The Use of PCNA Immunohistochemistry in the Evaluation of Choroid Plexus Tumours

FİGEN SÖYLEMEZOĞLU, KAMİL ÖGE, BEHSAN ÖNOL, AYKUT ERBENGİ

Hacettepe University, Medical School, Departments of Pathology (FS, BÖ) and Neurosurgery (KÖ, AE), Ankara, Türkiye.

Abstract: In diagnostic neuropathology, the histological distinctions between normal choroid plexus, typical and atypical choroid plexus papilloma and choroid plexus carcinoma may cause difficulties. Immunohistochemistry was applied for proliferating cell nuclear antigen on 7 samples of normal choroid plexus, 12 typical papillomas, 10 atypical papillomas, and 3 choroid plexus carcinomas to evaluate the use of this technique in differential diagnoses. Significant differences were found in the mean proliferating cell nuclear antigen labelling indices between normal choroid plexus (1.3 ± 1.2) and typical choroid plexus papilloma (3.1 ± 2.1), typical choroid plexus papilloma and atypical choroid plexus papilloma with p values of 0.021 and 0.0013, respectively. There is also a significant difference between typical and atypical choroid plexus papillomas (p=0.003). The amount of choroid plexus carcinoma cases in our series was low for a meaningful statistical analysis. Though no statistical analysis was carried out, the proliferating cell nuclear antigen labelling indices of 3 cases of choroid plexus carcinoma were relatively high with values of 17.7, 21.9 and 27.5%.

These results suggested that proliferating cell nuclear antigen labelling index can be of use to differentiate normal choroid plexus from choroid plexus papilloma in small, diagnostically difficult biopsy specimens.

Key words: choroid plexus tumours, normal choroid plexus, PCNA, immunohistochemistry, cell kinetics

INTRODUCTION

Choroid plexus tumours that account for less than 1% of all central nervous system (CNS) neoplasms, occur both in children and adults. In general they are divided into papillomas and carcinomas (5, 10). Recent studies attempted to classify these tumours as typical choroid plexus papilloma (TCPP), atypical choroid plexus papilloma (ACPP), and choroid plexus carcinoma (CPC) (7, 8). For the diagnosis of carcinoma, invasion of the adjacent brain tissue or presence of metastases are considered as definitive criteria. The distinction between TCPP and ACPP is less clear and mainly depends on the complexity of the arborization (7). The diagnoses of TCPP, ACPP, and CPC could be difficult in small biopsy fragments. Sometimes small tissue samples may lead to diagnostic difficulty between normal choroid plexus and papillomas.

Recently, several attempts have been made to predict the biological behaviour of brain tumours (1, 6). Proliferating cell nuclear antigen (PCNA) labelling index (LI) is one of the techniques that can be used to distinguish benign from malignant, and sometimes to highlight the grey zone.

PCNA, a 36 kD protein, is the auxiliary protein of DNA polymerase-α. It is present in G1 and S-phases, rarely present in G2/M phases and absent in quiescent cells. PC 10 is one of the commercially available monoclonal antibodies against PCNA which works on formalin-fixed paraffin-embedded tissue sections.

PCNA immunohistochemistry was performed to determine the potential of this method for distinguishing between normal choroid plexus and choroid plexus neoplasms.

MATERIAL AND METHOD

Selection of cases: Files from Hacettepe University, Department of Pathology were reviewed for biopsies of epithelial neoplasms of the choroid plexus and 25 cases were found. Seven samples of normal choroid plexus used as controls were incidentally obtained from several surgical

specimens that had been removed during surgery for CNS disease other than a choroid plexus tumour.

Immunohistopathological study: All the samples were fixed in formalin, embedded in paraffin and stained with haematoxylin-eosin (HE).
For immunohistochemical analysis, PCNA (PC 10, Dako; 1:50) monoclonal antibody was used. Antibody binding was visualised by an avidin-biotin-peroxidase method. Diaminobenzidine was used as the chromogen. Positive and negative control specimens were included.

The immunoperoxidase-stained slides were analysed and scored by one observer without knowledge of the diagnosis. Counts were done using a high-power (x40) objective and a video-camera. Cells with diffuse or granular nuclear staining counted as positive. In most cases the PCNA LI was based on counting 1000 nuclei from the areas of highest immunostaining and reported as a percentage.

Histological grading: HE stained slides were classified histologically as either TCPP, ACPP or CPC. Tumours with marked cytological atypia, pleomorphism, frequent mitoses, undifferentiated and solid growth pattern, necrosis, and microscopic invasion are classified as CPC (Fig. 3A). Tumours composed of arborising papillae that are lined by single-layered or pseudostratified epithelium are named TCPP (Fig. 1A). In the presence of one or more of the following histological features, the tumours were designated ACPP (Fig. 2A): multifocal cytological atypia, very rare mitotic figures and architectural complexity.

Statistical analysis: A nonparametric test, Mann Whitney-U test was used in the statistical analysis of the data as the number of samples did not fulfil the conditions for a parametric test.

RESULTS

The clinical and histological features of the tumours are summarised in Table 1. Twelve neoplasms were classified as TCPP (48%) with typical well-differentiated features. 10 out of 25 (40%) were classified as ACPP whereas 3 neoplasms (12%) were diagnosed as CPC.

The study group included 18 males and 7 females (M/F=2.6) with a mean age of 20.6 years, ranging from 6 months to 61 years. Tumours were located exclusively in the fourth ventricle (52%).

PCNA LI of the normal choroid plexus biopsies showed a mean of 1.3±1.2%. The PCNA labelling indices for the TCPP's ranged between 0.5% to 8.75% with a mean of 3.1±2.1% and the LI for ACPP's between 2.2% to 20.4% with a mean of 10.9±6.5%. The CPC revealed LI of 17.7, 21.9 and 27.5%.

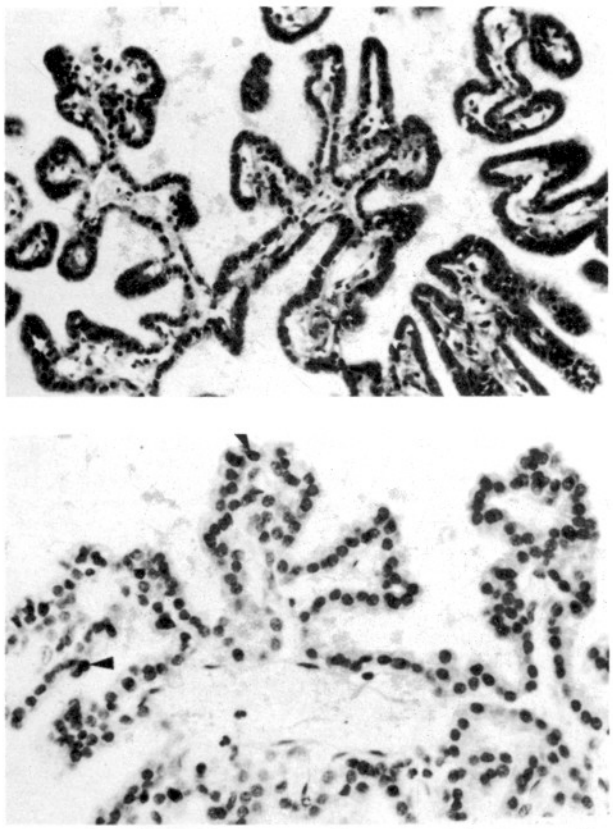

Fig 1. Typical choroid plexus papilloma. 1A. Arborizing papillae lined by single-layered epithelium (HE, x230), 1B. Immunolabelled nuclei (arrow heads) (PCNA, x200).

The difference between normal choroid plexus (NCP) and TCPP was significant (p=0.021, u=14.5). A highly significant difference (p=0.0013, u=2) was observed between normal choroid plexus and ACPP and between TCPP and ACPP (p=0.003 u=15,). Though the CPC's had high levels of PCNA labelling, the number was too low for statistical comparison.

Cases 15 and 19 were recurrent tumours that were classified as ACPP in the histomorphological examination with PCNA LI of 2.2 and 9.9%. Unfortunately primary tumours of those cases were not available for analysis. Case 17 recurred 7 years after the initial diagnosis, and we were able to examine both tumour samples. Though there was no obvious histological progression, proliferation potentials, as determined by PCNA LI, were 6.7% in the first and 30% in the recurrent tumour samples.

Figures 1B, 2B and 3B display examples of PCNA staining in TCPP, ACPP and CPC.

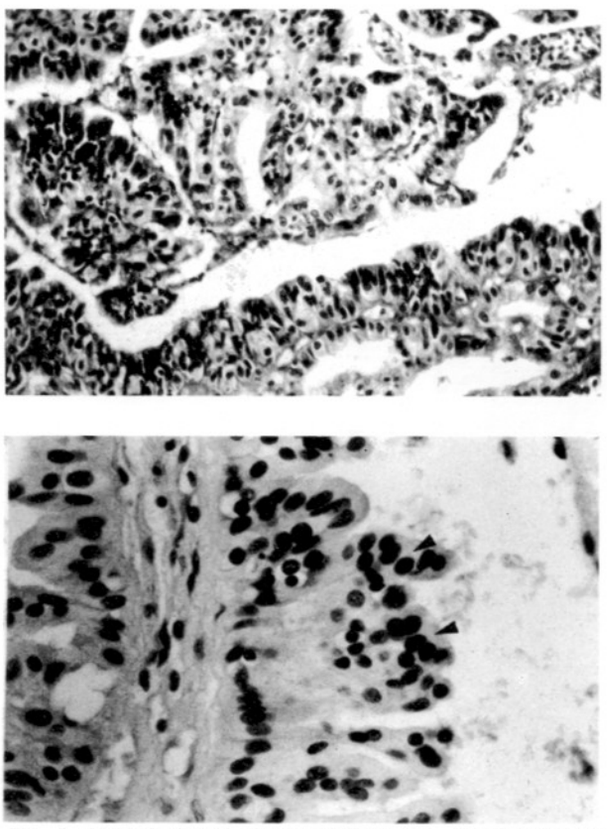

Fig. 2. Atypical choroid plexus papilloma. 2A. Papillae showing architectural complexity and lined by pseudostratified epithelium (HE, x230), 2B. Some immunolabelled nuclei (arrow heads) (PCNA, x230).

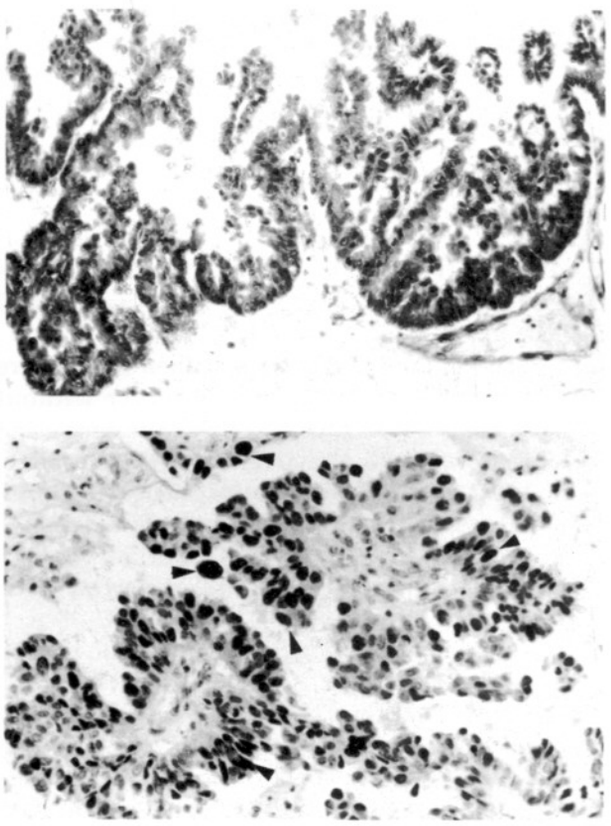

Fig. 3. Choroid plexus carcinoma. 3A. A papillary tumour with atypia, frequent mitoses and brain invasion (HE, x230), 3B. Many immunolabelled nuclei (arrow heads) (PCNA, x200).

DISCUSSION

Studies on the prognosis and in particular those that correlate prognosis with histomorphological features of choroid plexus tumours are quite rare. Though epithelial choroid plexus tumours are classified as papillomas and CPC (5, 10), there are only a few reports that apply a three-tiered grading system i.e., TCPP, ACPP and CPC (7, 8). McGirr et al. reported that histopathologically, the presence of occasional mitotic figures, microscopic infiltration, ependymal differentiation, or mild to moderate atypia was not correlated with complete resectability or tendency to recurrence (7). Inversely, Borcich and Davis showed that complex growth pattern, cytological atypia and mitotic activity were correlated with poor prognosis (2). This observation was supported by the findings of Paulus et al. who stated that the biological behaviour, as determined by fatal outcome or recurrence, correlated well with the histological features such as lack of S-100 and transthyretin immunoreactivity, presence of mitoses, brain invasion, absence of marked stromal oedema, and presence of necrotic areas (8).

Though we were not able to correlate our own findings with the patients' survival, the distribution pattern of PCNA LI of the study group clearly shows a wide range with several peaks. 52% of all cases showed a PCNA LI less than 5%, whereas 9 out of 25 had LI between 5-20% and 3 had LI greater than 20%. Three recurrent tumours in our study group were classified as ACPP in the histomorphological examination with PCNA LI of 2.2, 6.2 and 9.9%. In our opinion, these findings show necessity for three-tiered grading of epithelial choroid plexus tumours. Our results indicated that PCNA LI was reliable in distinguishing normal from epithelial neoplasms of the choroid plexus and ACPP from TCPP. In a recent study by Coons et al. a flow cytometric analysis of DNA and proliferation in choroid plexus tumours were reported (4). It was shown that evaluation of proliferation rates by means of S-phase fractions could help to predict the behaviour of choroid plexus tumours whereas the DNA ploidy measurements appeared to have no use. This result was similar to

Table 1. Clinical and histopathological data of 25 patients with choroid plexus tumours

Case no	Age	Gender	Location	Histological diagnosis	PCNA LI (%)
1	10/12	M	Lateral	TCPP	2.0
2	24	M	4th	TCPP	1.7
3	35	F	4th	TCPP	8.75
4	17	M	Lateral	TCPP	1.4
5	2.5	M	3rd	TCPP	4.2
6	1.5	F	4th	TCPP	4.2
7	18	M	Lateral	TCPP	3.6
8	6.5	M	3rd	TCPP	3.2
9	15	M	3rd	TCPP	2.6
10	16	M	4th	TCPP	2.8
11	24	M	4th	TCPP	0.5
12	9/12	M	Lateral	TCPP	2.6
13	35	F	4th	ACPP	16.4
14	8	M	Lateral	ACPP	19.8
15(1)	61	M	4th	ACPP	2.2
16	17	M	4th	ACPP	8.7
17(2)	17	F	Lateral	ACPP	6.2
18	38	M	4th	ACPP	13.8
19(3)	45	M	4th	ACPP	9.9
20	1.5	F	Lateral	ACPP	20.4
21	52	M	4th	ACPP	3.5
22	6/12	M	Lateral	ACPP	7.6
23	1	F	4th	CPC	17.7
24	3	M	4th	CPC	21.9
25	6/12	F	Lateral	CPC	27.5

Abbreviations: M; male, F; female, TCPP; typical choroid plexus papilloma, ACPP; atypical choroid plexus papilloma, CPC; choroid plexus carcinoma, PCNA LI; proliferating cell nuclear antigen labelling index.

1. 8 years ago a CPP was resected, material from the first operation was not available.
2. 7 years later a recurrent tumour was excised with a PCNA LI of 30%.
3. 10 years ago a CPP was resected, material from the first operation was not available.

the data of Qualman et al (9). In another study by Centeno et al., several techniques i.e., AgNOR technique, PCNA immunolabelling and DNA ploidy analysis were applied on choroid plexus biopsy specimens (3). It was concluded that the AgNOR and PCNA techniques can be used to distinguish normal choroid plexus from choroid plexus papillomas. Their results also confirmed that DNA ploidy analysis alone was not useful in distinguishing between papilloma and carcinoma.

Proliferation indices by means of PCNA immunolabelling have been used in several CNS tumours (1, 6) and particularly in choroid plexus neoplasms as mentioned above (3). In the present study the mean LI of normal choroid plexus was 1.3±1.2% whereas the mean LI were 3.1±2.1% and 10.9±6.5% in TCPP and ACPP, respectively. Though the intensity of staining varied among different nuclei, the nuclei were considered positive regardless of the intensity in our study. Also there was a labelling variation among the fields. We performed counts in the fields with highest labelling and LI was reported as a percentage. Statistically, there were differences between the mean PCNA LI of the normal choroid plexus, TCPP, and ACPP.

In summary, our data indicated that PCNA immunolabelling is a useful technique to distinguish normal choroid plexus from papillomas, and to differentiate TCPP from ACPP in surgical specimens.

Correspondence : Dr. Figen Söylemezoğlu
Yücetepe Ata Sokak, No:6/1
Ankara, 06580
Tel: (+312) 230 67 28

REFERENCES

1. Allegranza A, Girlando S, Arrigoni GI, Veronese S, Mauri FA: Proliferating cell nuclear antigen expression in central nervous system neoplasms. Virchows Arch A Pathol Anat 419:417-423, 1991
2. Borcich JK, Davis RL: Epithelial neoplasms of the choroid plexus- a study of 21 cases. J Neuropathol Exp Neurol 43:338, 1984 (Abst)
3. Centeno BA, Louis DN, Kupsky WJ, Preffer FI, Sobel RA: The AgNOR technique, PCNA immunohistochemistry, and DNA ploidy in the evaluation of choroid plexus biopsy specimens. Anat Pathol 100:690-696, 1993
4. Coons SW, Johnson PC, Haskett D, Rider R: Flow cytometric analysis of deoxyribonucleic acid ploidy and proliferation in choroid plexus tumors. Neurosurgery 31:850-856, 1992
5. Kleihues P, Burger PC, Scheithauer BW: Histological typing of tumours of the central nervous system. Berlin: Springer, 1993
6. Louis DN, Edgerton S, Thor AT, Hedley Whyte ET: Proliferating cell nuclear antigen and Ki-67 immunohistochemistry in brain tumors: A comparative study. Acta Neuropathol 81:675-679, 1991
7. Mc Girr SJ, Ebersold MJ, Scheithauer BW, Quast LM, Shaw EG: Choroid plexus papillomas: long-term follow-up results in a surgically treated series. J Neurosurg 69:843-849, 1988
8. Paulus W, Janisch W: Clinicopathologic correlations in epithelial choroid plexus neoplasms: a study of 52 cases. Acta Neuropathol 80:635-641, 1990
9. Qualman SJ, Shannon BT, Boesel CP Jacobs D, Finkens C, Hayes J: Ploidy analysis cerebrospinal fluid nephelometry as measures of clinical outcome in childhood choroid plexus neoplasia. Pathol Annu 28:305-321, 1992
10. Russell DS, Rubinstein LJ: Pathology of tumors of the nervous system. 5th Edition, London: Edward Arnold, 1989:394-420