



## Original Research

# Intraoperative Imprint-squash Methods in Central Nervous System Tumors

Canan Tanik, Fevziye Kabukcuoglu

Department of Pathology, Health Sciences University, Sisli Etfal Training and Research Hospital, Istanbul, Turkey

### Abstract

**Objectives:** Central nervous system (CNS) tumors constitute 1.3% of all cancers in adults and are the seventh leading cause of death in developed countries. CNS tumors are very soft and have a gelatin-like texture. Smear technique is a very simple and fast method for the diagnosis of brain tumors.

**Methods:** In this study, we evaluated the imprint and squash cytology of 100 cases sent to the pathology clinic. The sections of the paraffin blocks were prepared after the operation in the neurosurgery clinic of the SBU Hamidiye Şişli Etfal Training and Research Hospital. The accuracy rate was 90% in the differential diagnosis of malignant tumors from the benign ones.

**Conclusion:** Cytological samples were taken from 100 cases of intracranial tumors that were operated in the neurosurgery clinic of Şişli Etfal Hospital, and the paraffin sections prepared from the biopsy materials were examined. The cases with misdiagnosis were usually differentiated from solid-hard tumors, epithelial-grade cystic structures, and medulloblastoma localized in the posterior fossa, medulloblastoma and ependymoma. However, this method has been found to be very convenient in practice due to its ease technically, low cost and equipment savings.

**Keywords:** Central nervous system; histopathology; squash cytology.

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Central nervous system (CNS) tumors account for 1.3% of all cancers in adults and are the seventh leading cause of death in developed countries.<sup>[1–3]</sup> CNS tumors are very soft and have a gelatinous appearance.<sup>[1–3]</sup> These features of tumors make the squash method convenient because it provides easy viewing of cellular details in smears and facilitates squash. Intraoperative cytology was first performed by Eisenhardt and Cushing and by Badht in 1930 and 1936.<sup>[1, 2]</sup> Intraoperative squash smear technique is a very simple

and fast method for the diagnosis of brain tumors.<sup>[3–5]</sup>

This prospective study was designed with the coordination of Şişli Hamidiye Etfal Hospital Neurosurgery Clinic and the Pathology Laboratory to provide an emergency diagnosis, to assist the operation plan, to evaluate the accuracy of intraoperative squash and imprint smears in the diagnosis of space-occupying lesions in the central nervous system by imprint slides prepared from small tissue samples taken peroperatively.

**Address for correspondence:** Canan Tanik, MD. Sağlık Bilimleri Üniversitesi, Sisli Etfal Eğitim ve Araştırma Hastanesi, Tıbbi Patoloji Anabilim Dalı, İstanbul, Turkey

**Phone:** +90 532 446 91 13 **E-mail:** canantanik@yahoo.com

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## Methods

In our study, imprint slides and sections of paraffin blocks belonging to 100 patients who were operated in the Neurosurgery Clinic of SBU Şişli Hamidiye Etfal Training and Research Hospital and sent to the Pathology Clinic between 1994 and 1996 within two years were interpreted. The cases were reinterpreted according to WHO 2016 classification. Four of the six cases interpreted as oligoastrocytoma according to the classification in 1996 were reclassified as diffuse infiltrative astrocytoma and two of them as Grade II oligodendroglioma. Tissue samples of 0.22x0.2 mm thickness obtained peroperatively were delivered to our laboratory in gauze soaked with saline. The sample was placed between two clean glass slides and crushed with sufficient pressure, two slides of squash and 6-8 imprint slides were obtained. Smears were fixed in 95% alcohol for two minutes and then stained fastly with Hematoxylin&Eosin (H&E) and PAP, and the air-dried ones were stained with May Grunewald Giemsa and Diff-Quik stains. The results were evaluated considering their clinical and radiological findings; preliminary diagnoses were delivered to the neurosurgery clinic. Tissue sent for histopathological evaluation was evaluated in paraffin sections by the same pathologist after standard tissue processing procedures. Immunohistochemical procedures were also performed. When necessary, and the decided tissue diagnosis was compared with the intraoperative cytological diagnosis. Evaluation parameters of cytological materials were as follows:

1. The general accuracy level was determined as sensitivity and specificity.
2. Incompatibility parameters were grouped as false-positivity, false-negativity, downgrade or upgrade at tumor stage and difference in the histological specification.

**Specificity:** [total number of cases - number of false-positive cases]/total number of cases  
**Sensitivity:** [total number of cases - number of false-negative cases]/total number of cases

**Specification:** [number of cases upgrading histological grade + number of cases downgrading histological grade]/total number of cases X 100.

## Results

In the evaluation of the materials, information was firstly given about whether the tumor is malignant or benign, the tumor's origin and the tumor's surgical margins. Afterwards, paraffin processing was done for the tissue samples taken peroperatively and a definitive diagnosis was provided. Paraffin results were compared after imprint and standard tissue processing.

The patients had various clinical symptoms, such as headache, nausea-vomiting, vertigo, ataxia, epilepsy according to their tumor location. Seventy of the cases (70%) were localized in the cerebral lobes, 18 in the cerebellum (18%), and 12 in the spinal cord (12%) (Table 1). The youngest of our cases was 17 months old. The oldest was 73 years old, eight of them were children, and the mean age was 43.72 years. Fifty-four of them were female and 46 of them were male with a female/male ratio 1.2 (Table 2). 88 of the cases were malignant (88%), 12 (12%) were benign lesions. Diagnosis and grading of all cases are reported in Table 3.

In the series of 100 cases that we examined, 94% success was achieved in the distinction of malignant-benign tumors, which was our main target. Cases with different results with squash smears were as follows: two mesenchymal chondrosarcomas, one unspecified childhood tumor,

**Table 1.** Localization of the cases

Localization	Number	%
Cerebral lobes	70	70
Spinal cord	12	12
Cerebellum	18	18

**Table 2.** Number of the cases according to gender

Female	Male	Female/Male ratio
54	46	1.2

**Table 3.** Case groups according to the diagnosis

Tumor type	Number of cases	%
Glioblastoma	14	14
Oligodendroglioma	8	8
Astrocytoma grade 2-3	10	10
Schwannoma	7	7
Pituitary adenoma	11	11
Meningioma (atypic included)	12	12
Carcinoma metastasis	8	8
Primary cranial Mesenchymal tumors chondrosarcoma	2	2
Leiomyosarcoma metastasis	1	1
Malignant melanoma metastasis	1	1
Inflammatory and reactive formations	6	6
Malignant peripheral nerve sheath tumor	1	1
Epidermoid carcinoma occurred on epidermoid cyst ground	1	1
Medulloblastoma	4	4
Ependymoma	3	3
Hemangioma	1	1
Intracranial cysts, craniopharyngioma	8	8

one malignant peripheral nerve sheath tumor, one well-differentiated squamous cell carcinoma developing from an epidermoid cyst, and one case which was thought to be metastasis with malignant epithelial cells, but then, no malignancy detected in the sections prepared after paraffin processing.

When we compared the cytology samples, we examined to make a definitive diagnosis with paraffin sections, the diagnostic compatibility rate in tumor type definition and grading was 90%. Cases with diagnostic incompatibility were reported in Table 4.

## Discussion

Cytological identification studies by the squash and imprint methods, as well as the frozen section for emergency diagnosis, were developed by Eisenhard and Cushing (1930), Rusell (1937), Mc Nemey (1960), Jane (1962-1969) and Marshall (1973), respectively.<sup>[1-5]</sup> Fredericksen also performed DNA flow cytometry with these small tissues in 1978. In the light of all these researches, many centers have been implementing this practice in recent years.<sup>[10-22]</sup>

Tissue samples where the Squash and Imprint methods are technically valid are small particles. It is possible to confirm the diagnosis by preparing a large number of cytological preparations without tissue loss and by making paraffin sections and immunohistochemical studies from the re-

maining parts.<sup>[6,7]</sup>

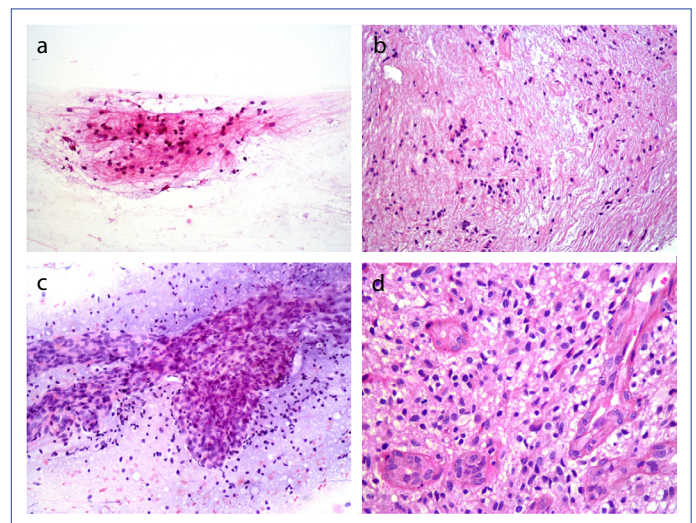
Another feature of the Squash-Imprint method that is available under our country's conditions is that preparation of samples without a cryostat, which is very costly for a pathologist working in limited technical conditions, and going for a diagnosis with Hematoxylin-Eosin, May-Grunwald-Giemsa and Diff-Quik staining techniques. In many studies in the literature, the imprint smear technique was compared with frozen-section, and the results were confirmed by paraffin processing. The rates obtained in many centers in the world can be listed as follows; 66%, 94% and 95%. The values obtained in our series are 90% and are compatible with the literature.<sup>[11-19]</sup>

In this study, which we used the Squash-Imprint method for diagnosis, we mostly observed astrocyte-derived tumors, and our diagnostic accuracy reached 98%. Fourteen of our cases in this group were glioblastoma (GBM) and showed marked pleomorphism, atypia, and high cellularity (Fig. 1c, d). A 65-year-old male patient was considered to have GBM with cytological features; the patient was diagnosed with adenocarcinoma originating from the colon in paraffin processing. Giant cells and a necrotic background, as well as highly pleomorphic shaped cells, were seen in this patient's cytological evaluation; however, since there was no clinical information about carcinoma metastasis in the preliminary evaluation of the patient, it was evaluated as primary GBM. The results of the paraffin sections examination showed that it was a metastatic carcinoma originat-

**Table 4.** Cases with diagnostic incompatibility

Six cases with incompatibility in malignant-benign distinction	6%
One case with malignant epithelial cells and thought to be a metastasis	1 case
No metastasis was detected in the paraffin	
Mesenchymal chondrosarcoma	
Evaluated in favor of meningioma in squash smears	2 cases
Spinal cord peripheral nerve sheath tumor, first defined as benign	1 case was defined as malignant peripheral nerve sheath tumor in paraffin
Evaluation in favor of simple cyst in the cranial midline in squash smears	One case of well-differentiated epidermoid carcinoma developing on an epidermoid cyst ground
Childhood tumor with an unconfirmed diagnosis	1 case

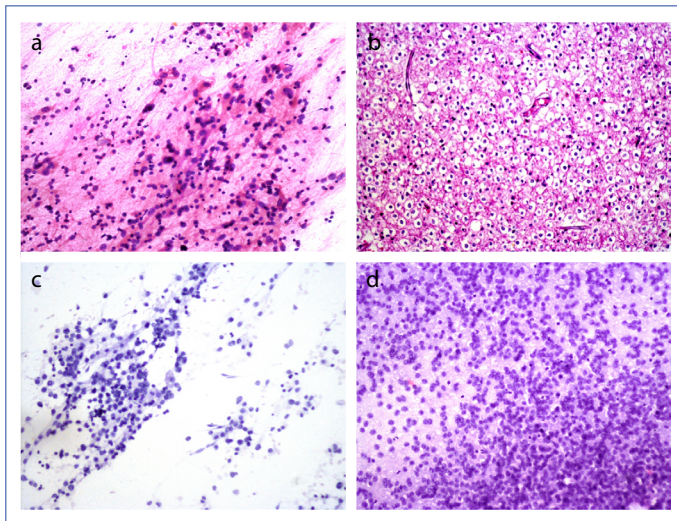
This study was presented as a poster presentation in Ankara/October 12<sup>th</sup> National Pathology Congress in 1996.



**Figure 1.** (a) Cell groups with fibrillar extensions in low-grade glioma, HEX100. (b) Images of the case with diffuse fibrillar astrocytoma after paraffin processing, HEX100. (c) Cytological view of the vascular structure conglomerated in the neurofibrillary matrix in a glioblastoma case, HEX100. (d) The appearance of the vascular structure conglomerated in the neurofibrillary matrix in the case of glioblastoma in paraffin sections, HEX200.

ing from the gastrointestinal system. In the 10 cases identified cytologically as Grade 2 astrocytomas, the diagnosis was confirmed in paraffin sections. Classical fibrillar matrix, eosinophilic globular bodies helped diagnosis in our cases, which were referred to as pilocytic astrocytoma in childhood (Fig. 1a, b). Eight cases were identified as oligodendroglioma in cytological preparations, and the diagnosis was confirmed in paraffin sections processing (Fig. 2a–d). Oligoastrocytoma definition was present in the 1993 CNS classification when this study was being conducted. The same cases were reevaluated with the morphological criteria, immunohistochemical and molecular definitions according to the 2016 classification we use today. Four of six cases were defined as diffuse infiltrative astrocytoma and two cases as oligodendroglioma.<sup>[15]</sup> The biggest changes in the 2016 classification were followed in this group.<sup>[15]</sup>

In our 12 meningioma cases, our cytological diagnoses were 100% compatible with paraffin processing results. Meningothelial cells had vesicular nuclei and prominent nucleoli, and they could be easily selected with their oval-round shapes; their whorl structure could also be observed in the cytological material. In one case of meningioma localized in the sphenoid wing, cellular atypia findings and mitosis were detected in paraffin sections and this case was evaluated as atypical meningioma. One of our cases was

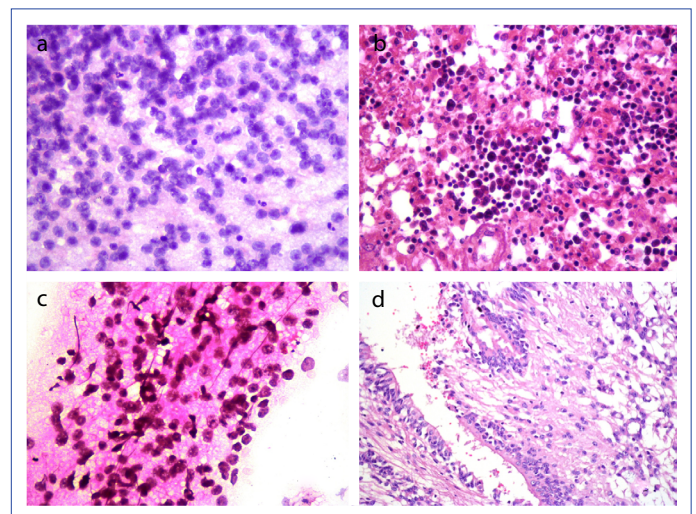


**Figure 2.** (a) Cytological appearance of thin-walled vascular structures and small normochromic round oligodendroglial cells in the neurofibrillary matrix in a case of oligodendroglioma, HEX100. (b) The appearance of thin-walled vascular structures with clear cytoplasm and small normochromic round oligodendroglial cells in the case of oligodendroglioma (eggshell finding) HEX100. (c) Cytological appearance of thin-walled vascular structures with clear cytoplasm and small normochromic round oligodendroglial cells in a case of oligodendroglioma, HEX100. (d) Hypercellularity in anaplastic oligodendroglioma case in smears, HEX100.

called angiomatous meningioma with widespread vascular structures and other findings seen in paraffin processing. Compared to other studies, similar results were observed with our study.<sup>[14–17]</sup>

In 12 cases evaluated as pituitary adenoma, cytological details consisting of small round-oval nuclei, narrow cytoplasm within cells and pseudorosette formation were observed. Necrosis, atypia and mitosis were not observed in these cases. Clinical history and radiological details were evaluated together, and results were given accordingly (Fig. 3a, b). A 100% accuracy rate was achieved compared to paraffin processing results. Similar results in the studies of Iqbal and Jaiswal have been noted in the literature.<sup>[11–16]</sup>

One of the cases that we defined as ependymoma in paraffin sections was a childhood posterior fossa tumor and was defined as a medulloblastoma with the imprint-squash technique. However, the result of paraffin processing was evaluated as ependymoma, and likewise, ependymoma was considered in smear sections in one of the four medulloblastoma cases. However, the paraffin block processing result was confirmed as medulloblastoma. When we examine these cases carefully, our reasons for misconception are that these two cases were childhood tumors and their localization was similar. In the case considered to be ependymoma, the appearance of nuclei in uniform appearance that stood one by one on a ground consisting of fibrillar material was noteworthy. Single-row pseudorosette



**Figure 3.** (a) Cytological appearance of thin-walled vascular structures and neuroendocrine cells with small normochromic round narrow cytoplasm in the pituitary adenoma, HEX100. (b) Paraffin section findings in a case of pituitary adenoma, HEX100. (c) Rosette formations in the neurofibrillary matrix in cytological samples in an ependymoma case at magnification, HEX100. (d) Arrangement of cells making ependymal rosettes in a well-differentiated ependymoma case in paraffin sections at magnification, HEX100.

formations were present occasionally; however, some cells were darker stained with small, narrow cytoplasm. In paraffin sections, it was revealed that these rosettes were very dense and the fibrillar ground was seen at the border of the tumor with the surrounding tissue, and it turned out that the hyperchromatic appearance that appeared due to the dye feature we used masked the diagnosis (Fig. 3c, d). Samples taken and the dye misled us. In the other case that we thought as medulloblastoma, it was evaluated in this direction because of the darkly stained pleomorphic shaped cells; however, when we examine it later, it was observed that this pleomorphism emerged as a result of disruption of the formation of cells during the application of the method. Diagnostic misinterpretation of both tumors creates confusion in terms of treatment protocols. While surgery is the first option in ependymoma, surgery-chemotherapy is recommended as a combination in medulloblastoma. The diagnostic confusion misleads the treatment and imposes a responsibility to the pathologist. In these cases, evaluation is required in the light of clinical and radiological information. Our accuracy rates in these cases are 50%, and this variability is also mentioned in the studies of Livniz, Bayindir, Marshall, Iqbal, in the literature, and the accuracy rates vary between 50% and 95%.<sup>[7-14]</sup>

In the 25-year-old woman, the mass adjacent to the falx was localized. It was macroscopically similar to the structure of meningioma. In our cytological evaluations, spindle-shaped cells were seen occasionally; no apparent atypia was detected. With these findings, meningioma with chondroid differentiation was considered. Afterwards, a tumoral structure consisting of cells with small round hyperchromatic narrow cytoplasm was seen around the common foci of chondroid differentiation in the sections of postoperative surgical materials. Structures similar to hemangiopericytoma and vascular clefts were observed occasionally. These findings were found to be compatible with mesenchymal chondrosarcoma when examined. When we examined the literature, it was observed that approximately 1/3 of the mesenchymal chondrosarcoma was localized in the head and neck and could originate from the falx. In the literature, patients who were diagnosed as meningioma first histologically, but later, by their recurrence, were identified as mesenchymal chondrosarcoma, were observed.<sup>[9-11]</sup> In a 60-year-old male patient, the case of mesenchymal chondrosarcoma was found to have been operated once and was diagnosed with meningioma with chondroid differentiation previously. However, when the recurrence of the tumor was examined one year later, the diagnosis of mesenchymal chondrosarcoma was confirmed. It is stated that these types of mesenchymal tumors may be confused with meningioma due to their cytological features and it

is stated that clinical-radiological features should be examined carefully.<sup>[10-14]</sup>

In two cases, cytological examination showed a large number of hypercellular epithelial cells that were located in the sellar region and on skull base and these cases were evaluated in favor of craniopharyngeoma. The other case was interpreted as a Rathke cleft cyst in paraffin sections. When these cases are examined in the literature, it has been shown that Rathke's cleft cyst and craniopharyngioma are embryologically related, both of which are developed from the remnants of Rathke's cleft.<sup>[11-14]</sup> Cystic structure covered with mature squamous epithelium and focal squamous carcinoma focus was observed in paraffin sections in the other case we considered as craniopharyngioma. It was evaluated as a well-differentiated squamous cell carcinoma developing on the squamous cyst ground. Clinical and radiological examinations did not reveal any other primary squamous cell carcinoma history and findings. The case was thought to have developed on the background of a primary cranial epithelial cyst.

When the cytological structures of these cases are examined, it has been shown that the presence of cystic structures should be considered in the differential diagnosis when interpreting in favor of craniopharyngioma due to the squamous epithelial cells we see in cytology and localization. Similar cases attract attention in the literature.<sup>[11-14]</sup>

During the evaluation of a total of eight cases defined as schwannoma and neurofibroma, their localizations, the stiffness of the material and clinical information were evaluated. Although these cases were confused with the findings of meningioma, the absence of whorl structures, the more spindle appearance of the nucleus and the palisatic sequence were evaluated in favor of schwannoma. One of our cases was taken into cytological evaluation in the first surgery. Schwannoma was diagnosed cytologically and as a result of paraffin processing. However, one month after the first operation, the tumor was found to grow again. The material taken was examined with direct paraffin block sections this time, and there was too much atypical mitosis in the tumor sections. With these findings, a diagnosis of malignant peripheral nerve sheath tumor was made. It was noteworthy that cytological samples in the first operation material had low cellularity and pleomorphism was moderate.<sup>[22-24]</sup>

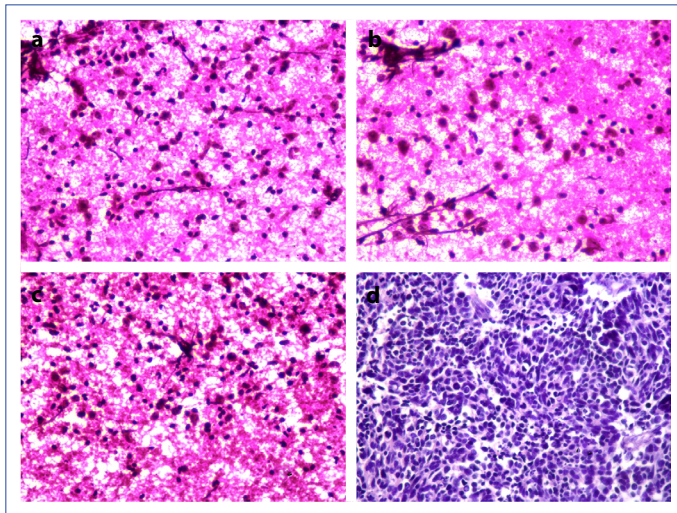
When we examined eight cases of metastatic carcinoma, it was seen that they showed an accuracy rate of 95% in tissue paraffin sections after the cytological examination. Seven cases were primary lung adenocarcinoma metastasis and one case was metastatic adenocarcinoma originating from the colon. In cytological samples, epithelial-look-

ing cells with large eosinophilic cytoplasm, loss of cohesion and accompanying necrosis, as well as polymorphonuclear leukocytes and other inflammatory elements, were important findings in the diagnosis (Fig. 4a–d). Tumors that metastasize to CNS are frequently seen in studies and in our institution.<sup>[26, 27]</sup>

In the imprint-squash method, this method is very helpful in the diagnosis in soft tumors. However, it is necessary to be careful in hard tumors, it should be emphasized that the details of the epithelial originated masses of the sellar region should not be overlooked, and all smears should be examined meticulously.<sup>[2–20]</sup> An immunohistochemical study should be performed in cytological preparations, if necessary.<sup>[12–15]</sup>

As a result, the advantages and disadvantages of the imprint-squash method are as follows:

1. Its primary advantage is saving time. On the other hand, it is very important that the technical team is few and the amount of tissue used is low. With adequate technical equipment, suitable operating room and frozen room conditions, 3-4 minutes is enough to prepare imprint-squash samples. In modern cryostats, this period is not less than 15 minutes.



**Figure 4.** (a) Small round cells whose atypical structure cannot be selected on the necrobiotic ground in cytological samples, HEX100. (b) Small round cells with a narrow cytoplasm, atypical and structure not fully selectable, showing nuclear pleomorphism in the necrobiotic ground in cytological samples and thin-walled vascular structures compatible with carcinoma metastasis, HEX100. (c) In cytological samples, small round cells with the atypical structure on necrobiotic ground that cannot be selected fully, thin-walled vascular structures, HEX200. (d) Findings compatible with primary lung-derived malignant small cell carcinoma metastasis consisting of small round cells that form groups/islands in paraffin sections at magnification, HEX100.

2. Protection of cytological detail is the 2<sup>nd</sup> important advantage. Nucleus and cytoplasmic detail and ground are preserved. Freezing artifacts in the frozen method may lead to confusion in the sections after paraffin processing.
3. Protection of the tissue: One of the most important advantages of this technique is that while a very small amount of tissue (Ex.: 0.1 mm) is required, the remaining tissue can be used in routine examinations and immunohistochemical studies. This is particularly important in small lesions, such as in the microadenomas of the pituitary gland and tissue samples taken from hard-to-reach areas.
4. Infection control: Since the pathology team does not use a knife in the imprint-squash method, they would not be at risk in suspicious cases, such as AIDS, viral encephalitis, tuberculosis and Jakob-Creutzfeld.

#### Disadvantages:

1. The smear is thick and may not evenly be distributed throughout. This may cause primary tumors to be evaluated as high grade. To standardize this technique, errors can be reduced by a single experienced person doing this.
2. When evaluating anaplasia, vascularity must be paid attention to. Endothelial proliferation helps differential diagnosis.
3. If sampling is done with a single slide, a false (+) or (-) result may be given. Sampling, in this sense, should be done with numerous slides.
4. Evaluation time should be kept in sufficient time. Slides should be fully scanned.
5. The disappearance of the tissue skeleton may not be identified if the lesion has a fibrous ground substance.

#### Conclusion

Cytological samples and paraffin sections prepared from biopsy materials taken from 100 intracranial tumors operated in Şişli Hamidiye Etfal Hospital Neurosurgery Clinic were examined in this study. In our series of 100 cases, the accuracy rate was 90% and very small tissue samples were studied. The misdiagnosis was faced generally during the differential diagnosis of solid hard tumors, epithelial cysts and localized medulloblastoma and ependymoma in the posterior fossa in childhood. However, this method was found to be quite convenient in practice due to its technical simplicity, low cost, saving on equipment, feasibility without a frozen device and no extra costs for hospitals.

## Disclosures

**Ethics Committee Approval:** The study was approved by the Local Education and Planning Committee of Şişli Etfal Training and Research Hospital in 1996.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** None declared.

**Authorship Contributions:** Concept – C.T.; Design – C.T.; Supervision – F.K.; Materials – C.T.; Data collection &/or processing – C.T.; Analysis and/or interpretation – F.K.; Literature search – C.T.; Writing – C.T.; Critical review – F.K.

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