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Case report

Cerebrospinal fluid and intraoperative squash cytology of childhood ependymoma

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Abstract. Ependymomas are glial neoplasms of central nervous system originated from the ependymal lining of the brain ventricles and spinal cord central canal, and rarely exfoliated into cerebrospinal fluid (CSF). In this case we report the cytomorphological and immunocytomorphological features of ependymoma in CSF and intraoperative squash preparations, confirmed by histology. *Case report:* The patient was a nineteen months old female presented at the University hospital of Heraklion, Crete, in a hemicoma, and was intubated. Computed tomography, scanning and magnetic resonance imaging (MRI), were performed and a mass in the posterior fossa was found. A sample of cerebrospinal fluid (CSF) was sent for cytologic evaluation. A diagnosis of ependymoma was rendered, followed by tumor resection, during which intraoperative squash smears for cytologic interpretation were obtained. Cytological consultation disclosed a grade II ependymoma (WHO grade II), with focally anaplastic features (WHO grade III).

Key words: Ependymoma, squash cytology, CSF cytology, immunocytology, histo-immunopathology

Introduction

Ependymomas are primary glial tumors of central nervous system with often very differing (varying, diverse) features, exhibiting ependymal differentiation, classified by WHO (2016) as subependymoma (WHO grade I), myxopapillary ependymoma (WHO grade I), classic ependymoma (WHO grade II), ependymoma RELA fusion positive (WHO grade II or III), and anaplastic ependymoma (WHO grade III) The latter two types are malignant with metastatic potential and have been described by Ellison et al (2016) (1).

Ependymoma in childhood ranks the third frequent tumor of CNS, following astrocytoma and medullonlastoma (2). and numbers about 2% of all pediatric cancers (3).

The vast majority of pediatric ependymomas are intracranial, two thirds of them are originated in the posterioe fossa including our case (4) The prognosis of pediatric ependymomas is poor. The 5-year outcome numbers from 39%-64% (5).

Treatment includes surgical excision, radiotherapy and chemotherapy.

Our case utilizing CSF cytology and squash cytology provides new insights in the intraoperative diagnostic approach of pediatric ependymoma.

Case report

A 19 months-old female, presented at the University hospital of Heraklion, Crete, in a hemicoma resulted from acute obstructive hydrocephalus. and was intubated. According to her parents she suffered from headaches, often worse in the morning followed by vomiting and eventually gait disturbance. They also re-

ported seizures. Prior to admission she had underwent funduscopy that showed papilledema. Additional prior neurologic examination revealed ataxia, dysmetria, and nystagmus, as also reported by parents. The patient was diagnosed by MRI with a tumor of the posterior fossa, demonstrating variable enhancement with contrast. The haematological, and biochemical parameters, were within normal limits.

Material and methods

Cytology-Immunocytology

1. CSF cytology.

A diagnostic paracentensis and CSF sample collection was performed. The CSF sample was sent for cytologic evaluation. Cytologic slides were prepared from the fluid after cytocentrifugion for 5 minutes. Five slides were fixed in 80% ethanol for Pap stain and five were airdried for Giemsa stain and immunocytochemistry.

Results.

The cytologic interpretation of the smears revealed the presence of isolated tumor cells with oval nuclei of medium size, without nucleoli or mitoses. The cytoplasm stained basophilic. Dysgerminoma, medulloblastoma, astrocytoma, ependymoma and lymphoma, were considered in the differential diagnosis. An immunocytochemical panel of antibodies was applied on air fried smears, which showed that the tumor cells were of glial origin: GFAP and S-100 positive. In contrast AFP, β-HCG, synaptophysin. LCA were nefative The cytologic diagnosis was of a glial neoplasm favoring ependymoma, based on the patient's age and anatomical site of the mass.

2. Squash cytology.

Intraoperative squash smears were prepared as follows: 1-2 mm^3 of fresh tissue from a specific area after gross evaluation was crushed between two slides to prepare smears as at first described by Adams et al (6), and reported by others (7-9).

One slide was immediately fixed in ethanol 80% for PAP stain. Additional smears were air dried for Giemsa stain, GFAP and S-100 immunostains.

Results

In squash cytology smears the tumor cells were abundant, arranged in papillary clusters or rarely rosettes (Fig. 1) or pseudorosettes (Fig. 2) with oval medium to large sized nuclei with atypia and scanty basophilic cytoplasm, GFAP (Fig. 3) and S-100 (Fig. 4) positive, confirming the CFS diagnosis of ependymoma.

Histology

Abudant material with many neuroepithelial neoplastic cells in rosette (Fig. 5)-pseudorosette. pattern with oval basophilic nuclei and mild atypia were demonstrated. Focally increased celllularity , severe nuclear atypia and mitoses were observed.

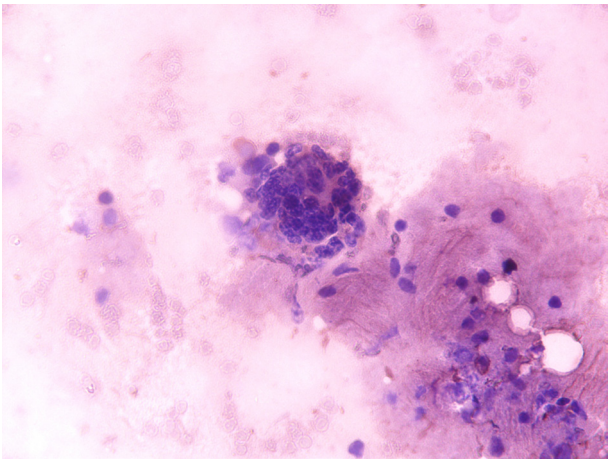

Figure 1. Pediatric ependymoma. Squash preparation. Rosette formation of neoplastic cells Giemsa stain X 400

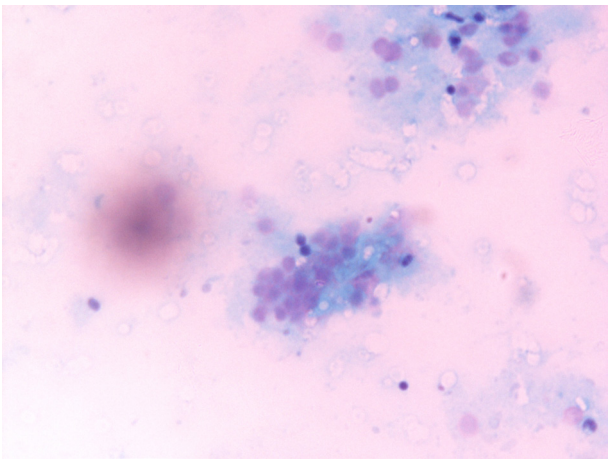

Figure 2. Pediatric ependymoma. Squash preparation. Pseudorosette formation of neoplastic cells. Pap stain X 400.

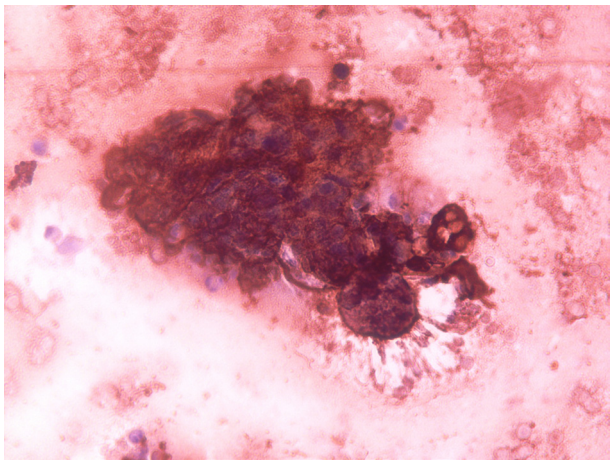

Figure 3. Pediatric ependymoma. Squash preparation. GFAP positive neoplastic cells. GFAP immunostain X400.

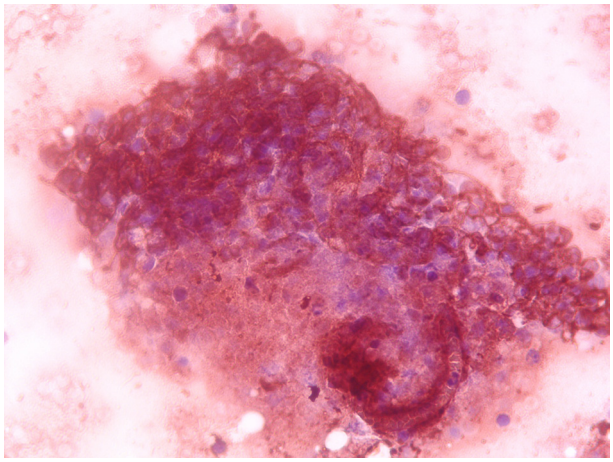

Figure 4. Pediatric ependymoma. Squash preparation. S-100 positive neoplastic cells. S-100 immunostain X 400.

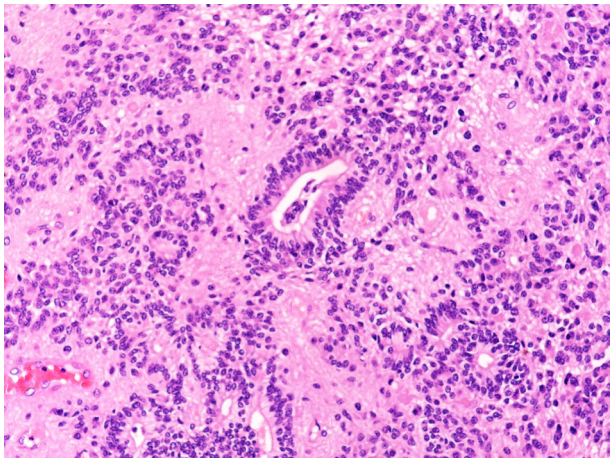

Figure 5. Pediatric ependymoma. Tumor section. Rosette pattern. Hematoxylene-Eosin stain X 200.

Immunohistology

The tumor cells were found to be positive for GFAP (Fig. 6), S-100 (Fig. 7), CD56 (Fig. 8) and negative for Synaptophysin, CD99, CKAE1/AE3. The ki-67(MIB-1) (Fig. 9) index was approximately 10%. Histologic diagnosis was of ependymoma WHO grade II, and focally WHO grade III (anaplastic ependymoma) (WHO 2016).

The patient received chemotherapy (first course) VEC (Vincristine 1,5mg/m2 Day 1, Etoposide 100mg/m2 D1-3, Cyclophosphamide 3000mg/m2 Day 1) (10).

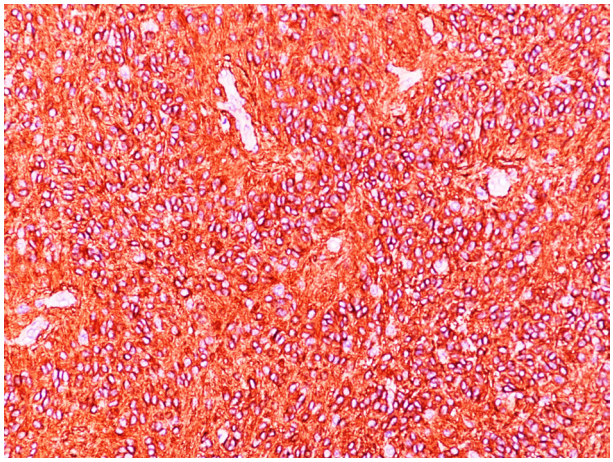

Figure 6. Pediatric ependymoma. Tumor section. GFAP immunopositive cells. GFAP immunostain X 200.

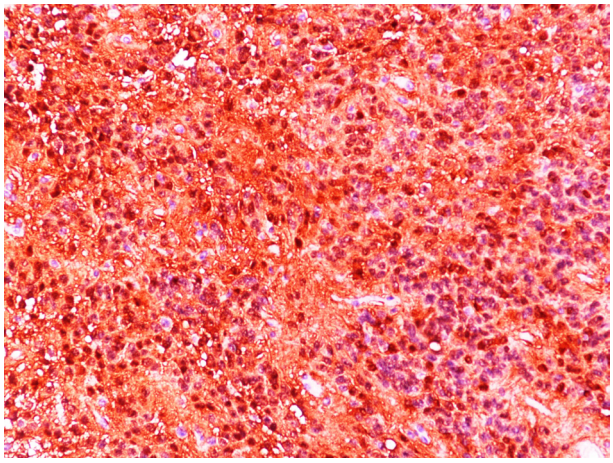

Figure 7. Pediatric ependymoma. Tumor section. S-100 immunopositive cells. S-100 immunostain X 200.

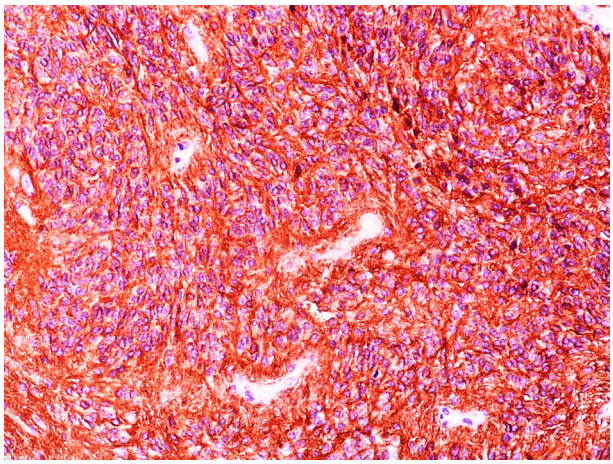

Figure 8. Pediatric ependymoma. Tumor section. CD56 immunopositive cells. CD56 immunostain X 200.

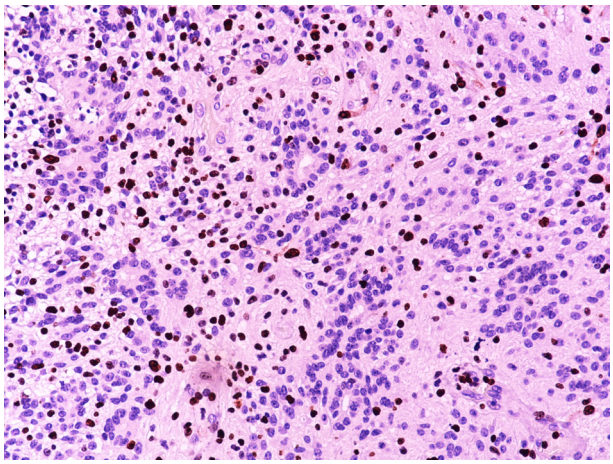

Figure 9. Pediatric ependymoma. Tumor section. MIB-1 immunopositive cells.(nuclear positivity) MIB-1 immunostain X 200.

Discussion

Ependymoma is the most common tumor in the posterior fossa of the brain. Intracranial pediatric ependymomas demonstrate significant histopathological variety between tumors and inside the same tumor. Histopathological grading is reported to be an independent outcome and predictive factor in several studies but this is not the case with other reports (11). WHO earliest classification (2016) has tried to address this important issue.

Cytopathological features of ependymomas in cerebrospinal fluid are not easily recognised in comparison to medulloblastoma and astrocytoma due to hypocellularity and failure of keeping CSF in good condition. Reports regarding the cytological diagnosis of exfoliated tumor cells from ependymomas are limited. (12). In our case single neoplastic cells with oval shaped medium sized nuclei and basophilic cytoplasm were observed. Immunocytochemical analysis showed GFAP and S-100 positivity while a negative staining was found with AFP, β-HCG, LCA and symnaptophysin. The diagnosis of a glial neoplasm favoring ependymoma based on the age of the patient and the site of the lesion was made. When papillary formations are observed in CSF a differential diagnosis should be made from choroid plexus papilloma (13).

Intraoperative cytological consultation of lesions by squash smear method is an axillary technique to help the neurosurgeon in the management of CNS tumors. It is efficient when applied upon minimal tissue pieces, provides the cytopathologist with cellular pattern (cohesive or non cohesive), cell (nuclear and cytoplasmic) morphology, and allows for immunocytochemical testing. More over it lacks the disadvantage of ice artefacts by frozen section analysis (14).

Squash cytology smear method has improved diagnostic efficacy and is an adjunct, reliable simple and cheap tool for the neurosurgeon in the operating theatre (14-17).

Squash smear cytology has gained attraction because of image guided stereotactic biopsies. A rapid diagnosis of nervous system lesions helps the neurosurgeon to plan the extent of surgery, The soft consistency of the CNS tumors is better suited for squash cytology, which in fact is an obstacle for frozen section. Squash smears are inexpensive and no special skilfulness is required to be obtained. No particular supplies are needed and minute tissue pieces can be employed, thus enough tissue is available for paraffin section examination. Failure to manage thickness, crushing artefacts, and inappropriate smearing are the limitations of squash smears.

Interpretation of the smears in our case showed the presence of medium sized cells with oval nuclei, scanty basophilic cytoplasm, organized in papillary aggregates and rarely forming pseudorosettes. GFAP and S-100 positive, findings consistent with ependymoma and thus enhancing our previously CSF diagnosis.

In our case the use of intraoperative cytology not only helped the surgeon in rapid diagnosis of epend-

ymoma, also it was ensured that minimum injury is caused to the normal brain structures surrounding the tumor

Hallmarks of ependymoma by cyto-histopathology are the presence of papillary like formations, vascular proliferation and the presence of rosettes or pseudorosettes, yet this is not always the case (18, 19). On the other hand there are CNS tumors that demonstrate rosette formation by cytopathology, and misdiagnosed as ependymomas like rosette forming glioneuronal tumors (RGNT) (20).

Histopathology in our case showed a neoplasm composed of neuroepithelial cells formind rosettes or pseudorosettes with basophilic rounded nuclei with little atypia. Foci of high cellularity and increased cellular atypia were demonstrated. Immunohistochemical study showed that neoplastic cells strongly expressed GFAP, S-100, and CD56 in contrast with negative expression by synaptophysin, CD99, and cytokeratin AE1/AE3. Proliferation index ki-67, by MIB-1, was uneven (varied) and in highly cellular areas measured up to 10%. Histological diagnosis was of ependymoma WHO grade II and focally WHO grade III (anaplastic).

In conclusion our case enhances the value of intraoperative squash smear cytology in the diagnostic approach of pediatric ependymoma when combining morphological and immunocytochemical features.

Conflicts of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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