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Touch-print Cytology of Brain Tumors and Its Correlation with Histological Features

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=Abstract=**A cytological preparation of neurosurgical biopsy material is one technique by which a rapid and reliable diagnosis can be made. Many specimens unfit for sectioning may be used, and the detailed cytological nature of the tumor can be evaluated, thus providing valuable information in differential diagnosis from other primary tumors or metastatic lesions. Touch-print specimens of 48 cases of CNS tumors collected during the last year from 1991 are reviewed and compared with paraffin sections of the same biopsies. Touch prints were prepared with specimens for frozed section diagnosis, just before they were utilized for frozen sectioning. Smears were stained both with Wright-Giemsa and Papanicolaou methods. Fourty eight cases of brain tumors consisted of 14 pituitary adenomas, 11 meningiomas, 7 oligodendrogliomas, 4 astrocytomas, 3 glioblastoma multiforme, 3 malignant lymphomas, 2 chordomas, 2 neurilemmomas, 1 pineocytoma, and 1 craniopharyngioma. The cytology and histology of pituitary adenoma and meningioma correlated best in this series. The most consistent findings were monotonous eccentric round nuclei and plump polygonal cytoplasm in the pituitary adenomas and meningothelial cell clusters with whorling pattern and psammoma bodies in meningiomas. The main dificulties encountered were differentiation between nonspecific glial hyperplasia and low-grade astrocytoma as well as grading of astrocytoma and oligodendroglioma. The remaining tumors revealed fairly consistent findings to be correlated with biopsy specimens.**

Key Words: *Touch-print, Cytology, Brain tumors, Correlation, Histology*

INTRODUCTION

The smear technique is well-known as a useful diagnostic tool in neurosurgical pathology (Balhuizen *et al.* 1974), which can be used as a substitute for frozen sections to obtain a rapid intraoperative diagnosis (Balhuizen *et al.* 1974; Marshall *et al.* 1973; Hitchcock *et al.* 1986). The cytodiagnostic accuracy of benign and malignant tumors is reported to be about 87-94% when adequate cell material was obtained

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(Marshall *et al.* 1973; Mouriquand *et al.* 1987; Willems and Alva-Willems, 1984; Liwniz *et al.* 1987) and it is also a great help is also obtained when sectioning of unfit specimens makes histologic evaluation hazardous (Mouriquand *et al.* 1987). It is obviously important particularly when craniotomy is not desirable but a precise pathologic diagnosis is necessary (Mouriquand *et al.* 1987). This type of cytology technique has recently become of interest. However the diagnostic cytologic findings have not yet been established in all CNS tumors. On this account, we thought it was important to conduct a review of touch-print samples of brain tumors which are histologically verified, trying to verify that imprints can complement frozen sections in the intraoperative diagnosis of tumors of the central nervous system (Reyes *et al.* 1991).

MATERIALS AND METHODS

Brain biopsy specimens of 48 primary tumors were obtained fresh from open craniotomy, stereotactic, or burr hole biopsies that were performed at Seoul National University Hospital during the last year. The tissue was held gently in forceps in such a way to avoid squashing or distorting it, and the cut surface was lightly touched onto the slides, leaving a mirror-image imprint (Mouriquand *et al.* 1987). Half of the slides were immediately immersed in 95% alcohol and stained with Papanicolaou procedure. The remaining smears are air dried as quickly as possible and stained with Wright-Giemsa stain. The use of both types of stains gives useful complementary information. The Papanicolaou stain, which contains phosphotungstic acid in its EA50 step, seems particularly fit for showing glial projections. Other cytoplasmic details (e.g. basophilia) are better demonstrated by the Giemsa stain. On microscopic observation, care was taken to scan each slide thoroughly since the biopsy may have collected material from normal as well as modified areas. All were compared with paraffin sections of the same biopsy material.

RESULTS

The distributions of 48 primary tumors are presented in Table 1. Their cytologic and histologic findings are as follows and summarized in Table 2 and 3.

1. Astrocytoma

The aspirate showed a slight but obvious increase of cellularity. The glial cells seldom preserved arrangement in the fibrillary eosinophilic background (Fig. 1). Several groups of cells showed ball-like aggregation without both specific arrangement and cytoplasmic projections of the individual cells. Rather, they round up and take a more or less lymphoblastlike appearance. The nuclear-cytoplasmic (N/C) ratio is high. Nuclei are round; the chromatin pattern is regular; the nucleolus, when present, is single and small. The cytoplasm, as seen in Wright-Giemsa stain, is blue and scanty all around the nuclear limits, with a fibrillary appearance. No particular development of a vascular bed is seen and no particular association of cells with the capillary vessels is observed. Necrosis is absent; edema is more or less obvious. The normal-appearing astrocytes were often included, showing their

Table 1. Final diagnosies made on paraffin sections in 48 cases

Diagnosis	No. of cases
Astrocytoma	2
Anaplastic astrocytoma	1
Glioblastoma	3
Mixed glioma	1
Oligodendroglioma	7
Pituitary adenoma	14
Meningioma	11
Chordoma	2
Pineocytoma	1
Neurilemmoma	2
Craniopharyngioma	1
Malignant lymphoma	3
Total	48

Table 2. Summary of cytologic findings of major CNS tumors

CNS tumors	Cytologic findings
Astrocytoma	Fibrillary background, Increased cellularity & N/C ratio, Cytoplasmic projections
Oligodendro-glioma	Monotonous egg-fried appearance, Arborizing tube-like capillaries, Calcium speckles
Malignant A. & GM	Marked pleomorphism with giant cells, Fibrillary fibrinous or granular background, Increased vascular component, Necrotic or inflammatory cells*
Pituitary adenoma	High cellularity with compact solid arrangement, Papillary clusters with vascular stalk, Uniform cuboidal to polygonal shape, Two types of cells (naked and plump cytoplasm), Intranuclear inclusions
Meningioma	Syncytial arrangement of spindle cells, Concentric whorls, Oval bland nuclei with vesicular chromatin, Psammoma bodies, Intranuclear inclusions

A: Astrocytoma GM: Glioblastoma multiforme
*: A finding of glioblastoma rather than malignant astrocytoma

Table 3. Summary of cytologic findings of minor CNS tumors

Chordoma	Hypocellular myxoid background, Large round vacuolated cells with small central nuclei
Cranio-pharyngioma	Squamoid cells with anuclear squames, Hemosiderin-laden macrophages
Malignant lymphoma	Monotonous atypical lymphoid cells around small vessels
Pineocytoma	Papillary clusters of small cuboidal to low columnar cells, Round to ovoid nuclei and finely vacuolated or granular cytoplasms
Neurilemmoma	Compact sheets of oval to fusiform nuclei in the fibrillary background, Indistinctness of cytoplasmic borders and nuclear palisading

characteristic fine fibillary processes (Fig. 2). Histologically, the tumor showed diffuse infiltration of mild to moderately hyperchromatic atypical astrocytes in the white and gray matters with no definable border, which was diagnosed as gliomatosis cerebri (Fig. 3).

2. Anaplastic astrocytoma

This tumor not only differs from the previous types by further increased cellularity, but also by the following changes: a definite increase of the capillary bed to which the cells are closely associated, frequent necrosis, and rather-frequent nuclear and nucleolar abnormalities (Fig. 4). Tissue section examination revealed an anaplastic astrocytoma, with occasional areas of unequivocally astrocytomatous component.

3. Glioblastoma multiforme

Highly cellular aspirate showed frequent abnormal malignant cells with conspicuous cellular changes, such as a great increase in size, and nuclear and nucleolar distortions. Many cells were multinucleated, and nuclear

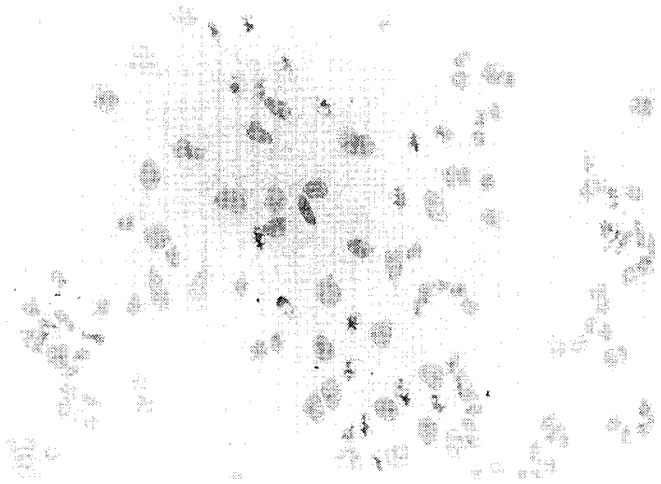

Fig 1. Slightly atypical astrocytes with increased cellularity in the fibrillary background (Papanicolaou, x100).

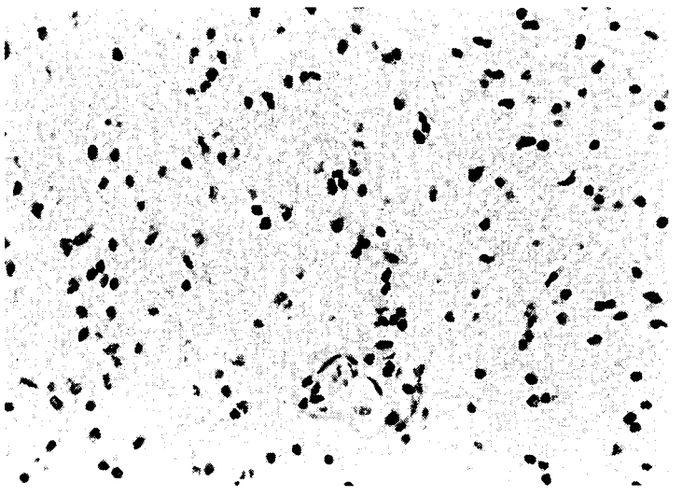

Fig 3. White matter showing diffuse infiltration of atypical astrocytes in the case of gliomatosis cerebri (H&E, x100).

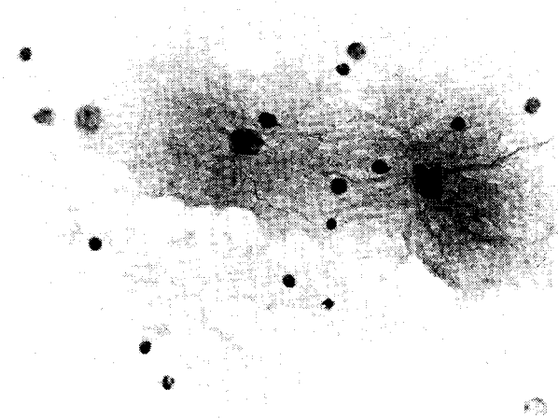

Fig 2. Normal astrocytes showing small round nuclei and stellate fibrillary processes (Papanicolaou, x 100).

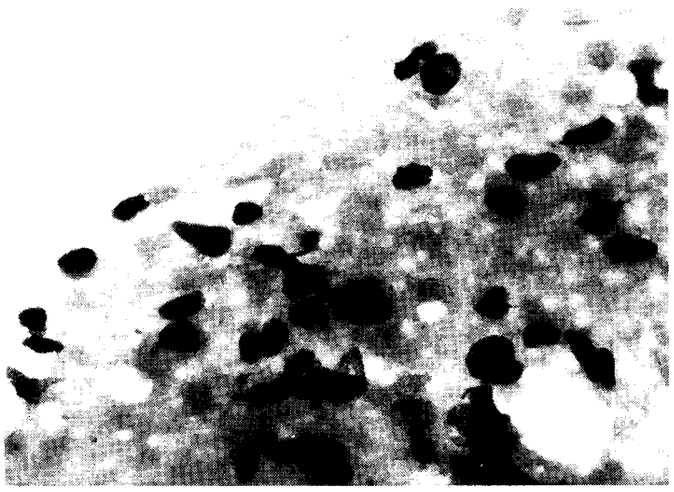

Fig 4. Highly atypical astrocytes with large hyperchromatic nuclei and ill-defined fibrillary cytoplasm in anaplastic astrocytoma (Papanicolaou, x200).

molding was seen (Fig. 5). Aspirate contained fibrin threads and fibrillary background. Mitotic figures were not observed in this aspirated material. Histologically, highly anaplastic giant cells were mixed with pleomorphic astrocytic tumor cells and numerous inflammatory cells.

4. Oligodendroglioma

The aspirates of oligodendroglioma showed a uniform population of round to slightly oval nuclei. The nuclei contained finely granular chromatin and small but distinct nucleoli. The nuclei were frequently rimmed by unstained halo, showing fried-egg appearance, which were scattered in the bubbly and fibrillary background (Fig. 6). The aspirates contained abundant capillaries and occasionally speckles of calcium. One case showed monotonous columnar appearance with eccentric nuclei and eosinophilic fibrillary cytoplasm, similar to gemistocytic astrocyte or pituitary adenoma cells. Histologic sections revealed proliferation of abundant arborizing capillaries and relatively monotonous round nuclei sometimes surrounded by halo. Four cases showed anaplastic areas

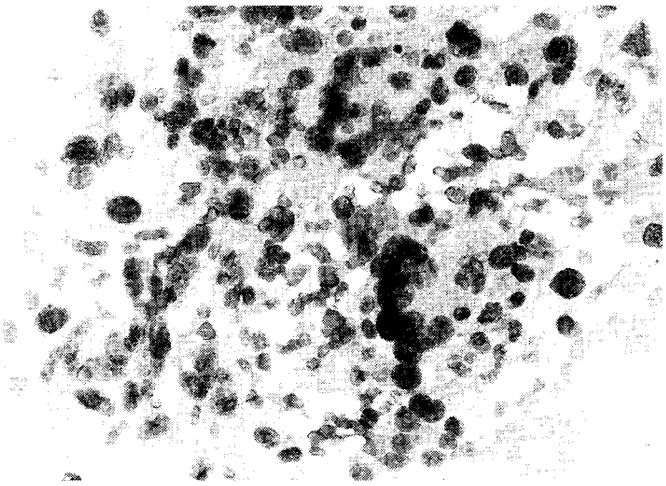

Fig 5. Many multinucleated giant cells and pleomorphic astrocytes in the fibrillary background were seen in the glioblastoma multiforme (Papanicolaou, x100).

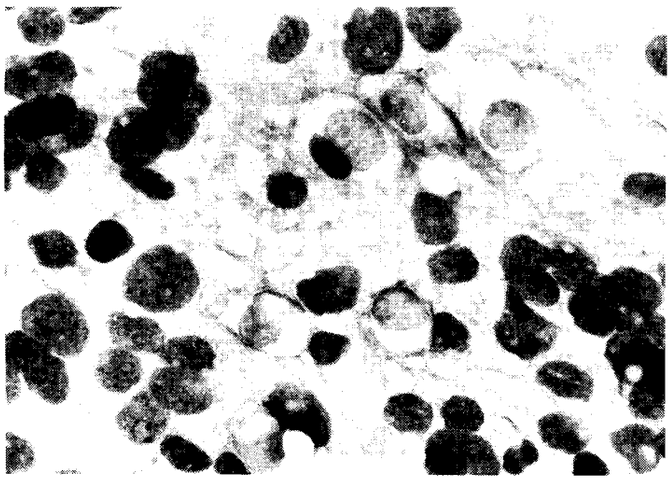

Fig 6. Highly cellular aggregates of oligodendroglia with large round nuclei and vacuolated or fibrillary cytoplasms showed sometimes fried egg appearances (Papanicolaou, x200).

comprised of more pleomorphic and poorly differentiated cells.

5. Pituitary adenoma

The aspirates were densely cellular at low magnification, with a thin layer of round to oval cells, sometimes arranged in clusters (Fig. 7). The clustered cells are divided in small solid nests by plentiful capillaries, thus often forming a hemorrhagic background. The dominant cell type consisted of large cells with well-defined cytoplasmic and nuclear outlines. The nucleus contained a distinct nucleolus and evenly distributed slightly coarse chromatin. The cytoplasm was pale stained with eosin, and showed a granular texture. Two types of cells appeared in some chromophobe adenoma cases, showing the larger cells with perinuclear halo and nuclei occupied by large inclusions to show a 'target' appearance (Fig. 8). Inclusions were generally single, had a glassy or finely granular appearance and were delimited by a dark halo. Innumerable naked dark nuclei appeared to be stippled or superficially

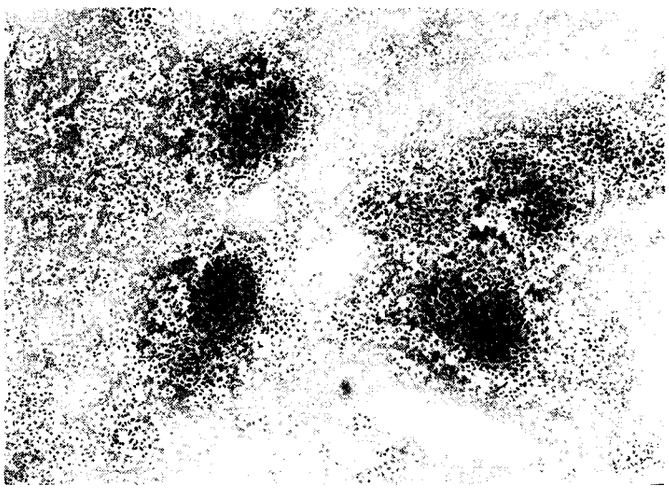

Fig 7. Low-power view of pituitary adenoma showed dense cellularity with small round tumor cell clusters (Papanicolaou, x20).

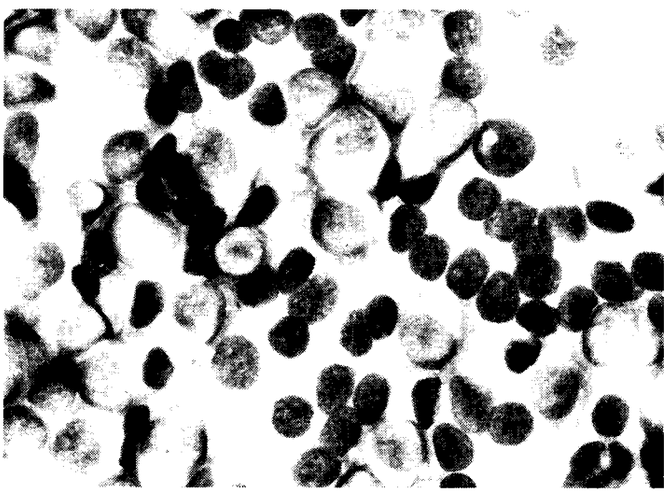

Fig 8. Pituitary adenoma cells showed two types of large pale and small dark nuclei (Papanicolaou, x200).

distributed in a pale ground substance. They were smaller and more hyperchromatic than others. Scattered mitoses could be detected easily. A few acidophilic type adenomas showed columnar cells with a uniform, fairly stainable eosinophilic cytoplasm and eccentric nuclei. Histologic examination revealed a highly cellular neoplasm made up of round or cuboidal cells, which are variably arranged in a solid, pseudoglandular, and papillary pattern. The tumors were histologically classified into predominantly chromophobe type and partly acidophilic type.

6. Meningioma

The tumor could be relatively easily identified on the cytologic preparations by the presence of whorl-like syncytial structures and psammoma bodies (Fig. 9). The aspirates of the syncytial type of meningioma showed polygonal or spindle cells with oval nuclei containing fine to coarsely granular chromatin and prominent small nucleoli. The cytoplasm had a poorly demarcated border and fibrillary appearance (Fig. 10). Often the nuclei showed pseudovacuoles and intranuclear inclusions (Fig. 11). In one angiomatous meningioma, tissue fragments with pleomorphic hyperchromatic nuclei were seen. Histologically, the most frequent type was meningotheliomatous menigioma and the remainder included transitional and angiomatous types.

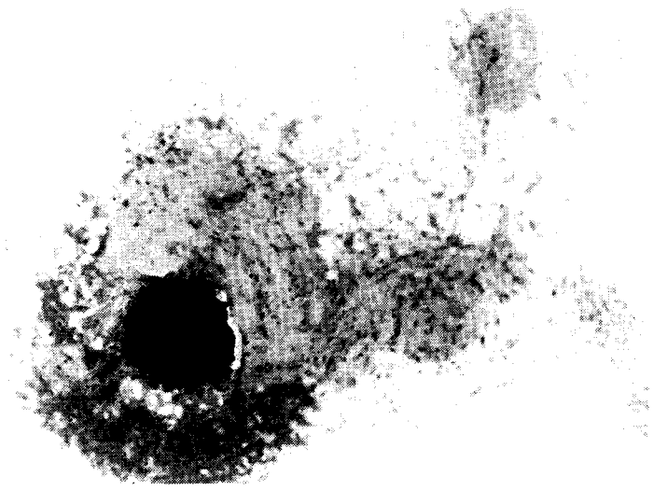

Fig 9. Psammoma bodies were scattered in the cellular touch print cytology of the meningioma (Papanicolaou, x33).

Fig 10. Meningothelial cells had fine fibrillary spindle cytoplasm (Wright-Giemsa, x200).

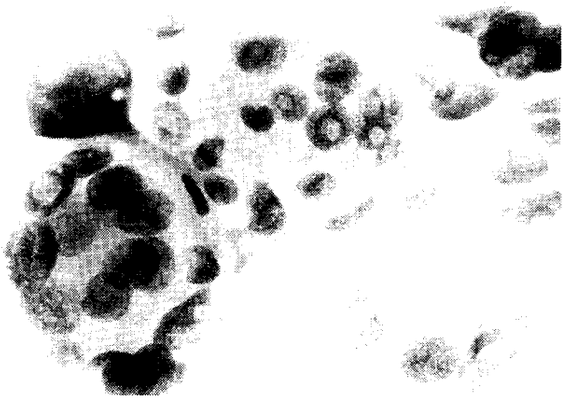

Fig 11. Cell ball clusters consisting of meningothelial cells with several intranuclear inclusions (Papanicolaou, x200).

7. Chordoma

Two cases of chordoma showed hypocellular aspirate in the bluish myxoid background. Small aggregates were composed of large round vacuolated cells with scalloped small central nuclei. The tumor cells were usually scattered or nested within the bluish amorphous material. Occasionally amorphous acellular vacuolated material was smeared in multifocal

areas. There was neither cartilage nor any chondroid element. Histological sections revealed vacuolated physaliferous cells in the fibromyxoid stroma.

8. Craniophyaryngioma

Aspirates of two cases showed squamous appearance of the cells and scattered squames. Areas of hemorrhagic material with many macrophages were seen. In some places, there were poorly cellular areas containing elongated fibroblasts and fibrillar material. Histologic sections showed squamoid epithelium-lined cystic lesions with inflamed fibrous stroma.

9. Malignant lymphoma

Three cases of malignant lymphoma were seen in the brain. The diagnosis could easily be made with Wright-Giemsa material, since the cells displayed the same characteristics as malignant lymphoid cells seen in extracerebral lymphoma (Fig. 12). The tumors cells had fairly uniform shape and size as compared to the bizarre nuclei and the great variations in cell size in malignant gliomas. Malignant lymphomas were all classified into diffuse large cell type by NCI working formulation in their histologic sections.

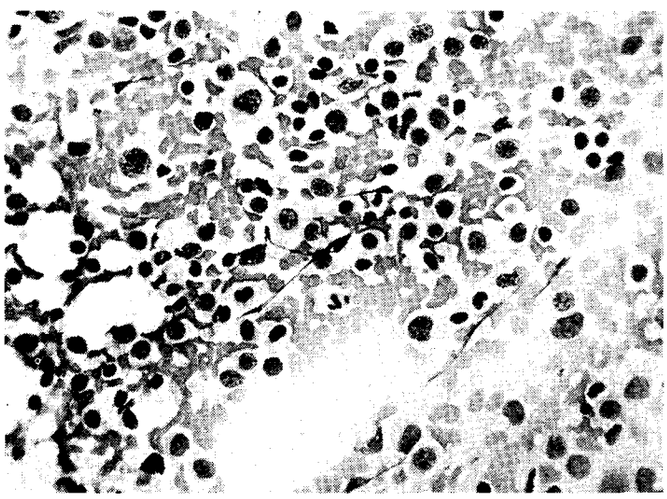

Fig 12. Malignant lymphoid cells scattered in the hemorrhagic background, which show relatively monotonous round nuclei with coarse chromatin and scanty cytoplasm (Papanicolaou, x200).

10. Pineocytoma

Papillary clusters were occasionally found among tumor cells that were small cuboidal to low-columnar. The cytoplasm was moderately abundant and finely vacuolated or granular. The nuclei were round to ovoid, with fine dispersed chromatin without nucleoli. Histologically, the tumor cells exhibited lobular architecture resembling normal pineal gland (Silverberg 1990).

11. Neurilemmoma

Touch-prints from two cases showed compact sheets of round to oval or fusiform nuclei in a fibrillary background. Some nuclei were markedly elongated or cigar-shaped; others were tortuous. The chromatin was fine and uniformly distributed or coarsely granular. Cytoplasmic outlines were indistinct. Fibrils in the background rarely formed compact bands, and they were loosely arranged with scattered empty spaces. It was difficult to find nuclear palisading and Verocay bodies in smears. Histology of two cases disclosed mixed and predominantly Antoni B type neurilemmoma with myxoid change, respectively.

DISCUSSION

The application of the smear technique as a means of obtaining a rapid diagnosis in neurosurgical biopsies has a long history (Marshall *et al.* 1973). Although frozen section diagnoses were clearly more accurate than smear or imprint diagnoses (Reyes *et al.* 1991), some advantage can be achieved by cytology technique. The principal advantages of the smear technique are its technical simplicity, the ease with which several small portions of a needle biopsy can be screened, and the clarity of the cytology on which the neuropathologist is dependent to establish the diagnosis (Marshall *et al.* 1973). The diagnostic accuracy of the cytology has been reported in the range of 87-94%, and it has been concluded in many studies

that imprints and smears could complement frozen sections in the intraoperative diagnosis of tumors of the central nervous system (Reyes *et al.* 1991). For the establishment of cytologic criteria for diagnosis, cytologic evaluation consistent with the established histologic classification has been performed (Liwniz *et al.* 1982).

Many brain tumors showed a distinctive cytologic picture in smears, allowing even conclusive diagnoses, particularly in cases of pituitary adenoma, craniopharyngioma, and meningioma in our study. Diagnosing pituitary adenomas was easy due to their characteristic cell picture. Two types of cells were noted in some chromophobe types: larger halo-type cells and smaller dark cells with round monotonous nuclei and fine granular cytoplasm. They were frequently nested. Craniopharyngioma could be recognized readily by their protein-rich poorly cellular fluid, containing cholesterol crystals and macrophages and occasional squamous cells. Meningiomas revealed two types of cuboidal meningothelial and spindle cells. The cuboidal cells might be confused with pituitary adenoma cells, although adenomas didn't reveal whorled arrangement, psammoma bodies, or poor cellular borders. The accuracy of cytologic diagnosis of these tumors was considered almost comparable to that of histopathologic examination. Neoplasms of the cerebral hemisphere are mostly of the astrocytic series. Analysis of the reliability of cytologic diagnosis of astroglial neoplasms revealed that errors were mainly due to two facts, i.e., sampling error and difficulties in microscopic interpretation (Willems and Alva-Willems 1984). When the biopsy was taken from the necrotic portion, gliosis or ordinary brain tissue, a reliable diagnosis couldn't be made cytologically. In general the error is detected much more frequently in the high-grade astrocytomas undergoing necrosis (Willems and Alva-Willems 1984). Difficulties experienced with the microscopic diagnosis on smears of astrocytic neoplasms are related to their degree of differentiation (Willems and Alva-Willems 1984). The distinguishing features of neoplastic astrocytes in one case of gliomatosis cerebri were higher cellularity, larger nuclei and irregularities of nuclear shape. These features could be the same diagnostic criteria of the gliomatosis cerebri in the brain tissue. High-grade astroglial tumors in adequate aspirates posed no problem for the cytologic diagnosis of malignancy because they showed conspicuous cellularity, pleomorphism, nuclear atypia, and mitotic figures. Glioblastoma multiforme cases, however, could not be well differentiated from high-grade astrocytomas because of similar pleomorphism and in cases of aspirating necrotic debris and failing to aspirate blood vessels with proliferation. One or two helpful distinctions are that glioblastoma multiforme on the Papanicolaou staining method reveals a higher degree of nuclear atypia and Giemsa staining is highly sensitive in demonstrating necrotic material of the tumor, giving a dark blue dirty appearance of the cells and the background. The only different cytologic feature between malignant and benign oligodendrogliomas was thought to be the cellularity. They were otherwise alike, due to monotonous polygonal cells sometimes with fried egg appearance. Arborizing capillaries with tube-like walls were infrequently seen. Chordoma, pineocytoma, and malignant lymphoma cases disclosed characteristic cytology comparable to their histologic findings. Neurilemmoma cases showed no diagnostic cytologic findings such as palisading pattern and degeneration except for scattered groups of degenerative changes or myxoid material. In general, neurilemmomas show pleomorphic, hyperchromatic or pyknotic nuclei and ill-defined cytoplasm with marked crushing artifact, irregular thick fragments, and only rarely pallisading pattern (Nguyen *et al.* 1988).

In conclusion, the cytologic diagnosis of various neoplasms of the brain could be made with relatively high accuracy and the cytologic study may complement the histologic information provided that adequate material is obtained.

However, all histologic findings were not seen in the cytologic samples and the discrepancies were always present. Technical improvements in the biopsy procedure, the cytopathologist's accumulated experience and immunohistochemical staining were thought to be important for the accurate cytologic diagnosis of the central nervous system tumors.

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