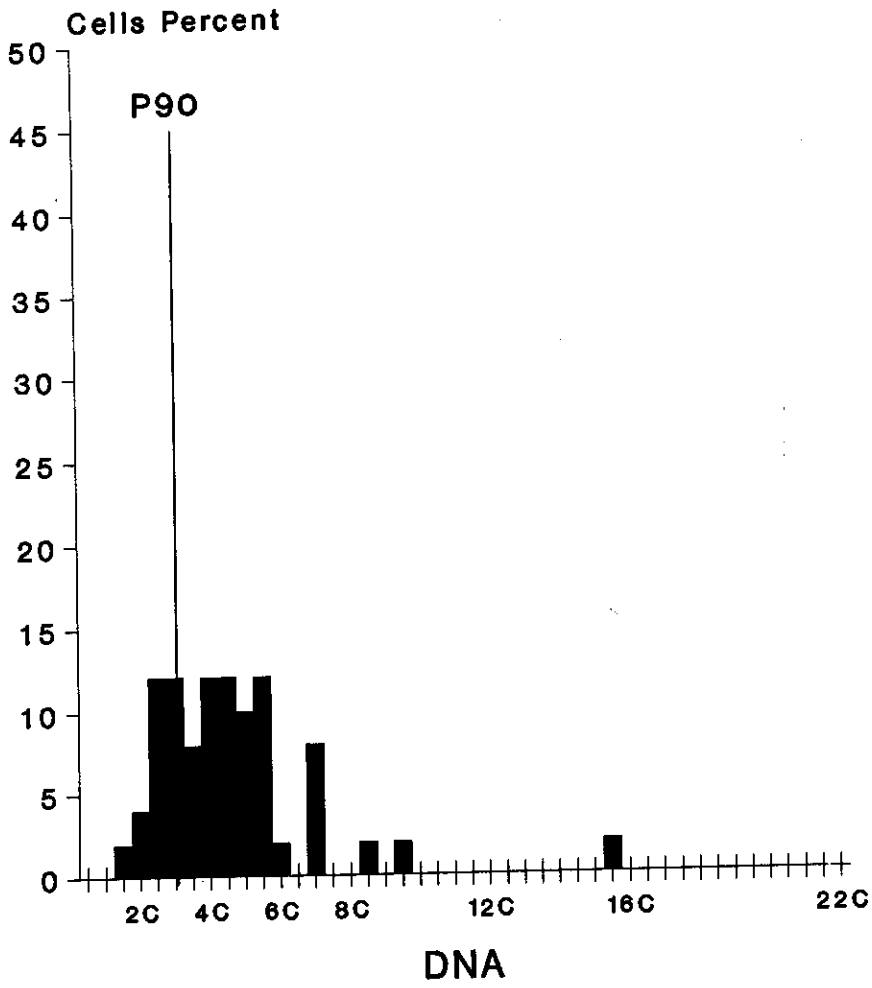


Figure 45. DNA distribution in choroid plexus papilloma patients.

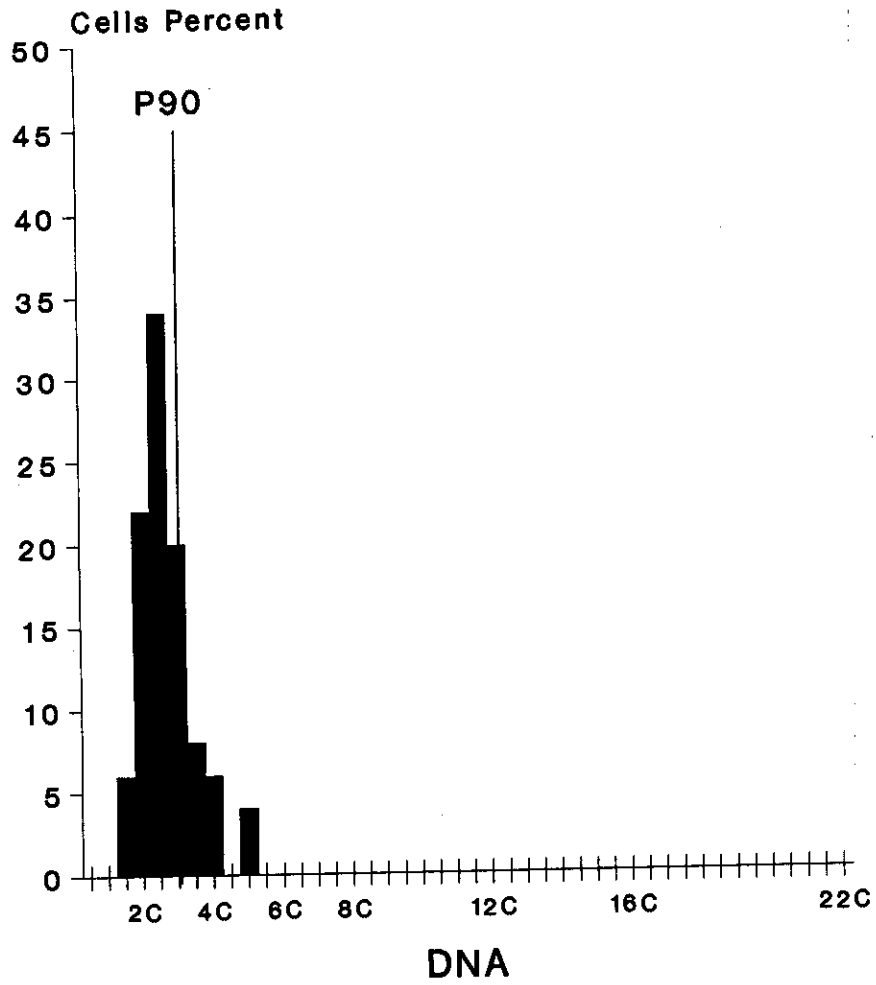
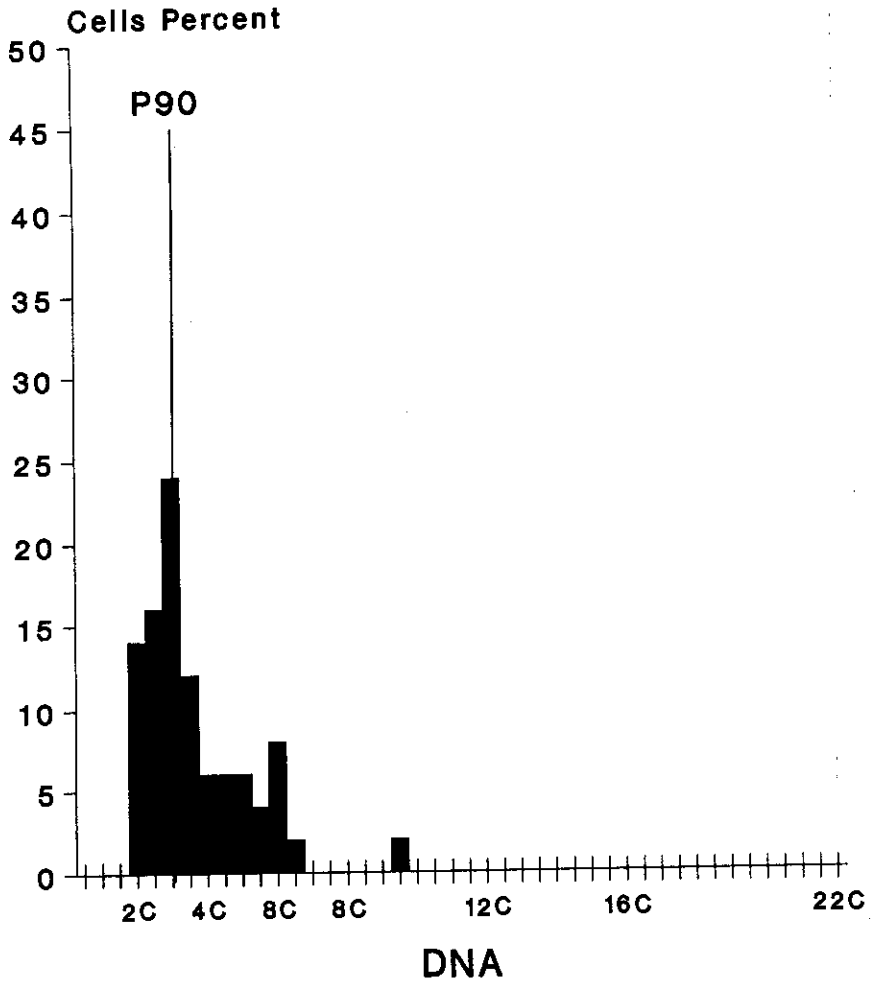


Figure 46. DNA distribution in oligodendroglioma patients.



## Ploidy

The forty cases of gliomas were divided into tetra aneuploidy and non tetra aneuploidy , this ploidy classification was compared to the pathological diagnosis of gliomas divided as low and high grades. ( **Table 16** )

A highly significant difference was encountered (P . = 0.018) with the tetraploid pattern mostly encountered in high grade gliomas

***Correlation between Ki67 labelling index and DNA content :***

The result of correlation shows that Ki67 labelling index and DNA content had poorly significant positive correlation Fig (47)

***Survival data :***

The duration of follow up of the patients ranged from 14-25 months except for two patients who died immediately after operations . The time lapsed before recurrence ranged from 3-16 months . The medulloblastoma case was the first case died after 3 months from the date of operation .The gliosarcoma was the first recurrent case after 3 months .

The recurrence rate in high grade gliomas (astrocytoma grade III , glioblstoma multiforme , medulloblastoma and gliosarcoma was higher than that in low grade glioms (**Table 17** ) .

*Statistical Analysis of ploidy.*

The 5 th percentile ( median P 50) of the DNA values of the control group was detrmined according to the technique of Kreicbergs et al ( 1982) . The 9 th percentile ( P 90) of the DNA value of the control group was calculated . This was used as an upper limit of the dibloid level . To detrmine whether a tumor cell population was diffrent from the corresponding control cell population with respect to ploidy , the percentage of cells with DNA values higher than P 90 was calculated using a Chi square test . Tumors were considerd aneuploid when 24 % of the cells exceeded P 90 of the normal control .

Histograms for control group and glioma group were prepared after estimation of DNA content and determination of the diploid value of the control group . ( 2 c ) .

The DNA distribution patterns were divided into non tetra aneuploidy and tetra aneuploidy according to the frequency of aneuploid cell population . When the frequency of cells with more than 6 c was less than 10 % , the tumor was defined as non tetra aneuploidy ; when it exceeded 10 % , the tumor was defined as tetra aneuploidy .

## Statistical Methods :

The Statistical Analysis System ( SAS ) was used data management and analysis and Harvard Graphics package was used for the figures .

Quantitative data were summarized as means and standard deviations. Qualitative data were summarized as proportions . Comparisons between means of two groups was done using Student's T-test or Mann-Witney test , a nonparametric equivalent of Student's T-test for small sample . When comparing more than two groups , a one way analysis of variance ( ANOVA ) or an equivalent of it for small sample size , Kruskal-Wallis test , was performed . The analysis of variance ( ANOVA ) which is a generalization of the T-test and appropriate for the comparison of the means for any number of groups ( Armitage and Berry , 1987 ) .

Comparisons between proportions were done by the Chi-square test or Fisher's exact test for small sample size ( Armitage and Berry , 1987 ) .

Spearman's correlation coefficients was used to measure the strength of the relationship between two quantitative variables .

All reported P-values are two-sided . P-values $\leq 0.05$ was considered significant .

Table 16. Pathological diagnosis and ploidy in intracranial gliomas.

| Diagnosis[a] | Tetra-aneuploidy | Non tetra-aneuploidy | Total |
|---|---|---|---|
| Low glioma | 7(33.3) | 12(66.7) | 19(100.0) |
| High glioma | 16(76.2) | 5(23.8) | 21(100.0) |
| Total | 23(57.5) | 17(42.5) | 40(100.0) |

P-value[b] = 0.018

a Diagnosis according to WHO classification:
Low = Grade I and II
High = Grade III and IV

b P-value for the difference between the groups, p-value <0.05 is considered significant, N.S.= Not significant.

Figure 47. Relationship between KI index and DNA content in brain tumor patients.

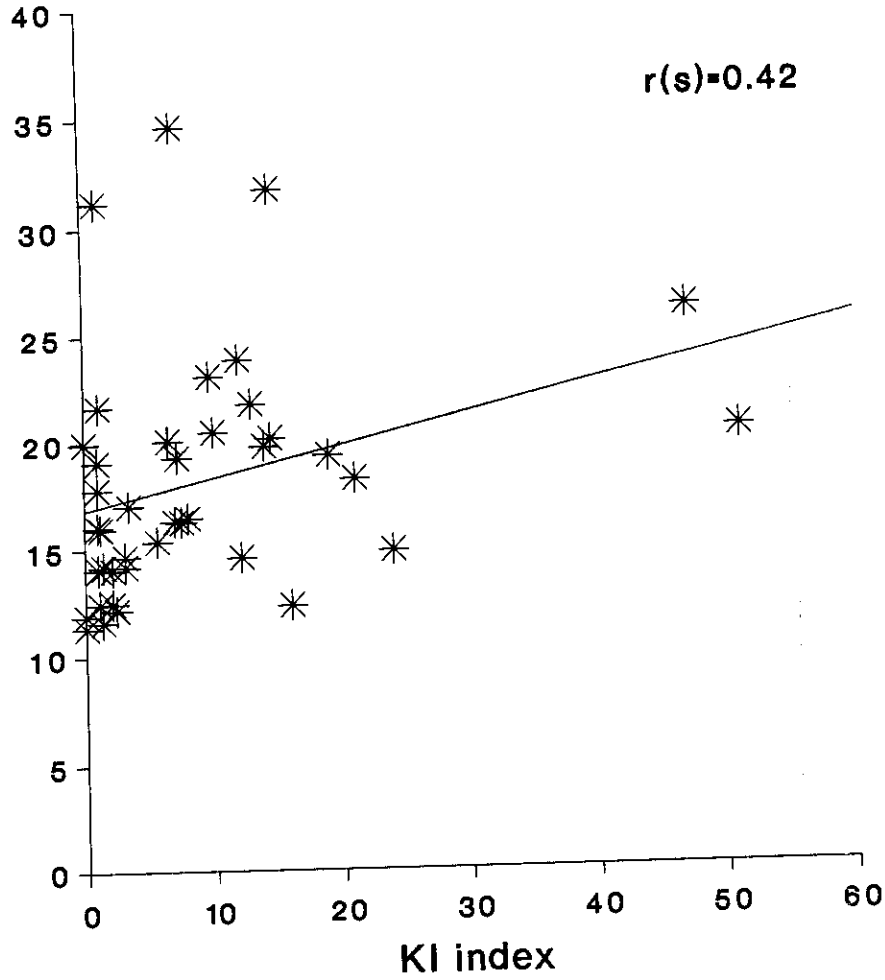


Table (17) : Recurrence rate in gliomas.

| Diagnosis | No of cases | No of recurrences | |
|---|---|---|---|
| Low grade gliomas | 19 | 4 | 21.5% |
| High grade gliomas | 21 | 8 | 38% |

No direct statistical significant correlation between disease free survival on one hand and lymphocytic infiltration , DNA ploidy or cell types on the other hand was encountered (Figs 48, 49, 50, 51, 52 ).

But in this correlation it may be thought that there is indirect correlation in an indirect way as follows :

* Recurrence rate was higher in high grade gliomas .

* Recurrence rate was higher with high labelling index of Ki67.

* High Ki67 labelling index was associated with high grade tumors .

* Tumors with high T lymphocytic infiltrates show high Ki67 labelling index .

* Tumors with supressor T lymphocytic infiltrates show high Ki 67 labelling index .

* From those we can conclude that . As recurrence rate was higher in high grade tumors & high grade tumor has high Ki-67 labelling index & Ki 67 labelling index has a correlation with high T lymphocytic infiltration especially suppressor, so , there is some indirect correlation between recurrence rate and T lymphocytic infiltration .

As regard the DNA content which showed high significant correlation with grade of tumors and from the positive high correlation between grade and Ki-67 labelling index , a good positive correlation may be present in an indirect way between Ki-67 labelling index and DNA content .At the same time a good correlation between DNA ploidy and recurrence rate was present .

The same result may be observed regarding the predominance of small anaplastic cells in high grade gliomas with also high Ki-67 labelling index .

From these observations , an indirect correlation between all these variables may be present .

Figure 48. Disease free survival in 40 patients with intracranial gliomas in relation to CD3.

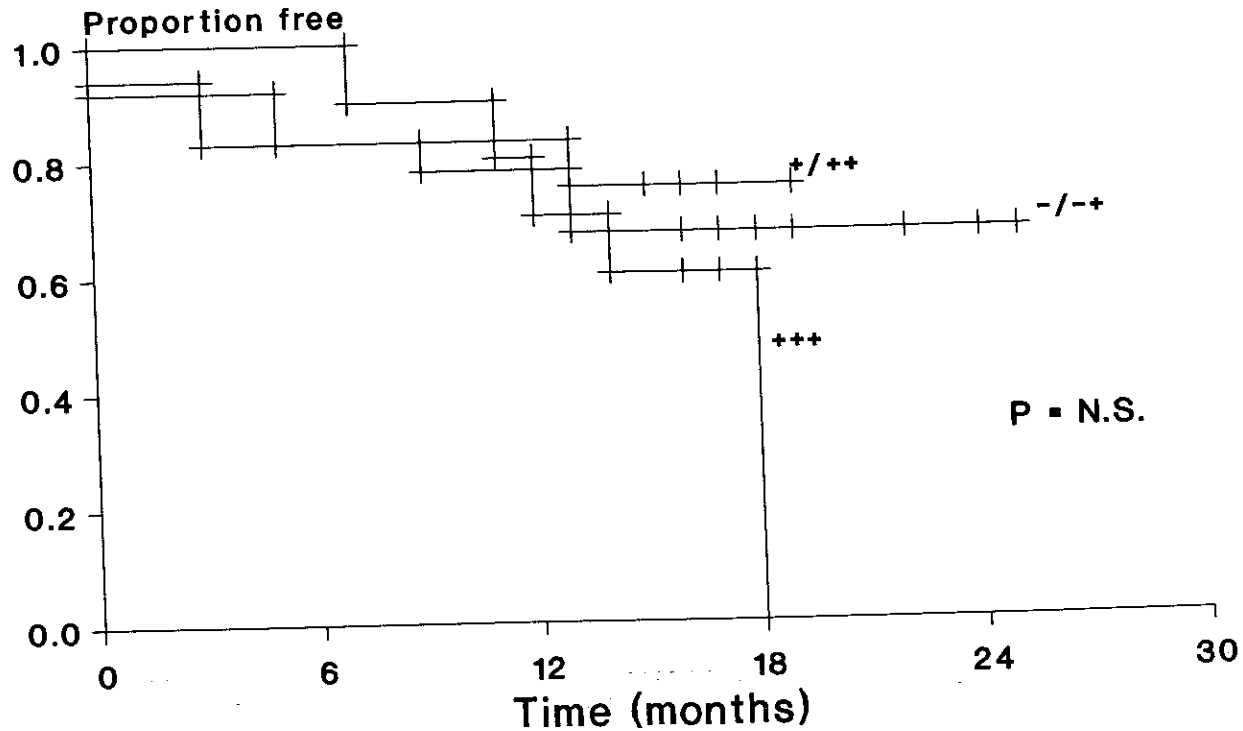


Figure 49. Disease free survival in 40 patients with intracranial gliomas in relation to CD4.

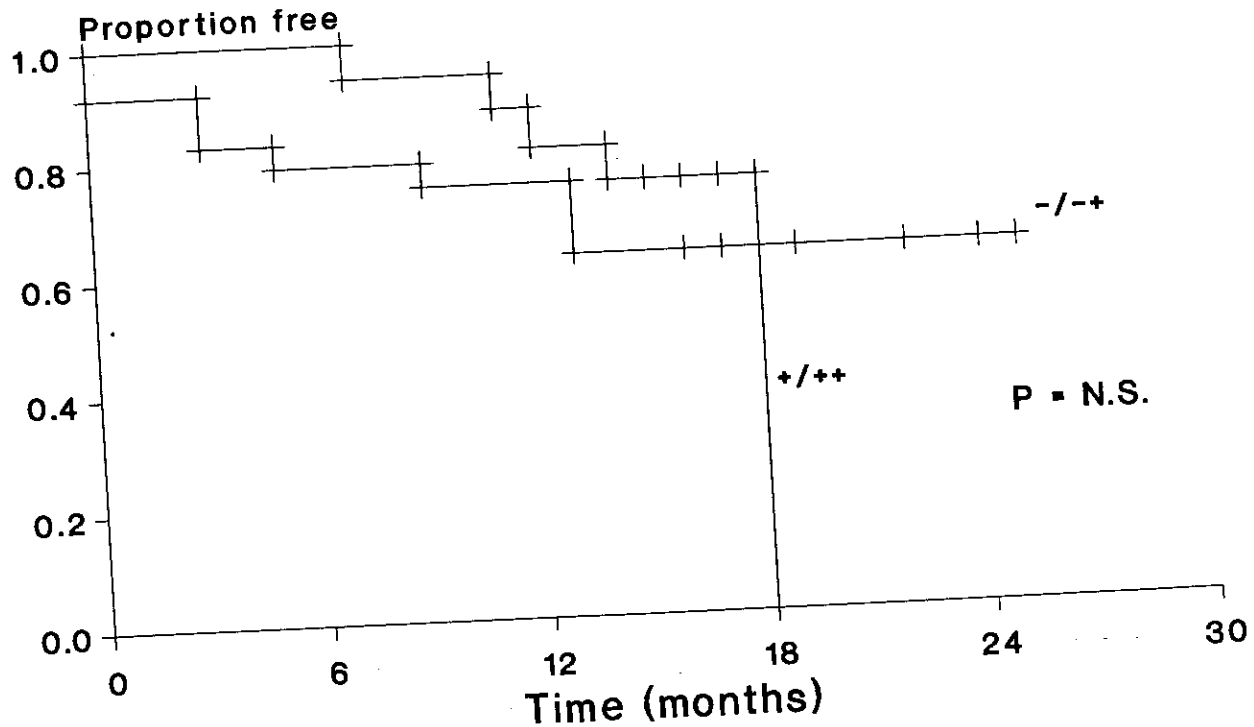


Figure 50. Disease free survival in 40 patients with intracranial gliomas in relation to CD8.

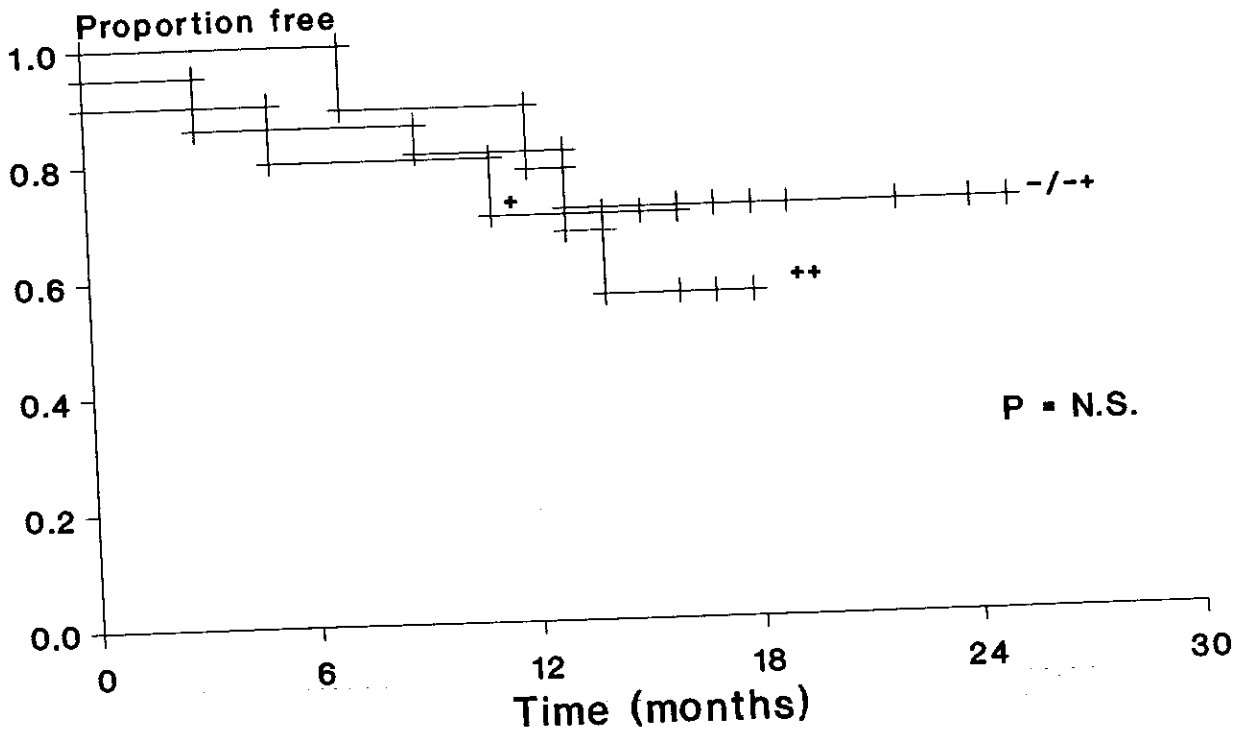


Figure 51. Disease free survival in 40 with intracranial gliomas in relation to tetraploidy status.

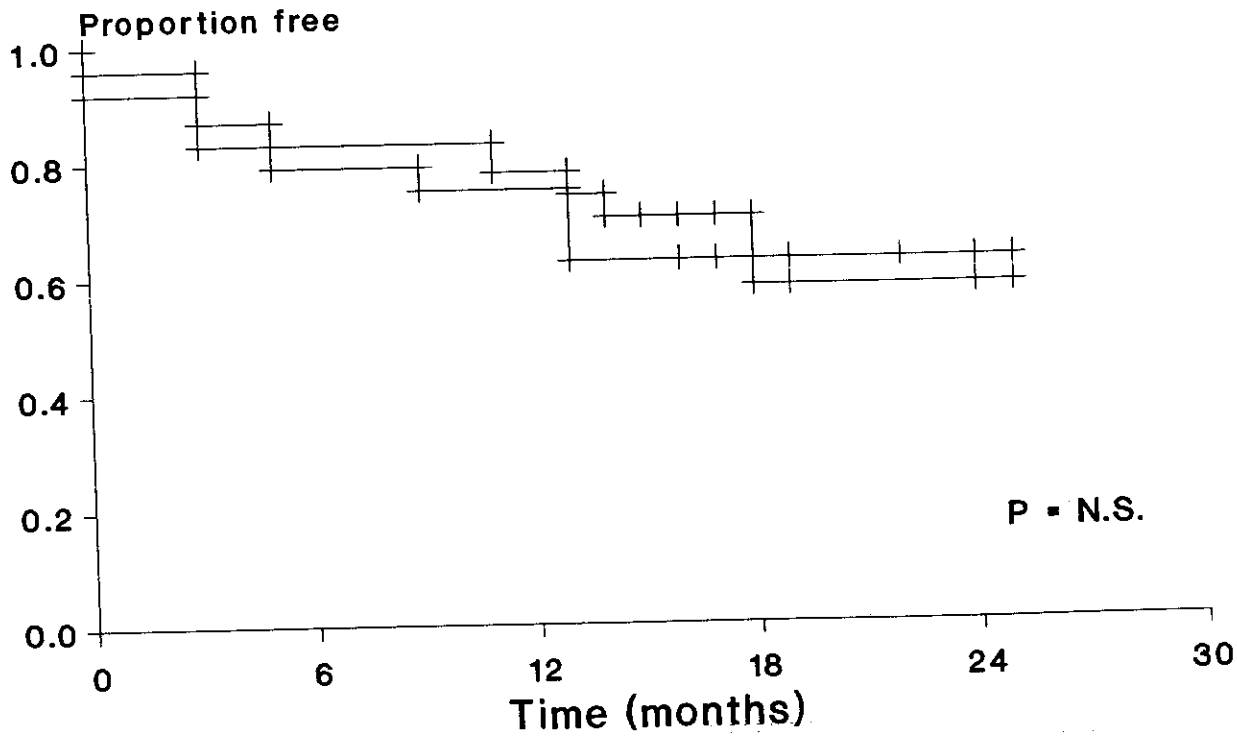


Figure 52. Disease free survival in 40 patients with intracranial gliomas by different cell types.