

Topoisomerase II alpha and p27; alternative markers to decide on the proliferation capacity of astrocytic tumors

Topoisomerase II alpha and p27; astrositik tümörlerin proliferasyon kapasitesini belirlemede alternatif immünohistokimyasal belirleyiciler

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ABSTRACT

Proliferation capacity is an important parameter which enables us to predict the prognosis of tumours. Many immunohistochemical studies were conducted to search the relation of proliferative capacity with different clinical and histological parameters. Ki67 is a well known immunohistochemical marker of proliferation and some standard values have been established for Ki67 indexes of astrocytic tumours. For this purpose, considering the roles of proteins in cell cycle, some immunohistochemical markers other than Ki67 can be suggested. In this study, expressions of topoisomerase II alpha, a nuclear protein in mitotically active cells and p27, a cyclin-dependent kinase inhibitor, were correlated with the grade and Ki67 indexes of 67 astrocytomas. Topoisomerase expressions demonstrated an increase with increasing grade. It also followed a parallel curve with Ki67. On the other hand, p27 had an inverse correlation with the tumor grade. The cut-off value for topoisomerase was calculated to vary 3.5% between low and high grade tumours. No cut-off value could be obtained for p27.

Key words: Ki67, topoisomerase, p27, astrocytoma

ÖZET

Proliferasyon kapasitesi, tümörün prognozu üzerinde yorum yapabilmeyi sağlayan önemli bir veridir. Ayrıca proliferatif indeksin, klinik ve histopatolojik farklı parametreler ile korelasyonunu araştıran çok sayıda çalışma vardır. Proliferatif aktiviteyi belirleme amacı ile en sık kullanılan ve yapılan çalışmalarla az çok standart eşik değerlere ulaşılmış olan immünohistokimyasal belirleyici Ki67'dir. Bu amaçla, özellikle hücre siklusundaki rolleri göz önüne alındığında Ki67 dışında başka immünohistokimyasal belirleyiciler de önerilebilir. Bu çalışmada Ki67 gibi, mitotik aktivite gösteren hücrelerde eksprese olan topoizomerez II alfa ve bir siklin bağımlı kinaz inhibitörü olan p27'nin ekspresyonları ile 67 astrositik tümörde histopatolojik derece ve Ki67 indeksleri arasındaki ilişki araştırılmıştır. Topoizomerez'in Ki67 ile paralel artış gösterdiği ve artan histolojik derece ile anlamlı olarak ilişkili olduğu saptanmıştır. p27 ise Ki67 ve topoizomerez II alfa ile ters bir orantı göstermiştir. Yüksek dereceli tümörler ve düşük dereceli tümörler arasında topoizomerez için %3.5 olan bir eşik değere ulaşılmış, ancak p27'de bir eşik değeri edilememiştir.

Anahtar sözcükler: Ki67, topoizomerez, p27, astrositom

INTRODUCTION

Although grading of astrocytomas is not ba-

sed on the proliferation index, it is still quite important in diagnosis and clinical follow-up. Many studies had been carried out to define the markers of proliferation and their relation with prognosis. Among these markers, Ki67 is a well-known one and took its place in routine practice. In this study, we aimed to investigate

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the reliability of two other markers in defining the proliferation capacity in astrocytomas, as compared with Ki67 values.

Ki67 and topoisomerase II alpha are the proteins expressed in mitotically active cells. Biologically, Ki67 and topoisomerase II alpha proteins are expressed in cells entering the cell-cycle so they are the markers of proliferation. On the other hand, p27 represents a contrary process. p27 is a cyclin-dependent kinase inhibitor and works to prevent mitosis. So in actively proliferating cells p27 is not expressed; p27 positive cells are the ones at G phase of the cell cycle. Thus knowing their biological role, Ki 67 and topoisomerase II alpha are expected to be expressed at high levels in tumors having high proliferative activity whereas p27 is not (1,2).

MATERIALS and METHODS

Histologic sections of 67 astrocytic tumors were retrospectively evaluated. The HE stained sections from formalin fixed-paraffin embedded tissues of operation materials were reexamined under light microscopy by two pathologists. On the basis of classification proposed by WHO (2007), nuclear atypia, mitosis, necrosis and endothelial proliferation were searched in each case (3). According to this grading system: Grade I, pilocytic astrocytoma (PA); Grade II, diffuse astrocytoma (DA); Grade III, anaplastic astrocytoma (AA); Grade IV, glioblastome multiforme (GB). Among 67 cases; 11 were PA, 29 were DA, 11 were AA and 16 were GB. Grade I and II tumors are examined in low grade category and high grade category was composed of grade III and IV tumors.

Immunohistochemical staining with Ki67, topoisomerase II alpha and p27 were applied for each case. For immunohistochemical staining; immunoperoxidase-AEC substrate chromogen system was used. For each case, 3 sections of 4µm thickness were obtained from paraffin embedded formalin fixed tissues. After deparaffinization, antigen retrieval was carried out in mic-

rowave oven for 30 minutes. The sections were let to cool in room temperatures, then put in H₂O₂ and washed by PBS. Ki67 (Clone MIB1, Dako), topoisomerase II alpha (clone JH2.7, Neomarker) and p27 (clone DCS-72.F6, Neomarker) antibodies were applied separately to the slides. Dilution rates and incubation periods were arranged according to datasheets. Slides were incubated with primary antibody for 1.5 hours. After incubation with primary antibody, the slides were washed with PBS. Then secondary antibody and AEC chromogen were applied. Mayer Hematoxylen was used as counter stain. For Ki67 and topoisomerase II alpha, the control slides were prepared from reactive lymphoid tissues. For p27 slides from infiltrative ductal carcinoma of the breast are used as control.

Slides stained with Ki67, topoisomerase II alpha and p27 markers were examined under light microscope. In evaluating Ki67, topoisomerase II alpha and p27, positively stained nuclei were counted in the most intensely stained areas of each slide. During immunohistochemical evaluation, intensity of nuclear staining was neglected. In most intensely stained area, 500 cells were counted and the ratio of positively stained nuclei to the 500 cells was accepted as labelling index of a given antibody (Figures 1,2,3).

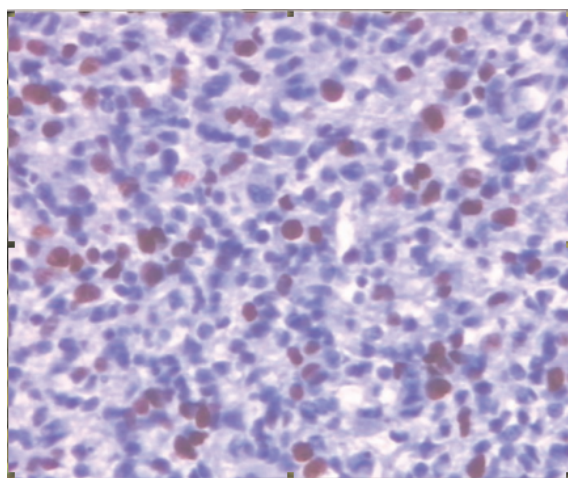


Figure 1. Nuclear staining with Ki67 in a grade II astrocytoma (x200).

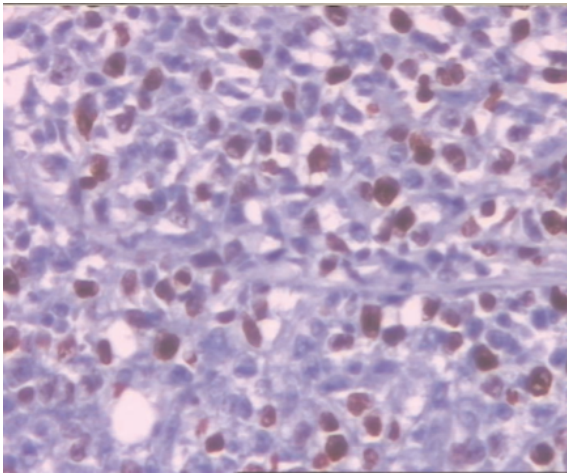


Figure 2. Nuclear staining with Topoisomerase II alpha in a high grade astrocytoma with restricted regions of oligodendroglial morphology (x400).

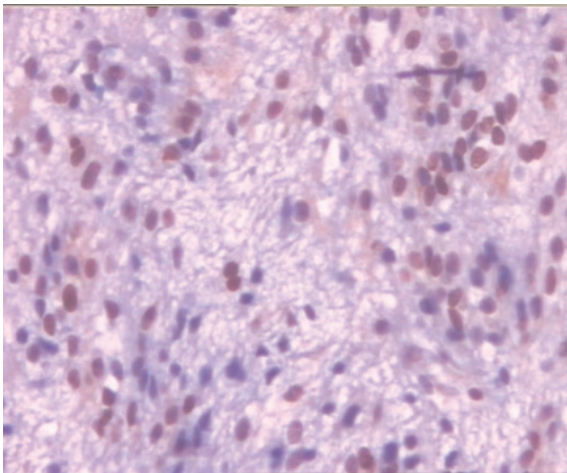


Figure 3. p27 staining in a case of grade II astrocytoma (x200).

For each antibody, the difference between low (grade I-II) and high grade (grade III-IV) tumors was statistically searched. In addition, percentage values for each variable (patient age, histologic grade, labelling indexes of antibodies) were calculated. The degree of correlation between labelling indexes of Ki67, topoisomerase II alpha and p27 was searched by Pearson correlation coefficient. The cut-off points for Ki67, topoisomerase II alpha and p27 were calculated by ROC curve.

RESULTS

Statistically significant difference was pre-

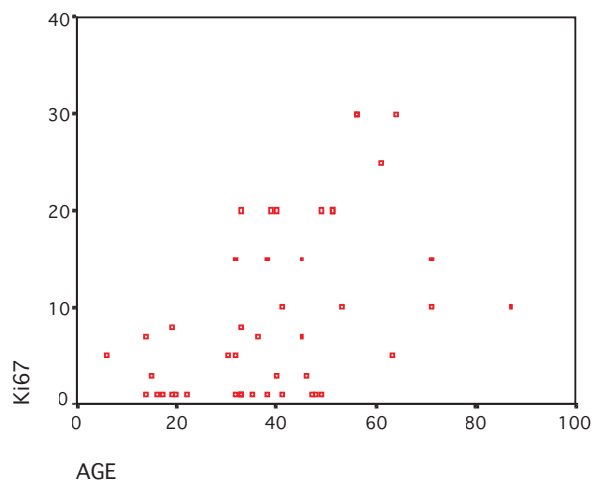
sent between histologic grades for age ($p < 0.01$). A statistically meaningful positive increase in Ki67 labelling indexes was detected between low and high grade tumors ($R = 0.557$, $p < 0.05$). In the same manner, topoisomerase expressions were also correlated with tumor grade ($R = 0.434$, $p < 0.05$). On the other hand p27 demonstrated an inverse correlation ($R = -0.341$, $p < 0.05$) (Table 1).

Table 1. Expression rates of Ki67, Topoisomerase II alpha and p27 according to grades.

Tumor Grade	Antibody	Minimum value	Maximum value	Mean Expression
I	Ki67	1	5	2.33
	Topo	1	3	1.67
	P27	15	90	51.67
II	Ki67	1	30	5.52
	Topo	1	50	7.52
	P27	1	100	43.52
III	Ki67	2	30	14.50
	Topo	3	25	11.37
	P27	3	90	16.50
IV	Ki67	2	30	15.15
	Topo	1	30	10.38
	P27	1	80	16.23

When patients' ages were considered, the sole statistical correlation was detected with Ki67 expressions ($R = 0.509$, $p < 0.01$) (Figure 4).

The relation between the labelling indexes of 3 antibodies was searched by Pearson correlation coefficient; Ki67 and topoisomerase II alpha followed parallel curves of expression,



while p27 expressions were completely opposite to them. For each antibody, the cut off points that discriminate between low and high grade astrocytic tumors, were calculated by ROC curve. A cut-off point of 4.5% was reached for Ki67 antibody with 0.80 and 0.72; specificity and sensitivity, respectively. Positive negative predictive values were 0.76 and 0.76, respectively. The cut-off point for topoisomerase was calculated as 3.5%, with specificity and sensitivity rates of 0.76 and 0.72, respectively. The positive and negative predictive values were 0.72 and 0.76, respectively. No cut-off value was obtained for p27.

DISCUSSION

Ki67/MIB1 labelling index was searched in malignancies of many organs such as breast, cervix, lung, lymph node and bladder as an indicator of proliferation capacity (4). In order to define the relation between Ki67 expression and the prognostic parameters, studies have been performed in glial neoplasms. By these studies, some cut-off point values have been calculated. In a study performed in astrocytomas by Zuber et al, a statistical correlation between the tumor grade and Ki67 expression was documented. They found Ki67 indices to be 0-4.5% in low grade, 0.7-7.4% in anaplastic astrocytomas and in 1.7-32.2% of glioblastome multiforme specimens. In the same study, they also proposed survival period of 40 weeks for patients with tumors having Ki67 index lower than 2.5% (5).

In a study performed by Parkins et al, a statistically meaningful increase in Ki67 indices was demonstrated with increasing tumor grade. The other studies reached the similar results and Ki67 antibody seemed to be gaining importance especially in predicting the survival (6).

In this study, a statistically meaningful correlation between the tumor grade and Ki67 labelling indices was demonstrated. This finding was in agreement with the literature. The mean Ki67 indices were 2.33% in grade I,

5.52% in grade II, 14.50% in grade III and 15.15% in grade IV tumors, respectively (7). ROC curve demonstrated a cut -off point value of 7.5% between low and high grade tumors.

Topoisomerase II alpha is an antibody proposed to be used to detect proliferative capacity in glial neoplasms. In a study of Holden et al., topoisomerase II alpha expression was found to increase with increasing tumor grade. In the same study, topoisomerase II alpha index was 2.1% in grade I and 39.5% in grade IV astrocytomas. Also a linear correlation between Ki67 and topoisomerase II alpha was demonstrated. In a study of Korshunov et al. among ependymomas, topoisomerase II alpha indices were found to increase with increasing tumor grade and a negative correlation between topoisomerase index and survival rate was documented (8,9). In the same study, a cut-off point value of 5% was accepted as the discriminative level between long and short survival times. In a study by Burstmann and Naude, topoisomerase II alpha, Ki67, PCNA and AgNOR values were compared and a correlation between topoisomerase II alpha and Ki67 was demonstrated. In another study performed by Bredel et al., high topoisomerase II alpha index values were found to be associated with anaplastic oligodendrogliomas but no association with survival was obtained. Korkolopoulou et al., found topoisomerase II alpha, as a single independent predictor of disease-free survival which provided useful prognostic information (10,11,12).

In our study, mean topoisomerase II alpha index in pilocytic astrocytomas was 1.67% and in glioblastome multiforme cases it was 10.38%. A cut-off point value of 3.5% was reached. When our findings were compared with the literature, they appeared to be lower than those found in the literature. Larger series would be important in detecting more reproducible results. When topoisomerase and Ki67 indices were compared, a statistically meaningful linear correlation was obtained. In lower grade tumors, mean Ki67 index was 5.32%, whereas mean to-

poisomerase index was 7.08% and in high grade tumors, they were 14.27% and 10.32%, respectively.

p27 is a cyclin dependent kinase inhibitor, lately it gained importance in glial neoplasms because of its role in predicting the cell cycle abnormalities. In a study performed by Nakayama et al., the brain tissue was mentioned to be quite rich of p27 protein. Tikko et al. also demonstrated p27 upregulation in glial precursor cells differentiating towards astrocytes (10). Korshunov et al. calculated mean p27 index as 41.3% in low grade ependymomas and 15.7% in high grade ones and no correlation was demonstrated between p27 expression with the grade and prognosis in meningiomas (13). In this study, a statistically significant negative correlation between tumor grade and p27 expression was demonstrated. No cut-off point could be attained for p27. Also in review of the literature, no such value was documented. Mizumatsu et al., on the other hand, demonstrated that p27 was inversely correlated with tumor grade and positively related to favorable outcome of patients with astrocytomas. So they suggested p27/Kip1 as a candidate for prognostic factor for astrocytomas (14,15).

In this study, Ki67 and topoisomerase expressions were found to correlate with the tumor grade. In addition, a statistically meaningful correlation was detected between expression rates of these two antibodies. Cut-off point values were calculated for Ki67 and topoisomerase II antibodies as 4.5% and 3.5%, respectively.

In this study, p27 was also shown to be an important immune marker to define tumor behaviour. It has a negative effect on tumor development and so its expression rates decrease as the grade of tumor increases.

We think that using these markers could help in planning treatment and predicting the prognosis in glial neoplasias. Further studies will establish standart values for routine use.

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