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**Research Article**

# Prognostic Significance of P53 Protein, Cyclin D1 and Ki-67 in Pineal Parenchymal Tumours

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## Summary

Pineal parenchymal tumours are very rare consisting less than 0.1% of all central nervous system tumours. The aim of this study was to investigate the prognostic significance of Ki-67, cyclin D1 and p53 protein expressions in pineal parenchymal tumours. Ten pineal parenchymal tumours were investigated: 2 pineocytomas, 5 pineal parenchymal tumour of intermediate differentiation and 3 pineoblastomas . Immunohistochemical staining was performed using avidin-biotin-peroxidase method. The number of mitoses ranged from 0-25 in pineal parenchymal tumour of intermediate differentiation and 2-30 in pineoblastomas. In general, Ki-67 was found between 0-53.5 %, cyclin D1 was found between 0-40%, p53 was found between 0-4% in pineal parenchymal tumors. In pineal parenchymal tumour of intermediate differentiation, positive staining rates for Ki-67, cyclin D1 and p53 were found as 4-17.5%, 2-30% and 0-4%, respectively. In pineoblastomas, Ki-67, cyclin D1 and p53 were found as 9.2-53.5%, 5-40% and 1-2%, respectively. The number of mitoses was not significant for the prognosis in pineal parenchymal tumors. The very low level of p53 protein made us think that it does not play an active role in the development of these tumours.

**Key words:** Pineal parenchymal tumors, Ki-67, p53, Cyclin D1

## Pineal Parankimal Tümörlerde P53 Protein,Siklin D1 Ve Ki-67'nin Prognostik Önemi

## Özet

Pineal parankimal tümörler çok seyrek görülürler,tüm santral sinir sistemi tümörlerinin % 0.1 den daha azını oluştururlar. Bu çalışmanın amacı pineal parankimal tümörlerde Ki-67, siklin D1ve p53 protein ekspresyonlarının prognostik önemini araştırmaktır. On pineal parankimal tümor araştırıldı: 2 pineositoma, 5 intermediyer differansiyasyon gösteren pineal parankimal tümor ve 3 pineoblastom. İmmunhistokimyasal boyamaları avidin –biotin peroksidaz yöntemi kullanılarak yapıldı. Mitoz sayısı intermediyer differansiyasyon gösteren pineal parankimal tümorlerde 0-25 ve pineoblastomlarda 2-30 arasında değişmekteydi. Genelde pineal parankimal tümörlerde Ki-67 yüzde 0-53.5, siklin D1 % 0-40, p53 % 0-4 arasında bulundu. İntermediyer differansiyasyon gösteren pineal parankimal tümorlerde pozitif boyanma oranları sırasıyla Ki-67, siklin D1ve p53 protein % 0.4-17, % 2-30 ve % 0-4 bulundu. Pineoblastomlarda sırasıyla Ki-67, siklin D1ve p53 protein % 9.2-53.5 , % 5-40 ve % 1-2 bulundu. Mitoz sayısı prognoz açısından önemli değildi. Bu tümörlerde çok düşük düzeyde p53 protein varlığı, bu tümörlerin gelişiminde p53 proteinin aktif rol oynamadığını düşündürdü.

**Anahtar Kelimeler:** Pineal parankimal tümor, Ki-67, p53, Siklin D1

## INTRODUCTION

Pineal parenchymal tumours are very rare. They consist less than 0.1% of all central nervous system tumours. Pineal parenchymal tumours make up 15-30% of pineal region tumours in the childhood[10,15]. Because of their rarity, classification of these tumours is controversial. Most recent classification of World Health Organization classified pineal parenchymal tumours as pineocytomas, pineal parenchymal tumour of intermediate differentiation (PPTID), pineoblastomas and papillary tumours of the pineal region. Pineocytoma is histologically classified as grade I, as pineal parenchymal tumour of intermediate differentiation grade II- III, pineoblastoma as grade IV and papillary tumour of the pineal region is classified as grade II[22]. It is not possible to predict the biological behaviour of pineal parenchymal tumours, particularly pineal parenchymal tumour of intermediate differentiation, due to both rarity and lack of specific histological criteria for diagnosis.

Presence of a correlation, if any, between tumour proliferation and clinical prognosis may be determined by proliferation markers. Ki-67 monoclonal antibody has been reported as a reliable marker for proliferative activity in central nervous system tumours[2]. Mutation of p53 has been reported to play role in the development of glial tumours[17-20,33-35]. But there are limited studies concerning the levels of p53 protein in pineal parenchymal tumours[14].

Cyclin-dependent kinases are a large group that regulates different phases of the markers. Cyclin D-Cdk4 and cyclin E-Cdk2 complexes regulate G1-S transition[6]. The cyclin D genes are over expressed in many cancers including those with the breast carcinomas, squamous cell carcinomas of the head and neck, bladder cancers and some subsets of lymphomas[26,29,30,32,36]. To our knowledge there is no report on the expression of cyclin D 1 in pineal parenchymal tumours.

The aim of this study was to determine whether Ki-67 proliferative index, cyclin D1 and p53 protein have prognostic significance in pineal parenchymal tumours.

## MATERIAL AND METHODS

Archive preparates of the patients diagnosed with pineal parenchymal tumours in the Pathology Department of Faculty of Medicine of Ege University between 1990 and 2001 were reviewed again by two pathologists according to WHO classification (2007). New sections were prepared from the paraffin blocks kept in the archive and immunohistochemical staining was performed. Antigen retrieval was obtained by microwave treatment. Primary antibodies were: neurofilament protein (clone 2F11, DAKO,dilution 1/100 ), cyclinD1 (cloneSP4, Lab Vision, dilution 1/100), Ki-67 (cloneSP6, Lab Vision, dilution 1/100) p53 (DO-7 monoclonal antibody, Lab Vision dilution 1/300), Glial fibrillary acidic protein (polyclonal, DAKO, dilution 1/500) and synaptophysin (SP11, Lab Vision, dilution 1/100). After peroxidase blocking, sections were incubated for 1 hour. Secondary biotinylated rabbit antimouse antibody and avidine-peroxidase complexes were added and identified using diaminobenzidine as substrate. For immunohistochemical analysis, a standard avidin-biotin-peroxidase technique was used: sections were counter-stained with hematoxylin. Appropriate negative and positive controls were included in the staining protocol.

Information about the patients was retrieved from their records or by interview with the patients or their relatives. Ki-67 proliferative index was established by detecting the number of cells with stained nuclei out of 1000 cells. Cyclin D1 and p53 were also evaluated in the same manner. Out of our 10 cases, 2 were

pineocytomas, 5 pineal parenchymal tumour of intermediate differentiation and 3 pineoblastomas. Three normal pineal glands obtained from autopsies were used for the control group.

## RESULTS

### Pineocytomas (PC):

Two male patients (34 and 64 years old) were pineocytomas. In one of the patients, magnetic resonance imaging (MRI) showed a cyst in the middle wall of the third ventricle. The tumour was evacuated through craniotomy. Biopsy was performed in the other case who had pineal mass and acute hydrocephalus. One of the patients did not receive radiotherapy or chemotherapy and he has been disease-free for 102 months since the operation. The other received radiotherapy and he died 9 months after the operation for shunt dysfunction. Histopathological examination of both cases revealed uniform tumour cells with round nuclei that showed pineocytematous rosette formation (Fig. 1). No mitosis or necrosis was observed. Immunohistochemically, synaptophysin was positive diffusely and neurofilament protein was positive (Fig. 2) while glial fibrillary acidic protein (GFAP) was negative. Ki-67 was 0%, cyclin D1 was between 0.5-1% and p53 was negative.

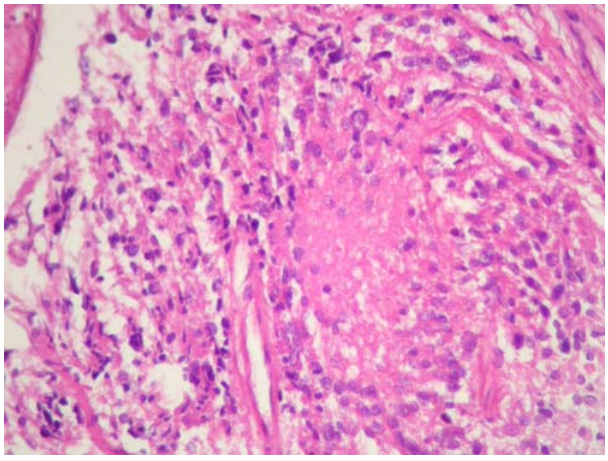

***Fig 1:*** *Pineocytoma (H&E; 400X)*

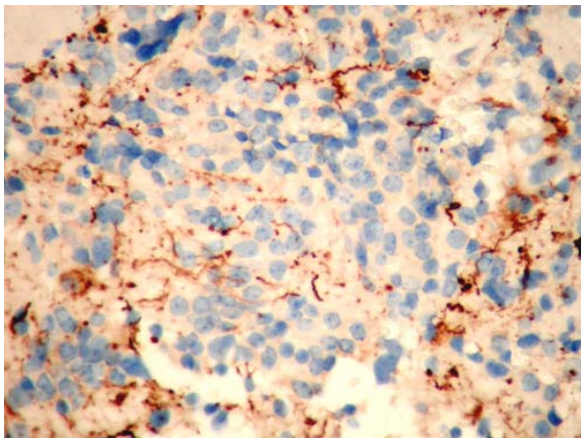

***Fig 2:*** *Expression of neurofilament protein in pineocytoma (neurofilament; 400X).*

### Pineal Parenchymal Tumours of Intermediate Differentiation (PPTID):

Five cases (3 female and 2 male) were PPTID. They were between 12-65 (mean 29.6) years old. The specimens were obtained from subtotal resections in three cases and the other two from biopsy materials. Histopathological examination showed fields resembling to both pineocytomas and pineoblastomas (Fig. 3). Those fields resembling to pineoblastomas were more cellular; cell nuclei contained large coarse chromatin and nucleoli were evident. Homer-Wright and Flexner-Wintersteiner rosettes were observed. Necroses were detected in 2 cases. Ten High-power field (HPF) mitoses were found between 0-25 (mean 5.8). Immunohistochemically, synaptophysin was focal or diffusely positive, neurofilament protein was positive (Fig. 4) in 2 cases. GFAP was reactive in one case. Ki-67 positivity was between 4-17.5 % (mean 6.4 %) (Fig. 5), cyclin D1 was 2-30% (mean 10.2%) (Fig. 6), p53 positivity was between 0- 4% (mean 1.3%). One of the patients had 25 mitoses in10 HPF, but survived for 94 months. One of the patients died 10 months after the operation for shunt dysfunction. The other died because

of spontaneous respiratory arrest. Three cases were free of disease for 94-98 months after the operation or the biopsy. Two cases received radiotherapy and chemotherapy. They were alive for 98 months after the operation.

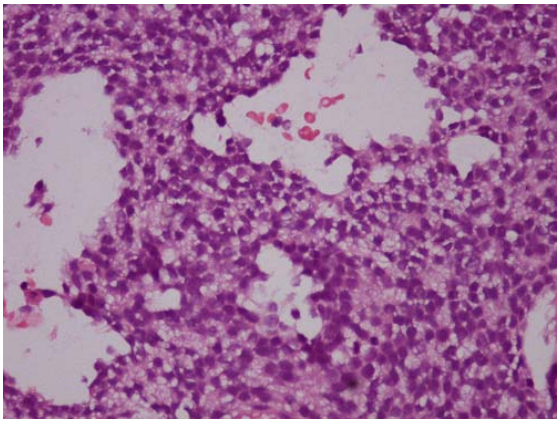

***Fig 3:*** *PPTID (H&E; 400X).*

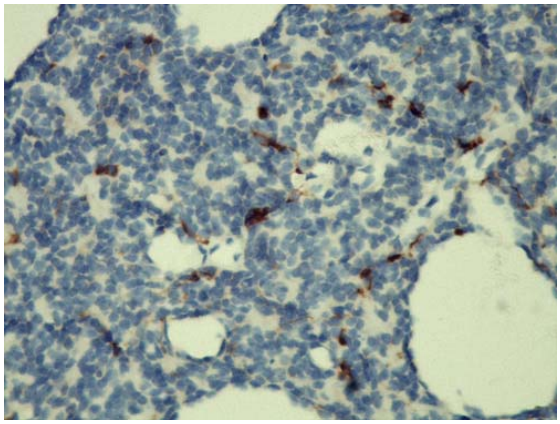

***Fig 4:*** *Expression of neurofilament protein in PPTID (neurofilament; 400X).*

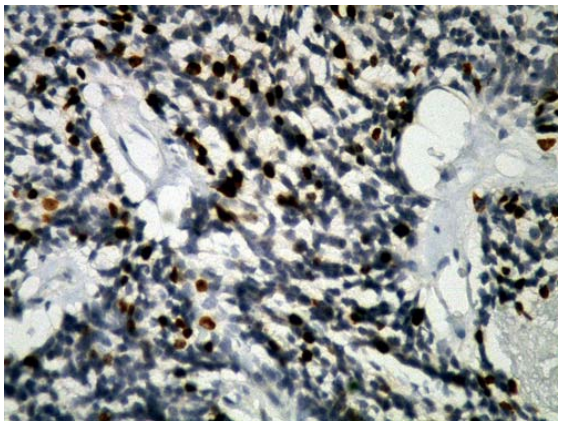

***Fig 5:*** *Ki-67 positivity was 17.5% in PPTID in case 4 (Ki-67; 400X).*

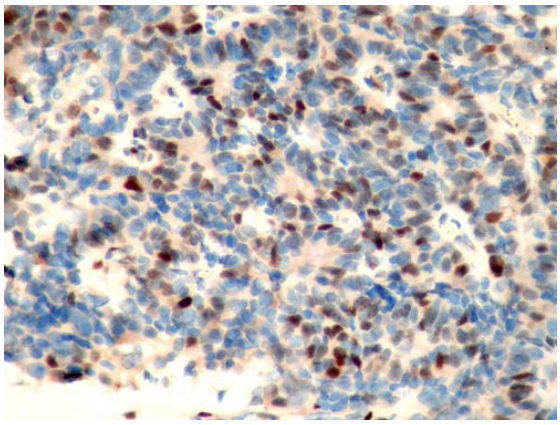

***Fig 6:*** *Cyclin D1 positivity was 30 % in PPTID in case 7 (cyclin D1; 400X).*

**Pineoblastomas(PB) :**

Three cases (2 boys and 1 girl) were PB. Their ages ranged 1 to 8 (mean age 3.7). Two cases presented with nausea and vomiting. The other presented with cephalomegaly. Histopathological examination showed undifferentiated cells with diffuse growth pattern and necrosis (Fig. 7). There were no rosette formations. Mitoses were observed between 2 and 30 in 10HPF (mean 15.6). Immunohistochemically, synaptophysin was positive, neurofilament protein was negative, GFAP was reactive in 2 cases and was negative in one case. Ki-67 positivity was between 9.2-53.5% (mean 35.2%) (Fig. 8), cyclin D1 positivity was found between 5-40% (mean 23.3%) (Fig. 9), and p53 positivity was found between 1-2 % (mean1.3%) (Fig. 10).

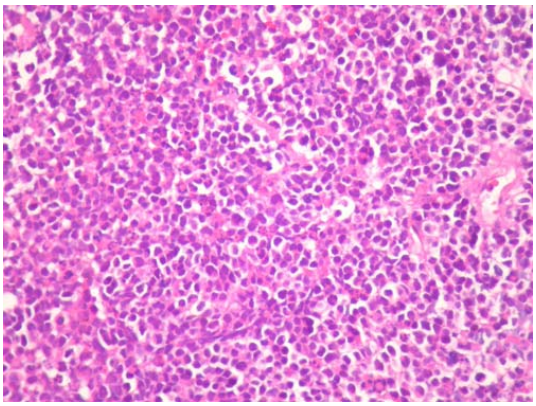

***Fig 7:*** *Pineoblastoma (H&E;400X).*

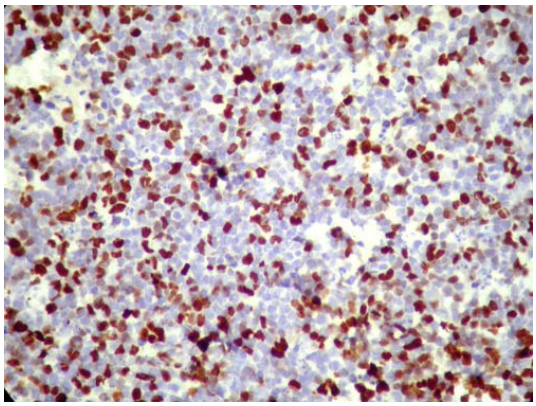

***Fig 8:*** *Ki-67 positivity was 43 % in pineoblastoma in case 8 (Ki-67; 400X)*

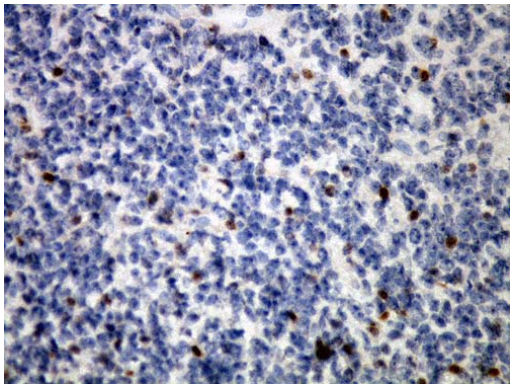

***Fig 9:*** *Cyclin D1 positivity was 5 % in pineoblastoma (cyclin D1; 400X).*

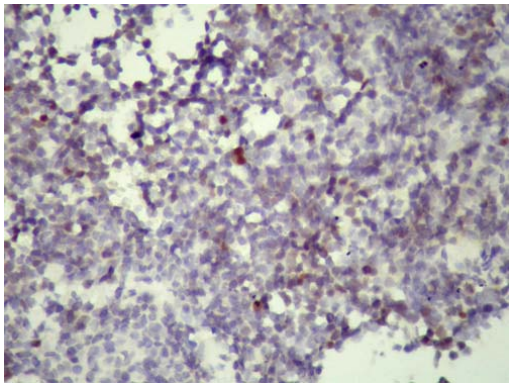

***Fig 10:*** *p53 positivity was 2 % in pineoblastoma in case 10 (p53;400X).*

In one of them, chemotherapy was performed before radiotherapy due to spinal seeding. Leptomeningeal spread was observed throughout the spinal cord. Pineal mass and metastases disappeared by radiotherapy but a residual mass persisted. Intrathecal chemotherapy was repeated because of malignant cells in serebrospinal fluid. No tumor was detected 6 months after radiotherapy. Later on leptomeningeal invasion developed, the patient deteriorated and died 27 months after the operation. The other one was re-operated 2 months after the first operation and received chemotherapy. He died 7 months after the operation. In the third case, the parents did not accept a second operation and the patient died 1.5 year after the operation.

Clinical, histopathological and immunohistochemical findings were shown in Table1 and 2 for our cases.

**Table 1** Clinical findings in pineal parenchymal tumors

| Case No | Age/Sex | Operation | Diagnosis | RT | CT | Follow-up (month) | |
|---|---|---|---|---|---|---|---|
| 1 | 34/M | Total resection | PC | (-) | (-) | 102 | Alive |
| 2 | 60/M | Biopsy | PC | (+) | (-) | 9 | Death, shunt dysfunction |
| 3 | 22/F | Subtotal resection | PPTID | (-) | (-) | 10 | Death |
| 4 | 65/M | Biopsy | PPTID | (-) | (-) | 94 | Alive |
| 5 | 21/M | Biopsy | PPTID | (+) | (+) | 98 | Alive |
| 6 | 28/F | Subtotal resection | PPTID | (-) | (-) | 0 | Death,respiratory arrest |
| 7 | 12/M | Subtotal resection | PPTID | (+) | (+) | 98 | Alive |
| 8 | 8/M | Subtotal resection | PB | (+) | (+) | 27 | Death |
| 9 | 1/F | Total resection | PB | (-) | (-) | 1.5 | Death |
| 10 | 2/M | Subtotal resection | PB | (-) | (+) | 7 | Death |

M: male, F: female
PC: pineocytoma
PPTID:pineal paranchymal tumour of intermediate differentiation
PB:pineoblastoma
RT: radiotherapy
CT: chemotherapy

**Table 2**. Histopathological and immunohistochemical findings in pineal parenchymal tumors

| Case no | Diagnosis | Mitosis | Ki-67 % | CyclinD1% | Follow-up (month) |
|---|---|---|---|---|---|
| Control | | 0 | 0 | 0 | |
| Control | | 0 | 0 | 0 | |
| Control | | 0 | 0 | 0 | |
| 1 | PC | 0 | 0 | 1 | 102 Alive |
| 2 | PC | 0 | 0 | 0.5 | 9 Death shunt dysfunction[a] |
| 3 | PPTID | 0 | 3.5 | 6 | 10 Death |
| 4 | PPTID | 25 | 17.5 | 10 | 94 Alive |
| 5 | PPTID | 1 | 9 | 3 | 98 Alive |
| 6 | PPTID | 1 | 7.9 | 2 | 0 Death, respiratory arrest |
| 7 | PPTID | 2 | 4 | 30 | 98 Alive |
| 8 | PB | 30 | 43 | 5 | 27 Death |
| 9 | PB | 15 | 9.2 | 25 | 1.5 Death |
| 10 | PB | 2 | 53.5 | 40 | 7 Death |

## DISCUSSION

Pineal parenchymal tumours are very rare tumours[10,15]. Histological criteria for diagnosis are not clearly delineated because of their rarity. Since the pineal region is a challenging and hazardous region for surgical intervention, biopsy has been shown to provide lower mortality compared to resection. Thus non-resective treatment has been proposed as an alternative to radical surgical resection in recent years. This approach increases the importance of stereotaxic, neuroendoscopic biopsies[1,8,23]. Materials obtained by biopsy complicate the task of the pathologist. Determining the proliferative activity of the tumour may be useful for diagnosis and prediction of the prognosis[1]. In our study, biopsy was performed for 3 cases, subtotal excision for 5 cases and total excision of tumour for 2 cases.

Jouvet et al reported histopathological features and patient survival of 66 pineal parenchymal tumours from12 neurosurgical centres. The number of mitoses that ranged from 0-16 in PPTID and 0-40 in pineoblastomas was significant for the prognosis[15]. In our study, the number of mitoses ranged from 0-25 in PPTID and 2-30 in pineoblastomas. One of the PPTID patients had 25 mitoses in10 HPF, this case resembled pineoblastomas in terms of the number of mitoses but lived for 94 months after surgery. This finding led the opnion that the number of mitoses are not significant for the prognosis in pineal parenchymal tumours.

Ki-67 is expressed during all phases of the cell cycle[7,11]. Expression of Ki-67 has been shown to be associated with histological grade and survival rates of central nervous system tumours[4,12,16,24]. In their study including 13 cases of pineal parenchymal tumours, Tsumanuma et al have demonstrated that Ki-67 proliferative index was considerably higher in pineoblastomas compared to pineocytomas and pineocytomas with anaplasia. However, no difference was observed between pineocytomas and pineocytomas with anaplasia. Neurofilament protein immune positive cases showed significantly lower Ki-67 proliferative indexes compared to negative cases[31]. Ki-67 proliferative index was between 0-53.5 % in our cases. Ki-67 proliferative index was 0% in pineocytomas. Neurofilament protein immune positivity was seen in all pineocytoma cases and in two PPTID cases. There is scarce data in the literature regarding the association of PPIDT with Ki-67 proliferative index. Ki-67 proliferative index reportedly ranges from 3-10%[13,23,31]. Fevre-Montagne et al. showed that Ki-67 proliferative index was between 3-7% in PPTID presenting with cytologic pleomorphism[10]. In 5 cases of PPIDT Ki-67 values ranged from 4 to 17.5%. In a study by Hopf et al that measured the proliferative index of central nervous system tumors by Ki-67 monoclonal antibodies, proliferative index of the pineoblastoma case ranged from 11.6 to 13.7 %[13]. Ki-67 values of our pineoblastoma cases ranged from 9.2 to 53.5 %. Numoto has stated that Ki-67 proliferative index was correlated with histologic malignancy and neuronal differentiation[23]. In a study that measured proliferative index by AgNOR, AgNOR score of pineoblastoma was shown to be higher than pineocytoma and mixed pineocytoma. But in these cases AgNOR scores showed no correlation with the prognosis of the tumour groups[21]. In our cases, Ki-67 proliferative index of pineoblastomas and PPTID were substantially higher than pineocytomas as seen in Table 2.

There is only one study that assessed p53 mutation in pineal parenchymal tumors. The study by Tsumanuma et al included 4 cases of pineoblastoma, and 5 cases of pineocytoma making up a total of 9 cases. None of these cases showed immunohistochemical staining with p53

protein (PAb1801 or DO-1 antibody). In these cases polymerase chain reaction-mediated single strand conformation polymorphous analysis failed to demonstrate abnormal migration exons in the 5th to 8th genes[31]. Six of our cases showed p53 (+) staining about 1-4%. The low level of p53 staining can be disregarded. These findings were correlated with the study of Tsumanuma et al. This finding led us think that the expression of p53 protein had no role in tumour genesis in pineal parenchymal tumors.

Over expression of cyclin D1 was observed in glial tumours[3,36], but no information was found in the literature with the over expression of cyclin D1 in pineal parenchymal tumors. In this study, cyclin D1 was between 0.5-1% in pineocytomas, 2-30% PPTID and 5-40% pineoblastomas. No expression of cyclin D1 was observed in pineal gland in control group. This finding led us think that cyclin D1 might have a role in tumour genesis in pineal parenchymal tumours.

In the literature, the five year survival rate of the cases with pineocytomas was 86-100 %, with PPTID 39-74% and with pineoblastomas 58%[5,9,27,28,35]. In our study; out of pineocytomas, one case was alive for 102 months, the other died because of the shunt dysfunction. Out of PPIDT, three cases lived for between 94-98 months, one case died 10 months later. All pineoblastoma cases died within 1.5-27 months.

As the result of this study, we have reported the clinical, histopathological and immunohistochemical findings in pineal parenchymal tumours. According to our findings, we suggest that the number of mitoses are not significant for the prognosis in pineal parenchymal tumours. p53 protein had no significance in these types of tumours and cyclin D1 might have a role in tumour genesis in pineal parenchymal tumours. However, our results are limited because, the number of the cases is small and the group is heterogeneous in terms of treatment and approach. Thus, large scale multicenter studies are required to learn more about these rare tumors.

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